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- The Journal of Clinical Pharmacology - January 2004
Drug-drug interactions are of great concern in clinical practice and can be manifested by an effect of one drug on the exposure to a second drug (i.e., a pharmacokinetic interaction) or an effect of one drug on a second by alteration of the response to the second drug (i.e., a pharmacodynamic interaction). While examples of the latter exist, the preponderance of interactions is of the former type. Instances of interactions that arise via the inhibition or induction of drug-metabolizing enzymes have been very frequently described in the scientific literature, on drug product package inserts, on drug labels, and even in the popular press. In the vast majority of these cases, the drug interaction occurs via alterations in the activity of cytochrome P450 enzymes; that is, one drug (the “perpetrator”) inhibits or induces the activity of a specific cytochrome P450 enzyme, thus causing an effect on the metabolic clearance of a second drug (the “victim”). If the second drug has a wide therapeutic margin, the interaction may be clinically insignificant in that the increase in exposure will not result in deleterious effects. However, if the victim drug possesses a low therapeutically index, then the decreased metabolic clearance and resulting increase in exposure may manifest itself in a clinically relevant interaction.

While cytochrome P450 enzymes have been and continue to be a major focus of drug interactions, alterations in the activities of other drug-metabolizing enzymes can also be an underlying mechanism of drug-drug interactions. However, these other enzymes have rarely been examined for their susceptibility to inhibition or induction by frequently used drugs.

Aldehyde oxidase is a molybdenum cofactor-containing soluble enzyme present in the liver and other tissues of several mammalian species. Despite its name, aldehyde oxidase is involved not only in the oxidation of aldehydes to carboxylic acids but also in the oxidation of nitrogen-containing heterocyclic compounds and the reduction of nitro-aromatic compounds, isoxazole, and isothiazole ring systems. In the metabolism of drugs, it has been shown to be involved...
in the metabolism of the antiviral agent famciclovir, the hypnotic agent zaleplon, the antiepileptic agent zonisamide, and the antipsychotic agent ziprasidone. It has also been shown to be involved in the conversion of nicotine ∆\(^{1(5)}\) iminium ion, an intermediary metabolite in the conversion of nicotine, to cotinine, as well as the metabolism of the intermediary aldehyde metabolites of citalopram and tamoxifen, among others. In humans, aldehyde oxidase is represented by a single gene product, although three inactive pseudogenes for this enzyme are also present in the human genome. Compared to the cytochrome P450 system, this offers a much simpler situation in which to address potential drug interactions using in vitro approaches.

The potential for drug interactions due to inhibition of human aldehyde oxidase has not been systematically examined. In characterizing the biochemistry of this enzyme in humans and animal species, several compounds, including some drugs, have been identified as inhibitors. These include drugs such as chlorpromazine and hydralazine and the endogenous hormone β-estradiol. Some agents cause inhibition of aldehyde oxidase across several species, such as menadione, while others appear to be selective inhibitors of aldehyde oxidase from a single species (e.g., methadone and rat aldehyde oxidase). Only one clinical drug interaction, between cimetidine and zaleplon, has been ascribed to inhibition of aldehyde oxidase. However, it should be noted that cimetidine is a very weak in vitro inhibitor of human aldehyde oxidase.

An objective of the studies described in this report is the in vitro assessment of aldehyde oxidase inhibition by more than 200 frequently prescribed drugs and related compounds. These inhibition data are intended to be of use by clinical pharmacologists in the design of in vivo studies to address the potential role of aldehyde oxidase inhibition in drug interactions. A second objective is to gain an understanding of structural determinants important for aldehyde oxidase inhibition. The development of an assay for human liver aldehyde oxidase–catalyzed phthalazine oxidation (Figure 1) and its utilization in the measurement of inhibition are described. Previous reports have described the characteristics of phthalazine as a substrate for aldehyde oxidase and led to the selection of phthalazine oxidase as a suitable marker activity in human liver cytosol.

**METHODS**

**Materials**

Phthalazine, 1-phthalazinone, and 4-methyl-1-phthalazinone were obtained from Aldrich Chemical Co. (Milwaukee, WI). The 239 drugs and chemicals tested as inhibitors were obtained from one of the following sources: Sigma Chemical Co. (St. Louis, MO), Aldrich Chemical Co. (Milwaukee, WI), RBI (Natick, MA), Biomol (Plymouth Meeting, PA), Research Diagnostics (Flanders, NJ), Apin Chemical Co. (Abingdon, UK), and Sequoia Research Products (Oxford, UK), or they were from the chemical sample bank of Pfizer, Inc. (Groton, CT). Human liver cytosolic fraction, pooled from 10 individual donors, was purchased from BD-Gentest Corp. (Woburn, MA). Other reagents and chemicals were obtained from common commercial suppliers.

**In Vitro Incubation Conditions**

Incubation mixtures consisted of human liver cytosol (0.05 mg protein/mL) and phthalazine (1.0-200 µM) in 25 mM potassium phosphate buffer, pH 7.4, containing 0.1 mM ethylenediaminetetraacetic acid (EDTA). The incubation volume was 0.2 mL. Inhibitors were added as DMSO stocks to a final solvent concentration of 1% (v/v). Initial experiments demonstrated that this concentration of DMSO had no effect on phthalazine oxidase activity, and all subsequent control incubations contained this solvent concentration. Phthalazine solutions (0.1 mL), with or without inhibitor, were preincubated at 37°C, and reactions were commenced with the addition of a mixture of cytosol, EDTA, and buffer (0.1 mL) that had been prewarmed to 37°C. An initial determination of the linearity of reaction velocity with protein concentration and time revealed that after 2.5 minutes, reaction velocities began to diminish; therefore, all incubations were conducted for 2.5 minutes. Formic acid (0.05 mL, 1 M), containing 4-methyl-1-phthalazinone as an internal standard (Figure 1), was added to terminate the reactions. Terminated incubation mixtures were subject to filtration through a Millipore Multiscreen-HA 0.45-µm mixed cellulose ester 96-well membrane vacuum filtration module into 96-well plates. The resulting filtrate was analyzed by HPLC-MS.

![Figure 1. Reaction of phthalazine to 1-phthalazinone.](image-url)
Analysis of 1-Phthalazinone by HPLC-MS

Filtered incubation mixtures were injected (0.02 mL) onto an Aqua reverse-phase C18 column (2.5 x 50 mm; 5 µ; Metachem, Torrance, CA) preequilibrated in 20 mM acetic acid (adjusted to pH 4.0 with NH₄OH) containing 5% CH₃CN at a flow rate of 0.5 mL/min. The HPLC system consisted of two Shimadzu LC-10AD pumps (Shimadzu, Columbia, MD), a CTC PAL autoinjector (CTC Analytics, Carrboro, NC), and a Micromass Ultima tandem quadrupole mass spectrometer (Micromass, Beverly, MA) operated in the multiple-reaction monitoring mode. The mobile phase was maintained at initial conditions for 0.5 minutes followed by a linear gradient to 77% CH₃CN at 3 minutes. The effluent was introduced into an ion spray source, and tune file parameters were as follows: capillary, 3.0; cone, 30; source temperature, 135°C; desolvation temperature, 350°C; cone gas, 190; desolvation gas, 750; entrance, –5; collision, 30; and exit, 1, with other potentials optimized to maximize the signal. 1-Phthalazinone was monitored with the transition m/z 147 to 90, and the internal standard was monitored with the transition m/z 161 to 102. Retention times for analyte and internal standard were 1.6 and 1.7 minutes, respectively. Quantitation was accomplished by extrapolation from a standard curve ranging from 1.0 to 100 ng/mL (6.85-685 nM). Assay standards were within ± 15% of nominal values. An example chromatogram and a representative standard curve are shown in Figure 2.

An alternate method was used in some cases in which all aspects were similar to the above except that detection was accomplished with a PE-Sciex API100 mass spectrometer (Thornhill, Ontario, Canada) following ions m/z 147 and 161 for analyte and internal standard, respectively. The HPLC pumps were Agilent 1100 (Agilent, Palo Alto, CA), and the gradient consisted of 14% CH₃CN for the first 3 minutes, followed by a linear increase to 77% CH₃CN over 0.5 minutes and reequilibration over the next 6.5 minutes. Retention times for phthalazinone and internal standard were 1.1 and 2.0 minutes, respectively. Mass spectrometer settings were as follows: source potential, 4500; source temperature, 450°C; orifice voltage, 35; ring potential, 100; and Q1, –5, with other potentials optimized to maximize the signal. Assay standards were within ±15% of nominal values. The activity measurements in control incubations were the same using either method of mass-spectrometric detection (single or tandem quadrupole instruments).

Data Analysis

Enzyme kinetic parameters and IC₅₀ values were obtained by nonlinear regression using SigmaPlot v8.0 (SPSS, Chicago, IL). The following equation was used to fit the phthalazine substrate saturation data:

\[ v_i = \frac{V_{max} \cdot [S]}{[S] + K_{M} + \left(\frac{[S]^2}{K_s}\right)} \]

where \( K_M \) represents the apparent Michaelis constant, \( V_{max} \) is the maximum reaction velocity, and \( K_s \) is the inhibition constant for phthalazine at high concentrations.
RESULTS

The enzyme kinetics of aldehyde oxidase–catalyzed conversion of phthalazine to 1-phthalazinone in pooled human liver cytosol were determined to select an appropriate concentration of substrate at which to test compounds for inhibition. A substrate saturation curve is plotted in Figure 3. The reaction exhibited kinetic behavior consistent with substrate inhibition at high substrate concentrations. Enzyme kinetic parameters were determined as follows: $K_M = 2.6 \mu M$, $V_{max} = 1.8$ nmol/min/mg cytosolic protein, and $K_s = 280 \mu M$. Thus, all inhibition determinations used a phthalazine concentration of 2.0 µM to remain $\leq K_M$ and easily identify competitive inhibitors. The well-characterized aldehyde oxidase inhibitor, menadione, was examined for inhibitory potency versus oxidation of phthalazine, and the IC$_{50}$ value was determined to be 0.20 µM (Figure 4). Therefore, all subsequent examinations of inhibitors included menadione as a positive control.

Initially, 239 drugs and other xenobiotics were examined for the potential to inhibit human aldehyde oxidase activity at a test concentration of 50 µM in order to identify those compounds meriting further evaluation. The data are listed in Table I. Compounds demonstrating 80% or greater inhibition include compounds from a variety of drug classes, including phenothiazines, steroids, tricyclic antidepressants, estrogenic agents, atypical antipsychotics, calcium channel blockers, and some other agents such as ondansetron, metoclopramide, loratadine, and tacrine. In some cases, a moderate apparent stimulation of activity was observed (e.g., fluconazole, nitrofurantoin, buspirone, etc.).

Thirty-six compounds exhibited 80% or greater inhibition at an inhibitor concentration of 50 µM, and thus a determination of IC$_{50}$ was made. These values are listed in Table II, and the structures of these compounds are shown in Figure 5. Most of the drugs that are the potent inhibitors of aldehyde oxidase are agents used for disorders of the central nervous system (e.g., antipsychotic and antidepressant agents). As a class, phenothiazine drugs potently inhibited aldehyde oxidase, with perphenazine, thioridazine, trifluperazine, promethazine, chlorpromazine, and promazine exhibiting IC$_{50}$ values between 0.033 and 1.6 µM. Estrogens and selective estrogen receptor modulators were also potent inhibitors (raloxifene, ethinyl estradiol, estradiol, and tamoxifen). Of the 239 compounds...
### Table I

Effect of 239 Drugs and Other Xenobiotics at a Concentration of 50 µM on the Oxidation of 2 µM Phthalazine Catalyzed by Human Liver Aldehyde Oxidase

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication or Use</th>
<th>% of Control Activitya (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen (paracetamol)</td>
<td>Analgesic/antipyretic</td>
<td>64 ± 6</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>Analgesic/antipyretic</td>
<td>100 ± 20</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>Antiviral</td>
<td>110 ± 9</td>
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<tr>
<td>Albuterol (salbutamol)</td>
<td>Bronchodilator</td>
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<tr>
<td>Alendronate</td>
<td>Antiosteoperatic</td>
<td>93 ± 18</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>Antihyperuricemic</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>Anxiolytic</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>Amantadine</td>
<td>Antiviral</td>
<td>92 ± 10</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>Antiarrhythmic</td>
<td>39 ± 6</td>
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<tr>
<td>Amlopiptyline</td>
<td>Antidepressant</td>
<td>9.4 ± 4.7</td>
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<td>Amlodipine</td>
<td>Antihypertensive/antianginal</td>
<td>12 ± 6</td>
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<td>Amodiaquine</td>
<td>Antimalarial</td>
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<td>Amoxacillin</td>
<td>Antibacterial</td>
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<td>Atenolol</td>
<td>Antihypertensive</td>
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<td>Atorvastatin</td>
<td>Antihyperlipidemic</td>
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<td>Azithromycin</td>
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<td>Benzonolate</td>
<td>Antitussive</td>
<td>118 ± 19</td>
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<td>Bisoprolol</td>
<td>Antihypertensive</td>
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<td>Bupropion</td>
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<td>Buspirone</td>
<td>Anxiolytic</td>
<td>136 ± 8</td>
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<td>Butalbital</td>
<td>Sedative/hypnotic</td>
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<td>Butorphanol</td>
<td>Narcotic analgesic</td>
<td>93 ± 25</td>
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<td>Caffeine</td>
<td>Stimulant</td>
<td>102 ± 9</td>
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<td>Candesartan</td>
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<tr>
<td>Clozapine</td>
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<th>Drug</th>
<th>Indication or Use</th>
<th>% of Control Activity (Mean ± SD)</th>
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<td>Codeine</td>
<td>Narcotic analgesic/antitussive</td>
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<td>Colchicine</td>
<td>Gout suppressant</td>
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<td>Cyclobenzaprine</td>
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(continued)
### Table I (continued)

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<th>Drug</th>
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<th>% of Control Activity (Mean ± SD)</th>
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<tr>
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(continued)
### Table I

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<th>% of Control Activity (Mean ± SD)</th>
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<td>Naltrexone</td>
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<tr>
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(continued)
Table I  (continued)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication or Use</th>
<th>% of Control Activitya (Mean ± SD)</th>
</tr>
</thead>
<tbody>
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<td>Salmeterol</td>
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Values represent the average of triplicate determinations. Italicized values represent those exhibiting 80% inhibition or greater.

a. The control reaction velocity (with 1% v/v DMSO) was 0.80 (± 0.11) nmol 1-phthalazinone formed/min/mg cytosolic protein. Data represent the mean ± SD for incubations conducted in triplicate. For data reported in italics, the data represent n = 6 incubations, except erythromycin (n = 3). For menadione, n = 24.

b. A value for hydralazine could not be calculated. Hydralazine yielded a small quantity of 1-phthalazinone, which interfered with the assay.
pounds examined, raloxifene was the most potent aldehyde oxidase inhibitor, with an IC$_{50}$ of 2.9 nM. Most tricyclic antidepressants were potent inhibitors, as well as other compounds that possess similar tricyclic substituents such as the atypical antipsychotic agents olanzapine, clozapine, and quetiapine, as well as other agents such as loratadine and cyclobenzaprine. The dihydropyridine calcium channel blocker felodipine was a potent inhibitor (IC$_{50} =$ 0.30 $\mu$M), amlodipine was weaker (IC$_{50} =$ 5.5 $\mu$M), and nifedipine was weaker still (79% inhibition at 50 $\mu$M). The only other cardiovascular drugs demonstrating substantial inhibition of aldehyde oxidase were verapamil and propafenone. Fused cyclic analogs of 4-aminopyridine demonstrated potent inhibition (tacrine, amodiaquine, and quina-
crine).

**DISCUSSION**

Unlike the cytochrome P450 enzymes, aldehyde oxidase has not been the focus of intense investigation of inhibition or drug interactions. This report describes the most comprehensive and systematic investigation to date on the potential of commonly used medications to inhibit aldehyde oxidase. Of the 239 compounds investigated, numerous agents were found to inhibit aldehyde oxidase, as assessed through measurement of phthalazine oxidation in pooled human liver cytosol.

Most of the drugs used in this investigation were selected on the basis of frequency of use rather than on the basis of structural identities, although many different chemical entities are represented. It can be seen from Table II and Figure 5 that no common physicochemical properties distinguish the most potent inhibitors from the weaker inhibitors listed in Table I. However, most of the compounds in Figure 5 contain a weakly basic substituent, whereas acidic compounds did not exhibit inhibitory activity. The inhibitors cannot be classified as substrate analogs, unlike compounds such as hydralazine, isovanillin, and amscarcine, which have been identified as potent aldehyde oxidase inhibitors in previous studies. However, lipophilicity appears to be an important factor in determining inhibitor potential. This has been noted previously for the binding of aldehyde oxidase substrates. In the present study, raloxifene was the most potent inhibitor, with an IC$_{50}$ value of 2.9 nM. Compounds of the similar therapeutic class—tamoxifene, ethinyl estradiol, and estradiol—were also inhibitors but not as potent as raloxifene. Many other steroid molecules did not show appreciable inhibition, and it appears that phenolic steroids are inhibitors whereas sterones are not, consistent with previous observations on a more limited set of compounds.

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC$_{50}$ (µM) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raloxifene</td>
<td>0.0029 ± 0.0003</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>0.033 ± 0.011</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>0.16 ± 0.07</td>
</tr>
<tr>
<td>Menadione</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>Trifluperazine</td>
<td>0.24 ± 0.08</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>0.26 ± 0.07</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td>Felodipine</td>
<td>0.30 ± 0.08</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>0.48 ± 0.17</td>
</tr>
<tr>
<td>Loratadine</td>
<td>0.49 ± 0.13</td>
</tr>
<tr>
<td>Promethazine</td>
<td>0.51 ± 0.26</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>0.57 ± 0.15</td>
</tr>
<tr>
<td>Etylinal estradiol</td>
<td>0.57 ± 0.15</td>
</tr>
<tr>
<td>Norclomipramine</td>
<td>0.60 ± 0.14</td>
</tr>
<tr>
<td>Amodiaquine</td>
<td>0.74 ± 0.07</td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>0.85 ± 0.46</td>
</tr>
<tr>
<td>Maprotiline</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>Promazine</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>2.2 ± 1.5</td>
</tr>
<tr>
<td>Loxapine</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td>Propafenone</td>
<td>2.5 ± 1.0</td>
</tr>
<tr>
<td>Domperidone</td>
<td>3.0 ± 1.4</td>
</tr>
<tr>
<td>Cyclobenzaprine</td>
<td>3.1 ± 1.2</td>
</tr>
<tr>
<td>Quinacrine</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>3.5 ± 1.6</td>
</tr>
<tr>
<td>Verapamil</td>
<td>3.5 ± 1.5</td>
</tr>
<tr>
<td>Clozapine</td>
<td>4.4 ± 1.8</td>
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<tr>
<td>Tacrine</td>
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<tr>
<td>Amodipine</td>
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<td>Erythromycin</td>
<td>15 ± 6</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>31 ± 1</td>
</tr>
</tbody>
</table>

Values represent the mean ± SE of triplicate determinations.
Figure 5. Structures of human liver aldehyde oxidase inhibitors.
sant agents and drugs possessing ring systems similar to the tricyclic antidepressants but with different indications (i.e., loratadine and cyclobenzaprine) were inhibitory to aldehyde oxidase. Some atypical antipsychotic agents that also possess a tricyclic system were aldehyde oxidase inhibitors (olanzapine, clozapine, quetiapine, and loxapine). In addition, several compounds of unrelated structures demonstrated weak inhibition, such as amodiaquine, ondansetron, ketoconazole, erythromycin, and others. Some agents caused a moderate stimulation of phthalazine oxidase activity (e.g., fluconazole, buspirone, nicotine, nitrofurantoin, etc.). As aldehyde oxidase is a redox enzyme, stimulation of oxidase activity may be observed if the compound added can serve as an alternative to molecular oxygen as an electron acceptor under the in vitro conditions employed in this study. However, the implications of these results to any clinical drug interaction would require further experimentation.

Clinical drug interactions occurring via inhibition of aldehyde oxidase have yet to be well established. This may be due to the fact that few drugs are cleared primarily by aldehyde oxidase–mediated metabolism. Demonstration of the inhibition of aldehyde oxidase in vivo has been accomplished by treatment with hydralazine in guinea pig and rabbit.23,26 Aldehyde oxidase is involved in the intermediary metabolism of several agents, such as conversion of nicotine Δ(1′)′ benzisoxazole, and benzisothiazole ring systems.8,9,29 Catalyze reduction reactions on aryl nitro substituents, oxidase,5 and thus coadministration of zaleplon and a potent aldehyde oxidase inhibitor could result in exacerbation of the sedative effect of zaleplon. Indeed, a moderate pharmacokinetic interaction between cimetidine and zaleplon has been described in the product label for zaleplon. However, as described in this report, several more drugs are far more potent inhibitors of aldehyde oxidase than cimetidine, which could potentially cause a more profound interaction (e.g., raloxifene, phenothiazine antipsychotic agents, etc.). Aldehyde oxidase is required for the oxidative bioactivation of the antiviral prodrug famciclovir to the active metabolite penciclovir.5,24 Coadministration of famciclovir and a potent aldehyde oxidase inhibitor could reduce or abolish the antiviral efficacy. Finally, a hypothesis exists regarding a role for molybdenum cofactor enzymes in free radical generation and alcohol-related carcinogenesis,27 and thus inhibition of aldehyde oxidase in vivo could provide a means to test this hypothesis and potentially serve as a chemoprevention approach.

The in vitro data described in this report should be useful in the design of clinical drug interaction studies and aid in the selection of drugs to test as aldehyde oxidase inhibitors in vivo. Prediction of the extent of drug interactions from in vitro data is difficult, as it is not clear what parameters, in addition to in vitro inhibition potency, are required for accurate prediction. Other factors such as dose of the inhibitor, fraction absorbed, unbound fraction, fraction of the drug that is cleared via the affected enzyme, and so forth can also be important determinants in drug interactions. Combinations of in vitro inhibitory potency data and factors such as dose and free fraction would suggest that raloxifene should be examined for the potential to affect aldehyde oxidase–metabolized drugs in a clinical study. Circulating unbound concentrations of raloxifene (< 0.15 nM28) may not be high enough to cause substantial inhibition of aldehyde oxidase. However, concentrations in the liver, a primary site of aldehyde oxidase expression, after a standard 60-mg dose during the absorptive phase could easily exceed 2.9 nM (the IC50 determined for raloxifene and aldehyde oxidase in the present study) and affect drug interactions during first pass.

It should be noted that aldehyde oxidase can also catalyze reduction reactions on aryl nitro substituents, benzisoxazole, and benzisothiazole ring systems.8,9,29 However, the data described in this report only focus on aldehyde oxidase–catalyzed oxidation reactions. It cannot be assumed that compounds that demonstrate an effect on phthalazine oxidation, as a model reaction for an oxidative biotransformation, would necessarily have a similar effect on aldehyde oxidase–catalyzed reduction reactions. The potential for these drugs to inhibit aldehyde oxidase–catalyzed reduction reactions requires further investigation.

In conclusion, several drugs have been demonstrated to be human aldehyde oxidase inhibitors in vitro. These data will be of use in the development of structure-activity relationships for aldehyde oxidase inhibition. It remains to be determined whether aldehyde oxidase inhibitors identified using in vitro methods will be aldehyde oxidase inhibitors of clinical significance in vivo. These data should prove useful in selecting potential inhibitors for clinical investigation.

REFERENCES


Global Burden of Disease (GBD) for Hepatitis C

The Global Burden of Hepatitis C Working Group

Hepatitis C virus (HCV) infection is now a global public health issue. However, the global burden of disease attributable to HCV infection is unknown. The objectives of this WHO informal consultation included the following: (1) defining a strategy to estimate the global burden of disease (GBD) associated with HCV infection in terms of morbidity and mortality, (2) describing the natural history of HCV infection in terms of morbidity and mortality, and (3) identifying areas for which more research is needed. The GBD project is an attempt to examine all causes of morbidity and mortality using an approach common to all conditions. The World Health Organization (WHO) already has estimated the burden of disease associated with hepatitis B virus (HBV) infection and is now about to conduct the same analysis for HCV infection. A review has been conducted to estimate the prevalence of HCV infection by age, gender, and region. These figures can be used to estimate incidence, although there are a number of areas of uncertainty. Combined with natural history parameters, incidence estimates could be used to estimate the future burden due to current infections. However, the present model is not validated and requires calibration before it can be used. A consensus was reached over the strategies to be used to (1) estimate the current burden due to past infections and (2) estimate the future burden due to current infections. Provisional expert consensus was reached over natural history parameters and cofactors that influence them. However, systematic literature reviews and meta-analysis are preferable for obtaining estimates to be included in models. Areas deserving future research include (1) obtaining a better estimate of HCV infection prevalence by age groups, (2) characterizing the various morbidity states associated with HCV infection and their disability weights, (3) understanding the long-term natural history of HCV infection beyond 20 years after infection, and (4) estimating the prevalence (and numbers of) of HCV infection among the drug-using population worldwide. A working group was created to address unmet needs and to assist the WHO in estimating the GBD associated with HCV infection.

Keywords: Hepatitis C virus; global burden of disease; World Health Organization; morbidity; mortality; GBD project

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MODELING HEPATITIS C GLOBAL BURDEN OF DISEASE

A Global Perspective on Hepatitis C

Hepatitis C has become an issue of global importance. It is not only of concern to industrialized countries. In Egypt, for example, the impact of hepatitis C virus (HCV) infection exceeds that of HIV. This World Health Organization (WHO) informal consultation on the global burden of disease (GBD) caused by HCV infection was organized for two reasons:

- The WHO needs burden of disease estimates to make policy decisions.
- The world is concerned with hepatitis C and is eager for proper guidance.

In the past, the WHO estimated the prevalence of HCV infection worldwide and published the results in...
the Weekly Epidemiological Record. However, these estimates need to be revised. In addition, preliminary, unpublished estimations of the global burden of disease have been made but need improvement.

This meeting has addressed three key areas:

1. the strategy to estimate the global burden of morbidity and mortality associated with HCV infection;
2. the natural history of HCV infection, including “healthy individuals,” morbidity, and mortality; and
3. the areas for which more research is needed.

About the Global Burden of Disease

Rationale of the GBD Project

National and international health policies should be based on accurate and meaningful health information. However, much of the information collated cannot be directly translated into policy. Health data from routine statistics or epidemiological studies are often fragmented, frequently concentrate on fatal health outcomes, and may only be partially available. Studies that investigate particular conditions may exaggerate claims on mortality. This is largely a reflection of comorbidity, in which several coexisting pathologies contribute to and compete for the cause of death. Moreover, traditional statistics use a variety of different measures, which do not permit direct comparisons of the cost-effectiveness of different interventions. The GBD project addresses these problems using a single metric, the disability-adjusted life year (DALY).

GLOBAL BURDEN OF DISEASE FOR HEPATITIS C

DALY is the measure used to quantify the health gap. It combines years of life lost (YLLs) and years lived with disability (YLDs) for varying degrees of severity; time itself becomes the common metric for death and disability. Years of life lost are weighted according to age (because a year of life lost does not have the same value at all ages) and discounted by 3% per year. Considering weighting and discounting, the value of an early death is reduced to approximately 75% of its original value in 10 years. GBD does not take into account the fact that life expectancy may increase over time in the future. There is not enough evidence to modify that in future projections. Japan, the country with the longest life expectancy, is being used.

GBD estimates age-specific death rates by sex using a variety of sources of data on mortality (vital registration,* surveys,† and epidemiological studies). From these, life tables can be derived using standard methods. The number of deaths abstracted from the life tables provides a “mortality envelope,” which serves to limit the total number of deaths from all specific causes. GBD uses a grading system to estimate data quality and conducts uncertainty analyses to reflect this quantitatively.

Goals

The goals of the GBD project are to

- decouple evidence from advocacy,
- use both fatal and nonfatal measures of health outcomes, and
- use a single metric to estimate burden and cost-effectiveness.

Objectives

The objectives of the GBD project are to

- estimate mortality by age, sex, and regions for 130 causes of deaths and their sequelae;
- estimate other epidemiological parameters;
- evaluate risk factors; and
- project burden of disease into the future.

Classification of Outcomes

For the purpose of GBD, health outcomes are classified as follows:

1. communicable, maternal, perinatal, and nutritional diseases;

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* The quality of vital registration varies from region to region. This quality is high in established market economies and the former socialist republics of Eastern Europe but low in many other places. However, the trend is toward improvement.

† Including Demographic and Health (DHS) surveys.
2. noncommunicable diseases; and
3. injuries.

This classification is used to prevent diseases from being counted twice.

Currently, hepatitis C virus infection is only counted as “acute and chronic hepatitis” (in group 1) as the data have not yet been made available to divide the chronic outcomes (hepatocellular carcinoma and chronic liver disease that are in group 2) into various causes (e.g., hepatitis B virus [HBV] infection, HCV infection, alcohol, and others). This could be revised if data are made available. However, the model ultimately needs to be built up from both ends and report on incidence and prevalence using the natural history model.

Modeling Hepatitis B from the WHO’s Point of View

Methods Used

One of the main input parameters of the hepatitis B model is the prevalence of chronic infection. This decision was made because (1) no estimates of incidence were available, and (2) the majority of the burden of disease attributable to HBV infection occurs during adulthood as a consequence of chronic infection acquired early in life. Mortality and morbidity from hepatocellular carcinoma and cirrhosis were estimated among patients chronically infected (HBsAg positive). Age- and gender-specific mortality rates among chronically infected patients that were derived from Gambian studies were applied to the prevalence of HBsAg in the population by age, sex, and region. The Gambian project generated good-quality data based on extensive surveillance. The Taiwan province of China generated very similar estimates. In addition to the burden estimates for mortality associated with chronic infection, additional work is ongoing to estimate the burden associated with chronic morbidity and acute HBV infection.

Results

The GBD hepatitis B model estimates that, in 2000, there were approximately 360 million persons with chronic HBV infection, nearly 5.7 million cases of HBV-related clinical disease, and just over half a million of HBV-related deaths. This represents current burden due to past infections that should be differentiated from future burden due to current infections. Accounting for background “competing” mortality is important in the case of HBV and other chronic infections because after infection, death and disability occur later in life. This is particularly important in sub-Saharan Africa as the background HIV-related mortality is high (Figure 1) and will be increasingly important in Asia. In the absence of HIV infection, expected chronic HBV death rates in Botswana would approach that of Singapore.

In terms of mortality, hepatocellular carcinoma and cirrhosis dominate. In terms of morbidity, acute hepatitis dominates. However, this does not take into account chronic active hepatitis, and therefore morbidity may have been underestimated. The characterization of chronic morbidity in terms of annual episode incidence, duration, and level of disability is planned for the near future. The impact of cofactors of hepatocellular carcinoma, including aflatoxin, alcohol, and other viral hepatitis infection, has not been investigated but should be addressed using case control methodologies. However, it is unclear whether a better reflection of these factors would substantially improve disease burden estimates. Rather, expanding the geographical scope of research into HBV’s attributable fraction in cirrhosis and hepatocellular carcinoma is likely to be more productive given the lack of information in this area.

Modeling Hepatitis B in Switzerland

In Switzerland, the global burden of HBV infection was estimated to help guide decisions in the area of immunization. In contrast to the prevalence-based approach used for the WHO global model, a decision tree approach was used based on experience with the model developed for the United States by the Centers of Disease Control and Prevention (CDC). This model could
be applied to hepatitis C. However, (1) data are not yet available, (2) background mortality has not been considered, and (3) DALYs have not been calculated. Besides Switzerland, the model may have been used in Liechtenstein and by private economists. For use in Asia, the model would have to be updated to take into account mutants and perinatal infection.

Global Prevalence of HCV Infection

Objective

The objective of the study was to estimate the prevalence of HCV infection by age, gender, and region and to update the estimate previously generated by WHO.

Methods

A literature search was carried out using Medline and other sources. The results of studies that included patients whose risk of HCV infection was thought to be similar to the general population were incorporated in a database. Studies were considered regardless of whether supplemental testing was conducted. Both English and non-English publications were considered. Surveys done since the late 1980s were assumed to be representative of the year 2000. First, country-specific studies were reviewed to obtain an overall estimate where possible, placing greater weight on community surveys and studies including supplemental testing. Second, gender- and age-specific prevalences were estimated. Countries lacking data were associated with other countries on the basis of epidemiologic similarities. Regional estimates were derived by weighting the country-specific estimates by overall population.

Results

Data from more than 300 studies representing all of the regions were considered in deriving estimates of the prevalence of HCV infection. Overall preliminary results suggest that the prevalence of HCV infection is approximately 2.2% worldwide. While the individual estimates from the different regions have undergone some change, the overall picture is still similar, with the WHO African region and the WHO Eastern Mediterranean region having the highest prevalence of HCV infection (Figure 2). Future work will focus on uncertainty analysis.

Discussion

Approximately one-quarter of the studies used represented community surveys, while one-third represented blood donors. No single approach was used to adjust these estimates as the mode of recruitment of blood donors differs tremendously depending on the region. However, none of the regional estimates relied exclusively on studies conducted among blood donors. In selected industrialized countries, another approach has been previously used to (1) estimate the size of the population in high-risk groups (e.g., injection drug users), (2) estimate the prevalence of HCV infection among these high-risk groups, and (3) compile these estimates to adjust the estimated prevalence in the general population. For example, this approach was considered useful in Switzerland, where community-based studies did not capture injection drug users. While this approach could work in industrialized countries where community-based studies may underestimate prevalence by not including injection drug users, it may not be useful in developing countries where unsafe medical injections are a main risk factor of infection. However, because there are emerging outbreaks of injection drug use in a number of developing and transitional countries, a mechanism to include this factor into a sensitivity analysis should be identified. The Evidence and Information for Policy (EIP) WHO cluster has estimates of the number of injection drug users by region. These figures could be used to adjust the HCV infection prevalence estimates in certain areas. Ideally, data should include estimates of both current and prior injection drug use. In the United States, for instance, most of the prevalent HCV infections were associated with former rather than current injection drug use.

Modeling the Incidence of HCV Infection

Difficulties in Estimating HCV Infection Incidence

Precise estimation of the incidence of HCV infection is not possible given available data. Because direct mea-
measurement of incidence is difficult, there are few published studies in which this has been attempted. Estimation of incidence from available prevalence data is the most practical approach for estimating incidence on a global scale, although results are sensitive to assumptions, particularly those regarding past trends in incidence. In addition, age-specific estimation of incidence is not possible because of the paucity of precise, age-specific data.

Impact of Past Trends on Incidence

The incidence of HCV infection is probably a function not only of age but also of time. Following the Second World War, there was an increase of use of injections, blood products, and injection drug use. However, in the past 10 years, in some industrialized countries, the incidence of new infections has decreased, presumably reflecting a decrease in percutaneous exposures. These temporal trends need to be taken into account when estimating incidence from prevalence.

Other important sources of uncertainty in the incidence model include the rate at which seropositive individuals lose detectable antibody (seroreversion) and whether persons with HCV infection have higher underlying mortality rates. The first of these, seroreversion, will cause the model to underestimate incidence if not taken into account. The second, differential mortality rates, will have the same effect. To take these issues into account, one should estimate survival functions for each of the birth cohorts that make up today’s population, as well as for the subpopulation of people infected with HCV, which is not practical. There is evidence, for example, that injection drug users have higher mortality rates than their non-drug-using counterparts. In the model proposed for this project, background mortality is assumed to be the same for infected and noninfected people.

Unresolved Issues

Key unresolved issues include the following:

1. Using or not using a seroreversion rate. A 1% seroreversion rate per year may be too high. There was a 7% loss of antibodies after 25 years in a transfusion study. Seroreversion may happen among nonviremic patients during the first 10 years after infection. If seroreversion does occur, then the prevalence in the population underestimates the proportion of the population ever infected.

2. Deciding on incidence trends.

3. Deciding on age-specific incidence estimates.

Natural History of HCV Infection

Revisiting the Natural History of HCV Infection

A decision tree can summarize the natural history of hepatitis C. However, an agreement needs to be reached regarding the parameters to use. A systematic review of natural history studies was conducted and published in 2001. Risk of cirrhosis varied according to the type of recruitment (highest for blood transfusion and liver centers, lowest for blood donors and community studies). Overall, it was suggested that for persons who acquire HCV infection in young adulthood, less than 10% are estimated to develop cirrhosis within 20 years. While higher estimates have been used in the past in many published models, the lower estimates probably reflect reality better at a population level and should be preferred. In essence, the model is homogeneous and averages the influence of cofactors.

Uncertainties beyond 20 Years

While natural history is reasonably known for up to 20 years, we need assumptions to go beyond that point. The assumption made was linearity of progression. Linearity may be the safest assumption to date in the absence of specific data. That the disease could accelerate was hypothesized in the studies published by Poynard et al. However, this interesting hypothesis was based on cross-sectional data, and there are no data to test it. Uncertainty about the long-term prognosis could be addressed using a number of approaches, including (1) population-based vital statistics data matched with incidence that we believed may have occurred in the past and (2) registry of infections based on notification of chronic cases matched with cancer registries.

The outcome of most discussions about the natural history of HCV infection is summarized later in “B. Natural History of HCV Infection, Including Morbidity and Mortality” (see p. 26).

Using DISMOD for Hepatitis C

Global Burden of Disease

The DISMOD computer software was created because (1) variables are observed with different degrees of reliability (mortality > prevalence > incidence), (2) data come from different sources, and (3) other disease characteristics may be stable across populations and thus be useful for the estimation of missing parameters.
DISMOD can be used to compute incidence on the basis of prevalence, check internal consistency or estimates, or change age groups.

**Limitations of DISMOD for HCV Infection**

DISMOD works best for chronic diseases (e.g., asthma). It is less adapted for infectious disease such as hepatitis C because HCV infection does not produce disability in itself. It is the complication (i.e., cirrhosis) that does.

**Using DISMOD for HCV Burden of Disease**

We decided to use two computer software programs—DISMOD to obtain incidence and the “HCV Natural History Programme,” a “homemade” MS-Access-based application—for the remainder of the modeling. The HCV natural history program includes a natural history model that is based on the parameters proposed by Dore et al4 (up to the stage of cirrhosis) and the parameters proposed by Sagmeister et al5 (after cirrhosis). These figures can be plugged into incidence estimates.

**Results**

Preliminary runs of the model suggest that 1% of cirrhosis prevalence in 2000 worldwide is caused by HCV infection. This is obviously a gross underestimation since the prevalence of HCV infection among patients with cirrhosis is much higher. We need to identify the cause of this discrepancy. Options include the following:

- Revise incidence trend scenarios. Because the incidence estimates were based on prevalence and because the incidence may have declined, thereby increasing the duration of infection, this trend scenario could have influenced the results and may explain the underestimation.
- Test the “acceleration” natural history scenario in the model.
- Calibrate the model on the basis of the fraction of chronic liver disease and hepatocellular carcinoma attributable to HCV infection in epidemiological studies.
- Introduce cofactors. However, there are few data to estimate the proportion of infected people in each cofactor category. A possibility would be to try a 100% cofactor-present case scenario as a starting point.

Models are more sophisticated in the postcirrhosis phase, but it is the pre-cirrhosis phase that is most important and that is subject to the highest source of uncertainty.

**Natural History of Hepatitis C in Japan**

The incidence of posttransfusion acute hepatitis C decreased in Japan in the early 1990s. However, the overall incidence of acute HCV infection has been stable, accounting for about 8% to 9% of acute hepatitis cases. Among patients dying from hepatitis C, 82% of deaths are caused by hepatocellular carcinoma. The average age of diagnosis for hepatocellular carcinoma is 62 years.6 Persons transfused in their 20s do not develop hepatocellular carcinoma before the age of 60. Factors associated with the development of hepatocellular carcinoma include initial age (older or younger than 50 years on diagnosis), stage of fibrosis, inflammation activity, and interferon treatment. Gender is not associated with risk. Among Japanese patients with hepatocellular carcinoma associated with HCV infection, 80% have cirrhosis. The incidence of hepatocellular carcinoma from all causes increased from less than 10,000 in 1958 to more than 30,000 in 1994, with the highest increase of incidence among persons between 60 and 69 years of age. The Japanese data differ from the experience in the rest of the world.1,7,8 This could suggest that ethnicity is a cofactor that explains the high rate of hepatocellular carcinoma among Japanese. However, there could be other factors of importance, including environmental ones.
3. Estimate the prevalence of HCV infection in 2000 by age, gender, and GBD region.
4. Estimate the fraction of chronic liver disease and hepatocellular carcinoma attributable to HCV infection on the basis of numbers 2 and 3 and on the basis of available attributable fraction studies.
5. Add to the mortality estimate a morbidity estimate based on the assumption that before dying, each patient has an average number of years alive with decompensated cirrhosis or hepatocellular carcinoma.*

Mortality and morbidity associated with acute hepatitis are considered negligible when compared to the burden associated with the chronic outcomes.

The Future Burden of Disease Due to HCV Infections in 2000

The proposed approach includes the following:
1. Estimate the prevalence of HCV infection in 2000 by age, gender, country, and GBD region.
2. Model the incidence of HCV infection in 2000 by age, gender, country, and GBD region on the basis of prevalence.
3. Estimate the future morbidity and mortality associated with the natural history of HCV infections acquired in 2000.
4. Include prevalence, incidence, natural history parameters, and background mortality into a DISMOD model to ensure internal consistency and estimate future mortality and morbidity in DALYs due to HCV infections in 2000.

B. Natural History of HCV Infection, Including Morbidity and Mortality

All these parameters are derived by experts using established methods:

| Proportion of Infected Persons Who Develop Chronic Infection (RNA Positive) |
|-------------------------------|------------------|
| Estimate                      | 75%\(^{11}\)     |
| Uncertainty                   | 50%-85%          |

This value tends to be lower among younger people and higher among older people.

<table>
<thead>
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</tr>
<tr>
<td>For persons infected younger than age 40 years</td>
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<tr>
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<tr>
<td>For persons infected age 40 years or older</td>
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<table>
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<tbody>
<tr>
<td>In contrast to the estimate for the two first decades, little evidence is currently available to document the natural history after the first two decades. Thus, this parameter is somewhat speculative. Expert consensus suggests that this parameter is age dependent:</td>
</tr>
<tr>
<td>For persons infected younger than age 40 years</td>
</tr>
<tr>
<td>Estimate</td>
</tr>
<tr>
<td>Uncertainty</td>
</tr>
<tr>
<td>For persons infected age 40 years or older</td>
</tr>
<tr>
<td>Estimate</td>
</tr>
<tr>
<td>Uncertainty</td>
</tr>
</tbody>
</table>

The effect of age at infection on the disease progression beyond 20 years is unknown. Assuming a linear inference to project the risk of progression to cirrhosis after 20 years implies that only age at infection matters. Under an alternative scenario, it is possible that the fibrosis progression in someone infected younger than age 40 years could accelerate as that person ages and reaches an age group for which progression to cirrhosis after 20 years of infection is higher.

---

* Use progression rates from transplantation lists to cross-check/validate progression rates from the natural history model.  
† Incidence estimates are not needed to estimate the current burden due to past infections that will appear in the next World Health Report. However, incidence is needed for the Global Burden of Disease (GBD) 2000 study report that will come out in 2003 and for the estimation of the future burden due to current infections.  
‡ Chronic and acute hepatitis C may need to be redefined. The traditional 6-month cutoff time may be outdated.  
§ The death to case ratio is close to 0%.
Annual Rate of Hepatocellular Carcinoma among Patients with Cirrhosis

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan and the Taiwan province of China</td>
<td>1.6%</td>
<td>1.5%-2.5%</td>
</tr>
</tbody>
</table>

Japan and the Taiwan province of China fall outside this range with a rate of > 7%.

Annual Death Rate among Patients with Hepatocellular Carcinoma

A number of studies suggest that this parameter depends on access to treatment:

In industrialized countries

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate</td>
<td>80%</td>
</tr>
</tbody>
</table>

In developing countries

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate</td>
<td>90%</td>
</tr>
</tbody>
</table>

The annual mortality rate of patients with HCV in Japan is significantly lower (~10%).

Annual Rate of Decompensation among Patients with Cirrhosis

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate</td>
<td>4%</td>
</tr>
</tbody>
</table>

Annual Death Rate among Patients with Decompensated Cirrhosis

A number of studies suggest that this parameter depends on access to treatment:

In industrialized countries

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate</td>
<td>15%</td>
</tr>
</tbody>
</table>

In developing countries

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate</td>
<td>30%</td>
</tr>
</tbody>
</table>

Factors that May Affect the Natural History of HCV Infection

Consistently normal ALT levels. Consistently normal alanine aminotransferase (ALT) levels are associated with slower fibrosis progression.

Steatohepatitis. Limited evidence suggests that steatohepatitis may affect fibrosis progression. Steatohepatitis, rather than obesity, seems to be the important cofactor. However, one intervention study from Brisbane, Australia, suggests that reducing weight reduces fibrosis progression.

HIV coinfection. The influence of HIV infection depends on CD4 count. The relative risk for the development of cirrhosis among HIV and HCV coinfected patients is around two.

HBV coinfection (HBsAg). Chronic HBV/HCV coinfection (HBsAg and anti-HCV positive) is uncommon globally, although it may be emerging in China. Coinfected patients have a higher risk of hepatocellular carcinoma than those who are only infected with one virus. However, it is unclear whether this high risk reflects a combined effect of the two viruses in the absence of interaction or some synergistic effect. The anti-HBc alone/anti-HCV serological profile is common. Some evidence suggests that the presence of anti-HBc alone might increase the risk of hepatocellular carcinoma among patients with chronic HCV infection.

Alcohol intake. Intake of more than 50 g alcohol/day accelerates progression to cirrhosis with a relative risk of about three.

Therapy. Globally, the proportion of viremic patients who undergo therapy is low, industrialized countries included. In Australia, for example, less than 10,000 people have been treated, while estimates suggest that there are 170,000 people infected. At the present time, effective treatment is not administered to all patients (e.g., in correctional facilities, developing countries). In addition, the proportion of viremic patients who will clear infection under treatment is variable, although improvements have been made for all genotypes, especially for genotypes 2 and 3. Sustained virological response is associated with improvement in necro-inflammatory lesions and fibrosis, but the effect on overall survival is still unclear. Thus, the impact of therapy is unlikely to affect the natural history of HCV infection at the population level at the present time. However, this analysis should be revised if improved treatment protocols become available and better therapy coverage is achieved.

Smoking. Preliminary evidence suggests that smoking may influence the development of hepatocellular carcinoma.

Factors that Probably Do Not Affect the Natural History of HCV Infection

Viral load. Evidence suggests that in general, viral load does not influence disease severity or progression.

Genotypes. Most studies suggest that in general, genotypes do not influence disease severity or progression.
C. Areas that Need Further Research

To better estimate the global burden associated with HCV infection, more research is needed in the following areas.

Estimates of HCV Prevalence

The quality and coverage of population-based estimates of HCV prevalence should be improved. The two critical elements for survey quality are (1) use of a representative sample and (2) use of accurate diagnostic tests. Because age-specific estimates of prevalence are important to estimate incidence trends and burden of disease, these surveys should attempt to estimate the prevalence of infection according to age. Stratification by gender should also be done.

Morbidity

Morbidity associated with chronic liver disease must be better characterized so that disability weights can be applied. This will allow a more precise estimation of DALYs. In that respect, the histological lesion of cirrhosis must be differentiated from (1) the disease that results from decompensated cirrhosis and (2) chronic hepatitis and/or chronic infection. The Australian system could be used as a basis for classifying health states of chronic hepatitis and its consequences (see Table I). In industrialized countries, knowledge of one's infection status is a major determinant of altered quality of life due to the uncertain progression of the disease and the increased anxiety caused by this uncertainty. Treatment may also need to be addressed. GBD needs a qualitative description of the range of potential symptoms, from severe to mild, that a patient may experience in one of the health states rather than a quantified estimate of disability. These figures will be useful to hepatitis B burden, too. Extrahepatic manifestations of HCV infection will not be addressed as they are uncommon (although the association between HCV infection and non-Hodgkin lymphoma needs confirmation by further studies to define the presence or absence of a causal relationship).

Natural History Parameters

Natural history parameters should be estimated using a systematic literature review and meta-analyses. In addition, research needs to better describe (1) the risk of fibrosis progression beyond 20 years, (2) the risk of fibrosis progression in developing countries, (3) spontaneous clearance of hepatitis C virus infection, and (4) the patients who do or do not progress to cirrhosis (e.g., normal aminotransferase levels, gender, ethnicity,* obesity, toxins, environmental factors).

Table I  Health States Used for Hepatitis C Virus Infection by the National Centre in HIV Epidemiology and Clinical Research of Australia

<table>
<thead>
<tr>
<th>Health State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild, chronic hepatitis, undiagnosed</td>
</tr>
<tr>
<td>Mild, chronic hepatitis, diagnosed</td>
</tr>
<tr>
<td>Moderate, chronic hepatitis, undiagnosed</td>
</tr>
<tr>
<td>Moderate, chronic hepatitis, diagnosed</td>
</tr>
<tr>
<td>Compensated cirrhosis, undiagnosed</td>
</tr>
<tr>
<td>Compensated cirrhosis, diagnosed</td>
</tr>
<tr>
<td>Liver failure</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
</tr>
</tbody>
</table>

* In the United States, there is a difference between African Americans and Caucasians. However, there are no data regarding Africans living in Africa. Genotype could be a cofactor. There is no information regarding Australian Aborigines.
REFERENCES


Putting Clinical Pharmacology in Context: The Use of Popular Movies

Magí Farré, Fèlix Bosch, Pere N. Roset, and Josep-E. Baños

Films have been used in medical schools for many years. Most of them were documentaries especially made for the classroom setting and usually bought from educational resource providers. Given their own nature, these productions are expensive and may be outdated in a few years. In contrast, the use of popular movies is not as frequent in medical teaching as in other disciplines such as, for instance, social sciences or medical humanities. This happens despite proven evidence that medicine and doctors are a frequent topic in popular movies.1

We define popular movies as those filmed to be exhibited in commercial theaters or on TV channels, and they are not made for their possible use as a teaching tool. For this reason, they are based on fictitious tales or are free adaptations of actual stories, in both cases using the rich resources of filmmaking companies. The high formal quality of such films makes them very convenient to be used in teaching college students. Among their advantages are the way they are made, their acceptability among young people, the good work of actors and actresses, and the way the story is developed, all of which make them believable to any spectator. Again, the huge amount of resources used in the production is greater than any of those used in common documentaries. Therefore, the tales of popular movies have a high level of truthfulness, which is difficult to achieve by other means. This is not surprising: A movie that is not credible is, of course, a bad movie.

There is no doubt that some movies are especially useful to show some aspects of medical duties. For instance, Dr. Ehrlich’s Magic Bullet (1940) is a good example to show how the first chemotherapeutic drugs were discovered by Paul Ehrlich, and it has an indisputable value in teaching the history of medicine and of infectious diseases. The Doctor (1991), inspired by a real story, is a touching movie that considers how patients are often victims of impersonal care by unmotivated physicians and how one’s personal experience may change this situation.2 Another one, Shadowlands (1985), is a magnificent example of how terminal cancer may affect the relationship of a couple. Outbreak (1995) is very helpful to show how an infectious disease may cause a national crisis, and Basketball Diaries (1995) is a good example of how drug addiction may strike young people. We believe that such films may motivate a young university student in a teaching set-

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Keywords: Popular movies; classrooms; pharmacology; teaching tools

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ting even more than when seeing it alone in a movie theater. In the case of pharmacology, this does not apply exclusively to medical students but to all of those following health sciences courses.

The usefulness of movies to illustrate the psychological and sociological conflicts of medical practice is widely recognized. However, the use of popular movies to teach less oriented medical sciences, such as pharmacology, is not so common. In fact, as far as we know, only Koren3 has suggested the use of a popular movie, such as *Awakenings*, to teach clinical pharmacology. Following his suggestion, we have been using several popular movies to teach clinical pharmacological principles to medical students. The activities were carried out under the title of “Films-Debate.” We would like to present our experience with the film proposed by Koren, *Awakenings*, as well as two others, *Lorenzo’s Oil* and *Miss Evers’ Boys*.

**AWAKENINGS**

In 1969, Calne et al4 reported that levodopa was able to improve the condition of some patients with postencephalitic parkinsonism, also known as encephalitis lethargica, or von Economo’s disease.5 These authors described an improvement in half of the 20 patients who received the drug, especially in the control of movement when walking. The following year, Oliver Sacks and his colleagues from Beth Abraham Hospital in New York described some of their own experiences with postencephalitic patients after administering them levodopa for 1 year.6 However, the full account of his experience was published 13 years later as a book, *Awakenings*.7 Earlier, Sacks tried to publish these experiences as original articles in medical and neurological journals, but all of them were rejected.8

Based on the book by Sacks,7 a popular movie with the same title (Columbia Tristar Home Video) came out in 1990, directed by Penny Marshall. The leading actors were Robin Williams as Dr. Malcolm Sayer (in fact, Oliver Sacks) and Robert De Niro as Leonard Lowe (the first patient to be “awakened”). The film shows how Dr. Sayer tries levodopa on a group of encephalitic patients and the early dramatic improvement that followed. The movie also shows how levodopa lost its efficacy and how adverse effects appeared, such as dystonias. The film also describes the doubts of Dr. Sayer in starting and following the trial and the difficulties he encountered both with the hospital staff and with some relatives of his patients.

Even considering the licenses that were taken in the presentation of the events and several important issues that are different from the true story reported by Sacks, *Awakenings* offers an engaging version of the facts. The movie may be used to illustrate several concepts, such as pathophysiology of parkinsonism, mode of action of levodopa and other antiparkinsonian drugs, design of drug studies, types of clinical trials, ethical misgivings, dose-response relationships, and adverse effects of agonists of dopamine receptors. Table I summarizes the general learning objectives that we assumed when using this movie with medical students and those receiving a general course on drugs, science, and society.9,10 We have also used this film in a training course for clinical research assistants, and some colleagues have tried *Awakenings* in teaching clinical pharmacology to physical therapy students (M. I. Martín, personal communication, 2002).

**LORENZO’S OIL**

*Lorenzo’s Oil* (MCA Home Video) is a film directed by George Miller, in 1993, about a couple, Michaela and Augusto Odone, whose 6-year-old son is a bright boy who becomes affected by adrenoleukodystrophy (ALD), a neurodegenerative disease that soon reduces him to an almost vegetative state. The film is based on true facts and shows how the parents, after the initial shock, are able to learn complex medical and biochemical facts that permit them to understand the disease and try to find a cure for it.11 The main actors of the film are Susan Sarandon (Mrs. Odone), Nick Nolte (Mr. Odone), and Peter Ustinov (Dr. Nikolais). The film is a really good work, but some of the facts stated, such as the success obtained with the oil, the conflicts between the parents and the medical establishment, and the portrayal of a parents’ organization, are considered as not completely true by some of the people who participated in the real story.11

The film shows how the disease appears and is diagnosed. After the despair due to the bad prognosis, the Odones start a long pilgrimage to try to find a treatment to stop the progression of the illness. Based on the lack of treatments, the Odones are asked to include the son in a clinical trial with experimental therapies. However, things are going badly; the child is not improving and is also experiencing severe side effects, so the Odones withdraw the boy from the study despite the opinion of the investigators. In a desperate search for solutions, Augusto Odone finds the existence of another experimental treatment in the Index Medicus not yet tested in humans but that seems to him to be a potentially effective therapy. The Odones contact the original researcher, organize a meeting between basic
and clinical investigators to discuss the feasibility of this treatment, and finally obtain the new product, erucic acid (Lorenzo’s oil), to be tested on their son. The child improves from his vegetative state, and the film ends giving the feeling that the efforts of the parents have been rewarded.

There is no doubt that the film is excellent, but some of the facts shown have been strongly criticized in scientific literature\(^{11-13}\) and even in the lay press.\(^{14}\) The clinical effectiveness of erucic acid was not confirmed through clinical trials,\(^{15}\) and it generated a strong controversy.\(^{16-18}\) A recent review of the clinical research carried out with erucic acid in ALD patients concluded that there is no proven efficacy of the oil in patients with the established disease.\(^{19}\) A tiny possibility remains that it may be of value in asymptomatic patients. Even though erucic acid may normalize the blood levels of the fatty acids that accumulate in ALD, its limited value to lessen the disease may be due to its inability to cross the blood-brain barrier.

The characteristics of Lorenzo’s Oil have been exploited as a teaching tool. It has been used to illustrate biological principles such as lipid structure, dangers of very long saturated fatty acids, the function of cell organelles, and inherited diseases.\(^{20}\) It may also show the problems associated with caring for people with a severe disability, families in crisis, or the doctor-patient relationship.\(^{21,22}\) We believe that the film may also be useful in the teaching of clinical pharmacology, as this movie considers several aspects of noteworthy interest to medical students dealing with the topic. Table II shows some pharmacological, methodological, and ethical aspects that can be discussed with this film. A good analysis of the multiple meanings of the movie and its interest for physicians and medical students has been published recently by Jones.\(^{23}\) A recent update of ALD may be found in Moser et al.\(^{24}\)

### MISS EVERS’ BOYS

This movie describes a true story that is well known in the United States, the Tuskegee experiment. Miss Evers’ Boys was filmed as a television movie (HBO Home Video) in 1997 following the play of the same title by David Feldshuh (New York: Dramatist’s Play, 1995). It was directed by Joseph Sargent, and its main actors are Alfred Woodward and Laurence Fishburne. The movie describes in a trustworthy way, but with fictional names of the main characters, the so-called Tuskegee Syphilis Study (TSS). This survey was initiated by the U.S. Public Health Service (PHS) in 1932 and was continued until 1972. The name refers to Tuskegee, a town in Macon County, Alabama. The movie began in 1972, with the statement before a committee by Nurse Eunice Evers, the fictional character of a genuine nurse (Eunice V. Rivers). In the movie, Miss Evers remembers how the TSS began and how it was developed until its cancellation many years later.

The TSS was a 40-year follow-up study of a group of 399 African American males affected by syphilis and 200 healthy peers who acted as controls. Those participating in the study were never given information about its existence, and they were not informed of the exact diagnosis of their illness. For describing it, the term bad blood was used as an unspecific name for different sicknesses ranging from anemia to infectious diseases, such as syphilis. The goal of the study was to know the “natural” evolution of the disease when untreated in African American males. Based on this premise, the PHS actively avoided any supposedly effective treatment, even when, in the late 1940s, penicillin was available and its ability to cure the disease was scientifically proven. Patients were cheated, and they only received tonic compounds, aspirin, or liniments for treating their ailments; they were appointed to periodic

### Table I  General Learning Objectives That Can Be Reached with Awakenings in the Domain of Clinical Pharmacology

- To discuss how changes in brain neurotransmitters can be followed by disease
- To explore the mode of action of levodopa and drugs used to treat parkinsonian patients
- To analyze how clinical trials with drugs may be designed after establishing a hypothesis
- To discuss the concept of the dose-response relationship in a clinical setting, both for therapeutic and adverse effects
- To debate the difficulties of using drugs in new indications
- To exchange different points of view on the ethical principles involved in research with human beings
- To introduce the basis for adverse drug effects
- To analyze the differences between therapeutic treatment and therapeutic research
- To comment on the risks of high expectations in medical treatments

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\(^{32}\) • J Clin Pharmacol 2004;44:30-36
visits of follow-up. To ensure their participation, pa-
tients received free meals, pocket money, diplomas,
and burial insurance. From 1936 until 1973, the results
of the TSS were published without concealing the
study design, and many papers started with the words
“the Tuskegee Study of Untreated Syphilis.” At the end
of the 1960s, some scientists tried unsuccessfully to
stop the TSS, so they decided to explain the topic to a
journalist, who unveiled the story on the front page of
the Washington Star and the New York Times on July
26, 1972. As expected, the consequent scandal caused
the creation of a subcommittee in the Senate to investi-
gate what actually was happening. Officially, the TSS
ended on November 16, 1972. Affected people re-
ceived economic compensation, but neither U.S. offi-
cials nor the government were ever accused or con-
demned. However, the controversy urged the creation
of a special commission by mandate of the Congress,
the National Commission for the Protection of Human
Subjects of Biomedical and Behavioral Research,
which set the ethical bases of biomedical research in
human beings. One of the most known works of this
commission was the Belmont Report (1979), which de-
fined three of the main principles of bioethics: respect
for persons (autonomy), justice, and beneficence.
Twenty years later, President Clinton publicly begged
the pardon of the survivors and their families in the
name of the government of the United States during a
reception at the White House on May 16, 1997.25-27

The movie is of interest in the teaching of health
workers, as it has many medical, historical, psycholog-
ical, and sociological implications. We have used it to
illustrate some aspects of clinical research and clinical
pharmacology to medical students (Table III).

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**Table II** General Learning Objectives That Can Be Reached
with Lorenzo’s Oil in the Domain of Clinical Pharmacology

- To apply the concept of competitive antagonism to develop new drugs
- To understand what is a surrogate variable and how this is not always correlated with clinical outcomes
- To learn how metabolic pathways may be modulated by exogenous substances
- To discuss the concept of orphan drugs and why these should be protected by law
- To know the difficult topic of asking parents why they authorize their children to be used as subjects of a clinical trial
- To discuss the bioethical principles of autonomy and beneficence
- To observe the difficulties of transferring basic research data to the clinical field
- To contrast the data of a single clinical observation, open clinical trials, and double-blinded clinical trials
- To understand the difficulties of refusing popular beliefs by means of scientific evidence
- To discuss broadly the validity of the scientific method to reach valid answers in therapy

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**SOME RECOMMENDATIONS WHEN USING POPULAR MOVIES AS A TEACHING TOOL**

Finally, in a few words, we would like to comment on
what we have learned from our experience using popu-
lar movies in teaching clinical pharmacology prin-
ciples (Table IV). Popular movies should not be used as a
form of “entertainment” for students: it is preferable
that the teacher attends the film’s projection with his or
her students. Although students might view the film
alone before the tutorial session, viewing the film to-
gether adds some advantages. For instance, the
teacher may outline some scenes and even stop the
projection to make some comments or to answer some
doubts or questions of the students. The size of the
group of students may change depending on the set-
ting. Our experience works with 20 to 50 students, but
other authors have used popular movies to be dis-
cussed in a large format.³

Giving written guidance to focus the interest of stu-
dents on some aspects of the film may also help to make
optimum use of this teaching aid (Table V). This guide
may contain some relevant questions that students will
discuss after viewing the film, as suggested by Koren.³
Questions should be adapted to the previous training
of the students. The depth of the discussion will greatly
differ depending on the students’ profile, and the goals
to be achieved with these films will be different in each
group. Once the film ends, an open discussion among
all students and the teacher may help to clarify and en-
rich the experience. After the film session is over, stu-
dents are evaluated using a questionnaire that they fill
in and give to the teacher during the following week.
Their responses are reviewed by the teacher, marked
accordingly, and considered in the final evaluation of the student. An example of such a questionnaire used to evaluate Lorenzo’s Oil is included in Table V. Informal comments of students showed that the activity had a high acceptance and was considered more useful than other teaching activities (practical courses, seminars) to understand clinical pharmacology principles.

The use of popular movies is not devoid of some potential disadvantages. Sometimes, films may not be of interest to the students, but in other cases, its own subject may distract the students from the, let’s say, pharmacological topic. For instance, The Fugitive (Andrew Davis, 1993) may be used to illustrate clinical research and fraud; however, the action of the film is overwhelming, and the medical subject is peripheral. Also, teachers need to prepare, direct, and guide the activity, and this requires a considerable effort, at least at the beginning. Finally, given the wide interests of the audience, films should be genuinely entertaining for almost everyone. In this respect, singular movies with an unusual screenplay or performance may be seen as bizarre and unable to trigger the interest of students.

Are popular movies giving an added value over traditional lecture-based learning? This aspect has not been formally tested, but we are quite sure that the increased motivation of students to understand what is happening and why it is happening in the movie may be easily translated into a driving force for an increased desire to learn. This is a good way to start their interest in clinical pharmacology.

The authors would like to thank Mr. Chuck Simmons, a native English-speaking university instructor of English, for the revision and correction of the manuscript.
Table V  An Example of the Students’ Guide for *Lorenzo's Oil*

**Background**

The film describes the real history of Lorenzo, a 6-year-old child who was diagnosed with adrenoleukodystrophy (ALD), a rare neurodegenerative disease with no known curative treatment. ALD is caused by the excess of long-chain saturated fatty acids that greatly affect the myelin and consequently affect the activity of the nervous system. The film describes the fight of Lorenzo’s parents to find a treatment that may help their son against ALD.

**Teaching goals**

After seeing and discussing the film, you should be able to

- understand how basic pharmacodynamic concepts (i.e., agonism, antagonism) may be applied to the discovery of new drugs,
- know the concept of a surrogate variable and the difficulty of establishing adequate endpoints in clinical research,
- learn the possibility of modulating the metabolism of endogenous substances by drugs,
- discuss the concept of orphan drug and orphan disease,
- understand the bioethical principles applied to clinical pharmacology,
- learn the importance of adequately designing clinical trials to obtain correct answers.

**Evaluation questionnaire**

In the week following this session, you should send an essay with the answers to the following questions to your tutor:

- Why are work hypotheses needed before starting a clinical study?
- Why should informed consent be obtained from those volunteering in research?
- How are bioethical principles applied to the research described in the film?
- Is it always necessary to follow the study protocol throughout the study? Why?
- What are the reasons for blinding the treatments?
- Define the concept of a pilot study.
- Discuss the conflict between scientific research and patients’ needs.
- Define a surrogate variable and identify which was in *Lorenzo's Oil*.
- Comment on the importance and limitations of basic research in the development of new drugs.
- Define *orphan disease* and its consideration by the drug industry.
- Why is clinical research sometimes funded and sometimes not?
- Comment on the impact of research in the media and vice versa.
- How is the dose of the new substances chosen?
- What does compassionate use of drugs mean?
- What are the reasons for lack of correlation between animal studies and clinical trials?
- Why do drugs need to be authorized by regulatory authorities before being marketed?
- What are the functions of institutional review boards in clinical research with new drugs?

**REFERENCES**

Risk-Benefit Value of Inhaled Glucocorticoids: A Pharmacokinetic/Pharmacodynamic Perspective

Shashank Rohatagi, PhD, FCP, MBA, Sireesh Appajosyula, PharmD, Hartmut Derendorf, PhD, FCP, Stanley Szefler, MD, PhD, Ruediger Nave, PhD, Karl Zech, PhD, and Donald Banerji, MD, FAAAAI

Inhaled glucocorticoids induce therapeutic and adverse systemic effects via the same types of receptors. Analysis of the pharmacokinetic/pharmacodynamic parameters of inhaled glucocorticoids generates a risk-benefit value (RBV). Targeted efficacy with minimal adverse effects helps to quantify an appropriate RBV. High lung deposition/targeting, high receptor binding, longer pulmonary retention, and high lipid conjugation are among the pharmacokinetic parameters to be considered for improved efficacy of the compound. Low or negligible oral bioavailability, small particle size and inactive drug at the oropharynx, high plasma protein binding, rapid metabolism, high clearance, and lower systemic concentrations are associated with low risks for adverse effects. Inhaled glucocorticoid potency is enhanced by solution inhalers, which result in higher pulmonary deposition and minimize local adverse effects. These properties, among others, determine the efficacy and safety of inhaled glucocorticoids. Currently available inhaled glucocorticoids do not provide the complete pharmacokinetic/pharmacodynamic parameters to optimize RBV, leaving room for improvement in the development of future agents.

Keywords: Inhaled glucocorticoids; risk-benefit value; pharmacokinetics; pharmacodynamics; asthma

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The potency of these inhaled glucocorticoids varies. In clinical trials, fluticasone propionate has shown the highest potency, followed by budesonide and beclomethasone dipropionate, which are approximately equipotent. Variation in potency arises from the many differences in pharmacokinetic and pharmacodynamic properties. A schematic of these properties and their physical importance is shown in Figure 1.

The risks and benefits of these drugs can be examined together. A risk-benefit value (RBV) compares the desired clinical effects with the undesired systemic effects of a (inhaled) drug. Factors that need to be taken into consideration, therefore, include not only the glucocorticoid activity of the drug but also the amount of drug delivered to the lung, as well as the drug’s pharmacokinetic properties (both pulmonary and systemic). A comprehensive understanding of a drug’s pharmacokinetic properties and the relationship between its pharmacokinetics and pharmacodynamics (Figure 2) provides a basis for evaluating the agent’s safety and efficacy and, therefore, risk-benefit value. Such an understanding also makes it possible to predict the drug’s behavior and effects upon administration.

This review examines the commonly available inhaled glucocorticoids and their pharmacokinetic properties. Specific properties that are important for the long-term treatment of asthma are also assessed, with a view toward identifying the ideal properties for effective and safe asthma management.

PROPERTIES IMPORTANT IN PULMONARY TARGETING

The properties discussed below are listed in Table I and summarized in Table II.

Bioavailability

A significant benefit of inhaled glucocorticoids is that the delivery method sends the drug directly to the lung for absorption. However, not all of the drug becomes available through pulmonary absorption. Instead, a majority of the dose is deposited in the oral cavity and swallowed, thereby leading to potential systemic absorption from the gastrointestinal (GI) tract. For currently available inhaled glucocorticoids formulated as suspensions and delivered via chlorofluorocarbon metered-dose inhalers (CFC-MDIs), the proportion of drug absorbed by the GI tract ranges from < 1% (fluticasone propionate) to 26% (17-beclomethasone monopropionate) (Table I). The absolute systemic bioavailability of a glucocorticoid is equivalent to the total amount of the dose delivered directly to the lung, combined with the amount of drug absorbed from the GI tract. Glucocorticoids are well absorbed in the lung; the vast majority of the drug that is delivered to the pulmonary site of action is absorbed and effective. Drugs with low oral bioavailability have less drug available in the systemic circulation due to the high first-pass/presystemic metabolism in the GI tissue and liver. Also, for drugs with higher respirable fraction, less drug is available in the gut, which undergoes first-pass metabolism, leading to low systemic exposures. Cer-
tain glucocorticoids, such as budesonide and fluticasone propionate, are extensively metabolized during their first pass through the liver and are therefore converted to inactive metabolites prior to entering the systemic circulation.\textsuperscript{22}

It is important to consider that while the amount of drug that is orally bioavailable is usually less than the pulmonary component, the oral component that ultimately enters the systemic circulation does not provide any beneficial effects but rather may only generate adverse events.\textsuperscript{14} Therefore, an inhaled glucocorticoid that has high pulmonary targeting should have greater potential for beneficial effects. The pulmonary bioavailability of the glucocorticoid is the more important factor and should be relatively high (> 30%), ensuring that a significant proportion is applied to the lung (Figure 3).\textsuperscript{14} Similarly, oral bioavailability should be limited to reduce systemic availability and thereby limit needless adverse effects.\textsuperscript{14}

**Formulation**

The overwhelming advantage of drug administration via inhalation for the treatment of asthma is that the medication is delivered directly into the airways. Commonly used aerosolized delivery systems include MDIs—both pressurized and breath activated—and dry-powder inhalers (DPIs).

Because DPIs are relatively easy to use, they encourage compliance and are preferred by patients. A number of inhaled glucocorticoids are available in this formulation, with fluticasone propionate available in two forms of DPIs.\textsuperscript{23}

Metered-dose inhalers deliver the glucocorticoid as a suspension in a carrier liquid or a solution. Currently, all inhaled glucocorticoids are available as suspensions propelled by CFCs. Since it has been recognized that CFCs deplete the ozone layer, these propellants are

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**Table I  Pharmacokinetic and Binding Parameters of the Currently Available Inhaled Glucocorticoids**

<table>
<thead>
<tr>
<th>Glucocorticoid</th>
<th>F_{oral} (%)</th>
<th>F_{inhaled} (%)</th>
<th>Receptor-Binding Affinity</th>
<th>Free Fraction (%)</th>
<th>Protein-Binding Fraction (%)</th>
<th>Total Clearance (L/h)</th>
<th>Volume of Distribution (L)</th>
<th>Half-Life (t_{1/2}; h)</th>
<th>IV</th>
<th>Inhaled</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-Beclomethasone monopropionate\textsuperscript{17,22,31,32}</td>
<td>26</td>
<td>36</td>
<td>1345\textsuperscript{a}</td>
<td>NA</td>
<td>NA</td>
<td>120\textsuperscript{b}</td>
<td>424\textsuperscript{b}</td>
<td>2.7</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Beclomethasone dipropionate\textsuperscript{16,17,22,31,32,37}</td>
<td>13\textsuperscript{c}</td>
<td>20\textsuperscript{c}</td>
<td>43-53\textsuperscript{a}</td>
<td>13</td>
<td>87</td>
<td>150</td>
<td>20</td>
<td>0.5</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Budesonide\textsuperscript{16,19,22,28,32,67,68,69-74}</td>
<td>11</td>
<td>28</td>
<td>258-935\textsuperscript{a}</td>
<td>12</td>
<td>88</td>
<td>80-84</td>
<td>183-217.5</td>
<td>2.2-4.5</td>
<td>2.0-3.5</td>
<td></td>
</tr>
<tr>
<td>Flunisolide\textsuperscript{16,18,22,28,32,35}</td>
<td>7</td>
<td>39</td>
<td>180-478\textsuperscript{a}</td>
<td>20</td>
<td>80</td>
<td>58-65</td>
<td>61-96</td>
<td>1.6</td>
<td>1.3-1.7</td>
<td></td>
</tr>
<tr>
<td>Fluticasone propionate\textsuperscript{15,16,22,28,31,32,36,74,75,76-78}</td>
<td>&lt;1\textsuperscript{d}</td>
<td>26\textsuperscript{e}</td>
<td>813-2062\textsuperscript{a}</td>
<td>10\textsuperscript{d, e}</td>
<td>90\textsuperscript{d, e}</td>
<td>65\textsuperscript{d, e}</td>
<td>318\textsuperscript{f}</td>
<td>7.8\textsuperscript{f}</td>
<td>10.1\textsuperscript{d, 14.4\textsuperscript{f}}</td>
<td></td>
</tr>
</tbody>
</table>

F_{oral}, oral bioavailability; F_{inhaled}, systemic bioavailability after inhalation; NA, not available.

- a. In vitro data.
- b. Apparent clearance or volume of distribution, based on the beclomethasone dipropionate (BDP) dose and assuming complete conversion from BDP to 17-beclomethasone monopropionate (BMP).
- c. Bioavailability data for total beclomethasone (beclomethasone dipropionate and 17-beclomethasone monopropionate).
- d. Fluticasone propionate dry-powder inhaler data.
- e. Fluticasone propionate metered-dose inhaler data.
- f. Fluticasone propionate IV data.
being phased out in favor of hydrofluoroalkanes (HFAs), in accordance with the Montreal Protocol (1987). In addition to HFA inhalers being more environmentally friendly, the switch of propellant provides an opportunity to improve the formulation.

Aerosolized suspensions of beclomethasone dipropionate delivered by CFC-MDI produce particles with a mean aerodynamic diameter of 3.5 to 4.0 µm.24 Average aerodynamic diameters for other inhaled glucocorticoids have been found to be 2.5 µm for fluticasone propionate and 2.4 to 4.0 µm for budesonide.25,26 In contrast, solution inhalers produce particles with an average diameter of 1.1 µm, a significant reduction from those produced by suspensions.25 As small airways have an internal perimeter of < 2 µm, solutions offer the potential for delivering a larger proportion of respirable particles. MDI solution formulations have therefore been developed with glucocorticoids, such as HFA–beclomethasone dipropionate.25 In both a clinical trial and a radiolabeled study, the percent of deposition of the solution form of HFA–beclomethasone dipropionate to the lung was more than 50%, compared with CFC–beclomethasone dipropionate, which was primarily deposited in the oropharynx.24,25,27 The increased lung deposition that is achievable with solution HFA-MDIs may therefore increase delivery to the smaller peripheral airways, the site of largest surface area of inflammation, and lead to an overall improvement in the RBV of inhaled glucocorticoids.28

Receptor-Binding Affinity

Glucocorticoids generate a clinical response by penetrating the phospholipid bilayer of the pulmonary cellular membranes and binding to the glucocorticoid re-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Efficacy</th>
<th>Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability</td>
<td>High pulmonary availability &gt; 30% allows for more drug at site of action.</td>
<td>Low oral availability &lt; 10% decreases systemic availability and the incidence of adverse events.</td>
</tr>
<tr>
<td>Formulation</td>
<td>Small particle size (&lt; 5 µm) leads to higher lung deposition (%)</td>
<td>Small particle size (&lt; 5 µm) decreases the incidence of local adverse effects.</td>
</tr>
<tr>
<td>Receptor-binding affinity</td>
<td>High binding strength is correlated with high anti-inflammatory activity.</td>
<td>Low binding strength is correlated with a lower incidence of systemic side effects.</td>
</tr>
<tr>
<td>Protein binding</td>
<td>Efficacy in the lung is not significantly affected by protein binding because of high local drug concentration in the lung.</td>
<td>Protein-bound molecules are not available to cause adverse effects.</td>
</tr>
<tr>
<td>Clearance</td>
<td>Rapid clearance does not affect pulmonary efficacy.</td>
<td>Rapid metabolism (~90 L/h) reduces the potential for systemic effects.</td>
</tr>
<tr>
<td>Half-life ($t_{1/2}$)</td>
<td>Longer pulmonary terminal $t_{1/2}$ is related to prolonged efficacy.</td>
<td>Shorter systemic elimination $t_{1/2}$ is related to lower systemic risk.</td>
</tr>
<tr>
<td>Prodrug structure</td>
<td>Active form with high receptor potency increases efficacy.</td>
<td>Specific targeting to the lung increases safety. Inactive parent form decreases systemic effects.</td>
</tr>
<tr>
<td></td>
<td>Rapid hydrolysis to active form in lung increases efficacy.</td>
<td>Inactive final metabolites increase safety.</td>
</tr>
<tr>
<td></td>
<td>Specific targeting to the lung increases efficacy.</td>
<td></td>
</tr>
<tr>
<td>Lipid conjugation</td>
<td>Longer retention time in lung allows for longer efficacy.</td>
<td>Lipid conjugation is not related to adverse effects.</td>
</tr>
<tr>
<td>Lipophilicity</td>
<td>Higher lipophilicity is positively correlated with increased retention in the lung and longer duration of action.</td>
<td>Lipophilicity is linearly correlated with $t_{1/2}$. Lipophilicity is inversely correlated with peak concentration.</td>
</tr>
</tbody>
</table>
PHARMACOKINETICS AND PHARMACODYNAMICS

Pharmacokinetics and Pharmacodynamics

The receptor-binding affinities for the glucocorticoids are usually given in comparison with an affinity of 100 for the standard, dexamethasone.13 With respect to the

Figure 3. Schematic availability of inhaled glucocorticoids with poor (A) and good (B) lung targeting. These figures describe the distribution of a 1000-µg oral inhaled dose (a typical daily dose of glucocorticoid) through the lung and the gut in the case of good and poor lung targeting (30% and 5% of the total dose, respectively). A: When lung targeting is poor, final systemic exposures following a total oral dose of 1000 µg are 14.5% and 5.95%. B: When lung targeting is good (30%), the final systemic exposures following a total oral dose of 1000 µg are 37% and 31%. Adapted with permission from Rohatagi S, Rhodes GR, Chaikin P: Absolute oral versus inhaled bioavailability: significance for inhaled drugs with special reference to inhaled glucocorticoids. J Clin Pharmacol 1999;39(7):661-663.

A: An inhaled corticosteroid [glucocorticoid?] with poor pulmonary targeting will have high systemic availability

B: An inhaled corticosteroid with good pulmonary targeting will have low systemic availability

Protein Binding

Because their binding affinity to plasma proteins in the systemic circulation varies, glucocorticoids can also be differentiated on another level. Unlike receptor binding, this variability in potency can have a direct effect on the therapeutic index, as it is the concentration of free drug that determines the systemic pharmacologic activity and the level of adverse effects (Figure 4). An understanding of the level of drug that is not bound to the plasma proteins is important because only free, unbound drug can bind to the glucocorticoid receptor. A glucocorticoid with a high affinity for plasma proteins is desirable because it results in a low proportion of unbound drug in the systemic circulation and, therefore, a reduced incidence of adverse events. Limiting the amount of systemic free drug in this way does not compromise efficacy because the drug’s pulmonary concentration will remain high.
With the glucocorticoids currently in use, a significant proportion of the systemic glucocorticoid dose tends to remain unbound. The percentage of unbound glucocorticoid ranges from 10% with fluticasone propionate to 20% with flunisolide (Table I). An increase in binding of just a few percent can translate into a significant reduction in the amount of systemic side effects. For example, if one glucocorticoid approached the ideal of complete binding in the systemic circulation (i.e., 99% binding), another that was only 98% bound would present double the unbound concentration and a higher incidence of side effects.

Clearance

Clearance is defined as the volume of blood or plasma that is completely cleared of drug over a unit of time. As a drug passes through multiple organs, each organ may extract a proportion of the drug, leading to an additive effect in clearance. A glucocorticoid with high and, therefore, rapid systemic clearance should result in reduced systemic side effects, leading to an improved RBV. The inhaled glucocorticoids currently used all have rapid clearance, and high extraction rates in the liver have been shown. The clearance rates observed with budesonide and fluticasone propionate approach the rate of liver blood flow. An elevated clearance rate is, therefore, a highly beneficial property for an inhaled glucocorticoid.

Volume of Distribution

When examining the pharmacologic properties of a medication as it passes through the patient’s body, volume of distribution at steady state (V_{dss}) is a useful measure that relates drug concentration in the plasma to the total amount of drug in the body. A greater volume of distribution corresponds to a greater amount of drug in the tissue and a lesser amount in the plasma. While an increased V_{dss} ensures greater tissue distribution and binding, it is important to note that V_{dss} does not necessarily correspond directly to pharmacologic activity; this depends on additional measurements, such as the degree of protein binding and potency. The reported values of V_{dss} vary significantly, with beclomethasone dipropionate up to 424 L for beclomethasone monopropionate (Table I). The value reported for fluticasone propionate is far greater than values observed for other currently available inhaled glucocorticoids and correlates with the lipophilicity of the drug.

Half-Life

The volume of distribution is linearly correlated with the elimination half-life ($t_{1/2}$) of the drug, which is also dependent on the rate of systemic clearance. An inhaled glucocorticoid that is present in small amounts in body tissue and is rapidly removed from the systemic circulation will have a fairly rapid $t_{1/2}$. Many of the currently available glucocorticoids have an elimination $t_{1/2}$ of about 2 hours, but the values range from 6 to 30 minutes (beclomethasone dipropionate) up to 7.8 hours (fluticasone propionate) (Table I). It should be noted that although $t_{1/2}$ values for beclomethasone dipropionate range around 6 to 30 minutes, the product information for QVAR® states that 17-beclo-methasone monopropionate (the active metabolite of beclomethasone dipropionate) has an elimination $t_{1/2}$ of 2.8 hours. With regard to clinical applications, it is beneficial for the elimination $t_{1/2}$ to be relatively low. If elimination is rapid, there is less opportunity for the free drug in the systemic circulation to contribute to systemic events.

When the glucocorticoid is delivered by inhalation, it is possible for the terminal $t_{1/2}$ to be different from the terminal elimination $t_{1/2}$ observed when the drug is administered intravenously. For this to occur, the pulmonary absorption of the glucocorticoid must be slow and must be the overall rate-limiting step. It is important from a therapeutic standpoint because a longer terminal $t_{1/2}$ after inhalation is associated with a
longer pulmonary retention time and greater pharmacologic activity than a longer terminal \( t_{1/2} \) after IV administration.13

**Prodrug**

Most inhaled glucocorticoids are dispensed in a pharmacologically active form and, as such, have the potential for immediate local side effects in the throat and oral cavity. One approach to reducing this risk is to rely on specific mechanisms that activate the drug when it is present in the target tissues. These so-called prodrugs have little or no intrinsic activity until localized at the target tissue and provide an excellent mechanism to improve the therapeutic ratio.13,21

A prodrug-inhaled glucocorticoid relies on the presence of mechanisms in the lung for activation (e.g., esterases) and will result in increased retention times in the lungs and airways.13,21 The structure of the activated prodrug should allow it to have increased receptor potency and little or no intrinsic value in the parent form, thereby limiting the risk of systemic side effects.

Beclomethasone dipropionate is an example of a prodrug-inhaled glucocorticoid because it is significantly less active than its respective monoester, 17-beclomethasone monopropionate. Beclomethasone dipropionate is metabolized to the more active form in the body.13,21 It should be noted, however, that beclomethasone dipropionate has been reported to have a receptor-binding affinity several times greater than endogenous cortisol, although this may be partly explained by the formation of the highly active metabolite, beclomethasone monopropionate, during the receptor-binding experiment.32 While the activation of beclomethasone dipropionate highlights the potential advantages of this type of drug structure, the nature of activation should also be considered. Beclomethasone dipropionate is cleaved by esterase activity in the body; this esterase activity has been shown to be expressed in human liver, colon, stomach, breast, and brain tissue.41 Therefore, systemically available beclomethasone dipropionate will be metabolized, leading to the presence of active product in the body. A prodrug that is specifically activated in the lung target tissue will have reduced potential for systemic side effects.

**Lipid Conjugation**

Another mechanism by which the therapeutic ratio of inhaled glucocorticoids can be increased is by conjugation to lipids. Once a glucocorticoid has been conjugated, the uptake of the drug into the target tissue is increased, leading to greater retention time and prolonged anti-inflammatory action.42 The increased uptake, in turn, acts as a slow-release reservoir from which the glucocorticoid gradually becomes available after hydrolysis by intracellular lipases.42,43 Due to the tissue-specific nature of this process, no increase in adverse events will occur through this mechanism.

A hydroxyl group at position 21 of the glucocorticoid is essential for conjugation to occur.32 The hydroxyl group at this position is a suitable substrate for microsomal enzymes involved in lipid conjugation.42,43 Some currently available inhaled glucocorticoids have the appropriate hydroxyl group; however, budesonide is the only glucocorticoid for which lipid conjugation has been demonstrated.42,43 The fatty acids involved in this reversible reaction are found in pulmonary and airway tissues. Little to no conjugation is observed in skeletal muscle or plasma.32,44

**Lipophilicity**

Another property important for the efficacy of an inhaled glucocorticoid is the lipophilicity, or degree of polarity, of the molecule.38 Inhaled glucocorticoids must penetrate the cell membrane’s phospholipid bilayer to bind to the intracellular glucocorticoid receptor. While the molecular size, configuration, and overall molecular charge help to determine a drug’s ability to pass through the phospholipid bilayer, the lipophilicity is of particular interest because it is directly correlated to \( V_{dss} \) and \( t_{1/2} \). Thus, an increase in lipophilicity is associated with higher tissue binding, increased pulmonary retention time, and, ultimately, a prolonged duration of action.

The more recently developed inhaled glucocorticoids, such as fluticasone propionate and budesonide, have been shown to have kinetic properties that are superior to early glucocorticoids (e.g., prednisone) because of their lipophilicity.32 Fluticasone propionate has the highest demonstrated lipophilicity of the ICS (Table I).28 Since lipophilicity also correlates with increased receptor affinity and hepatic first-pass inactivation,45 it is of significant importance when considering the clinical efficacy and safety of ICS.

**DISCUSSION**

The pharmacokinetic properties of inhaled glucocorticoids have a direct impact on their clinical effects. Inhaled glucocorticoids with a high RBV will lead to...
improved control of asthma. The desired properties of an inhaled glucocorticoid are summarized in Table II. A high relative receptor affinity (RRA) ensures efficacy but is also coupled with elevated levels of adverse events. Improved pulmonary availability ensures enhanced efficacy and limits oral bioavailability. A high degree of plasma protein binding minimizes the amount of free drug that is present in the circulation and able to cause systemic side effects. Rapid systemic clearance does not affect efficacy but does significantly reduce the possibility of systemic side effects. A high volume of distribution is indicative of greater tissue penetration. Extended pulmonary retention time, as indicated by a longer terminal t1/2 after inhalation, is also desirable.

The clinical importance of the pharmacokinetic properties discussed is significant. For instance, once-daily budesonide has been shown to be as effective in the treatment of mild to moderate asthma as the same total dose of budesonide given twice daily46,47 and also as twice-daily fluticasone propionate.48 From a pharmacokinetic standpoint, this finding may be attributed to the prolonged duration of action of budesonide arising from its ability to be reversibly esterified with fatty acids.42 Furthermore, topical budesonide has been reported to exert prolonged anti-inflammatory activity in comparison to fluticasone propionate.49

The reformulation of inhaled glucocorticoids as HFA-MDs is also important, as HFA-MDs allow an increased proportion of drug to be delivered to the lung. For example, a solution form of HFA–beclomethasone dipropionate deposits more than 50% of the dose in the lung.54,25,27 This increase in lung deposition translates into improved efficacy in clinical trials. One randomized parallel-group trial of 323 patients has shown that 2.6 times as much CFC–beclomethasone dipropionate would be required to achieve the same improvement observed with HFA–beclomethasone dipropionate.50 In patients switched from 400 to 1600 µg CFC–beclomethasone dipropionate daily, HFA–beclomethasone dipropionate has demonstrated the ability to control asthma over 12 months at approximately half the dose (200-800 µg daily).51 Similarly, an HFA formulation of flunisolide has shown 49% greater lung deposition than its CFC counterpart.52 Also, a randomized, double-blind, placebo-controlled trial examining HFA and CFC formulations of flunisolide showed that HFA-flunisolide displays an efficacy and safety profile comparable to approximately one-third the dose of CFC-flunisolide.53 These results were attributed, in part, to the beneficial effects of smaller particles that reach the smaller airways of the lung53 (as highlighted in the Formulation section of this article). Although HFA-MDI inhalers are being developed for many formulations, no single, currently available, inhaled glucocorticoid demonstrates all of the desirable properties described.16

While all of the inhaled glucocorticoids discussed in this article are effective, some concerns have arisen regarding the safety of their long-term use, especially at higher doses. Of primary concern is the potential for suppression of growth, bone formation, and adrenal suppression.5-9 Adrenal suppression associated with glucocorticoid inhalation is the result of a negative feedback effect on glucocorticoid receptors in the anterior pituitary gland and hypothalamus, which, in turn, suppresses levels of corticotropin-releasing hormone and corticotropin, the hormone that controls the adrenal glands.9

While fluticasone propionate has demonstrated greater dose-related adrenal suppression,9 minimal effects have been demonstrated with doses ≤ 500 µg.54-56 Furthermore, a 2-year study of adult patients with mild asthma examined the effects of twice-daily 500-µg fluticasone propionate DPI.57 No significant changes in morning plasma cortisol concentrations were observed. At the end of the study, only minor decreases in poststimulation mean peak plasma cortisol concentration and 8-hour plasma cortisol area under the curve (AUC) values were observed.57 This reduced level of side effects can be attributed to the low unbound plasma concentrations of fluticasone propionate. As shown in Figure 4, the amount of free drug from the lower dose will generate a number of side effects from a lower part of the curve. Still, inhaled glucocorticoids are being developed with reduced adrenal suppression potential.

Mometasone furoate, which has been available for several years in dermatologic and nasal formulations, has been investigated as an inhaled glucocorticoid in DPI and HFA-MDI (metered dose inhaler) formulations.58 A total daily dose of 400 µg of mometasone furoate DPI has been shown to be effective in patients with mild to moderate asthma,58 while twice-daily 400- and 800-µg regimens of mometasone furoate DPI have demonstrated significant improvement in lung function and asthma symptom scores in patients with severe asthma.58 The systemic effects of both formulations of mometasone furoate have been examined.58 Twice-daily administration of the 400-µg dose of the pMDI formulation produced no significant change in
mean cortisol. In contrast, the 800-µg dose resulted in a significant reduction compared with placebo, but not as marked as the reduction observed with 880 µg of fluticasone propionate.\textsuperscript{58} This difference in the side effects profile with respect to dose can be attributed to the low protein-binding property of mometasone (Figure 4). The reported free (unbound) fractions of inhaled corticosteroids in general use range from 10% to 13% (Table I). From a physiologic perspective, increases in protein binding lower the free concentration of drug, resulting in a lower potential to cause systemic side effects such as cortisol suppression since free concentration is what is available to the receptors.

One newer glucocorticoid, ciclesonide, has been shown to be effective in the treatment of asthma with no significant adrenal suppression. Ciclesonide is an inactive parent compound when inhaled and has an RRA of 12\textsuperscript{64} in relation to the RRA standard of 100 for dexamethasone. However, upon reaching the target tissue, the drug is activated to desisobutyl-ciclesonide (des-CIC) by esterases. des-CIC has an RRA of 1200\textsuperscript{64}, which is comparable to many potent glucocorticoids. A double-blind, randomized, parallel-group study showed improvement in asthma control with morning to evening administration of ciclesonide 160 µg (ex-actuator) delivered via HFA-MDI. Improved endpoints included evening peak expiratory flow, asthma symptom scores, and the number of acute exacerbations. In addition, ciclesonide was not shown to significantly affect the hypothalamic-pituitary-adrenal axis.\textsuperscript{63} A daily ciclesonide dose of up to 1280 µg (ex-actuator) has been shown to decrease hyperresponsiveness to adenosine compared with both placebo\textsuperscript{62} and fluticasone propionate.\textsuperscript{65} However, moderate to high doses of ciclesonide do not significantly suppress cortisol levels, unlike fluticasone propionate.\textsuperscript{65} Furthermore, ciclesonide’s formulation provides a high fraction of respirable particles. In one study, subjects who inhaled \textsuperscript{99m}Tc-labeled ciclesonide 40 µg and 400 µg (ex-actuator) using an MDI inhibited high lung deposition: 52% of the ex-actuator dose reached the lungs and was distributed evenly throughout the lungs.\textsuperscript{66}

In summary, an inhaled glucocorticoid that possesses optimal pharmacokinetic properties while remaining an inactive prodrug prior to delivery and that affords increased lung retention via lipid conjugation after local application would approach “ideal” performance. It would also have an excellent RBV and, as such, would effectively control asthma. Thus, through improved formulations and/or pharmacokinetic and pharmacodynamic parameters, the newest generation of inhaled glucocorticoids can be developed, leading to better benefits, reduced systemic effects, and, consequently, an improved RBV.

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Pharmacokinetics of Etoricoxib in Patients with Renal Impairment

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The effect of renal insufficiency on the pharmacokinetics of etoricoxib, a selective inhibitor of cyclooxygenase-2, was examined in 23 patients with varying degrees of renal impairment (12 moderate [creatinine clearance between 30 and 50 mL/min/1.73 m²], 5 severe [creatinine clearance below 30 mL/min/1.73 m²], and 6 with end-stage renal disease requiring hemodialysis) following administration of single 120-mg oral doses of etoricoxib. Even the most severe renal impairment was found to have little effect on etoricoxib pharmacokinetics. The low recovery of etoricoxib in dialysate (less than 6% of the dose) supports that hemodialysis also has little effect on etoricoxib pharmacokinetics, and binding of etoricoxib to plasma proteins was generally unaffected by renal disease. Single doses of etoricoxib were generally well tolerated by patients with renal impairment. Based on pharmacokinetic considerations, dosing adjustments are not necessary for patients with any degree of renal impairment. However, because patients with advanced renal disease (creatinine clearance below 30 mL/min/1.73 m²) are likely to be very sensitive to any further compromise of renal function, and there is no long-term clinical experience in these patients, the use of etoricoxib is not recommended in patients with advanced renal disease.

Keywords: Etoricoxib; pharmacokinetics; renal impairment; bioavailability; cyclooxygenase; COX-2

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Etoricoxib (5-chloro-6′-methyl-3-[4-(methylsulfonyl)phenyl]-2,3′-bipyridine) is a recent entry into the field of cyclooxygenase-2 (COX-2) selective inhibitors that has been developed for the treatment of osteoarthritis, rheumatoid arthritis, and pain. Agents that selectively inhibit COX-2 are expected to be as clinically effective as nonselective nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit both COX-1 and COX-2 enzymes, in the treatment of pain, inflammation, and fever but with a much reduced risk of gastrointestinal toxicity.2,4

Elimination of etoricoxib has been shown to occur primarily through metabolism, with less than 1% of an administered oral or intravenous (IV) dose recovered intact in urine over 24 hours postdose.9 Renal insufficiency would, therefore, not be expected to have any direct effect on the elimination of etoricoxib. Nevertheless, it was deemed important to examine the effect of renal insufficiency on the pharmacokinetics of etoricoxib because of the potential effect of renal disease on other aspects of etoricoxib pharmacokinetics, such as protein binding and volume of distribution, and because renally impaired patients are potential recipients of therapy with etoricoxib. In addition, for pa-
patients undergoing hemodialysis, establishing the extent to which this procedure contributes to the removal of etoricoxib from circulation is important. Therefore, two pharmacokinetic studies were conducted to determine the effect of renal insufficiency and hemodialysis on etoricoxib pharmacokinetics. Both of these studies involved administration of single oral doses of etoricoxib; multiple-dose studies were not conducted because the pharmacokinetics of etoricoxib are linear, and thus single-dose pharmacokinetics reasonably predict multiple-dose pharmacokinetic behavior. A subset of the renally impaired patients was also administered a single IV dose of etoricoxib to more closely examine the disposition of etoricoxib in these patients.

METHODS

Study Designs

To evaluate the effect of renal insufficiency and hemodialysis on the pharmacokinetics of etoricoxib, two clinical studies were conducted. The first study was a two-part study involving 14 patients with renal impairment (8 moderate to severe [3 moderate, 5 severe], 6 with end-stage renal disease requiring hemodialysis). In Part I, 6 patients with end-stage renal disease (mean age: 51 years; range: 35-69 years; 5 men, 1 woman) were given, in two separate periods, single 120-mg oral doses of etoricoxib both 48 hours prior to and 4 hours prior to their normally scheduled 3-hour hemodialysis session. In Part II, 8 moderate to severe renal insufficiency patients were given separate single 120-mg oral doses and single 25-mg IV doses of etoricoxib in a randomized, crossover fashion. Three patients had moderate renal insufficiency (creatinine clearance between 30 and 50 mL/min/1.73 m²; mean age: 58 years; range: 49-68 years; 3 women), and 5 patients had severe renal insufficiency (creatinine clearance below 30 mL/min/1.73 m²; mean age: 47 years; range: 18-67 years; 3 men, 2 women). A washout period of at least 2 weeks was allowed between periods in each part of the study.

Because data in moderate renal insufficiency patients were very limited (n = 3) in the first study, an additional study was conducted to gain more experience in patients with this degree of renal impairment. In the second study, 9 patients with moderate renal insufficiency (mean age: 49 years; range: 32-72 years; 9 men) were given single 120-mg oral doses of etoricoxib only.

All doses were administered in the fasting state. All oral doses were administered with 240 mL of water. All IV doses were administered by 15-minute infusion; the exact weights of the IV solution administered wererecorded for each patient, so actual administered doses were used in all calculations. Blood samples were collected in heparinized tubes at predose and 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 30, 36, 48, 60, 72, 96, 120, and 168 hours following all doses; additional blood samples were collected at 0.25 and 0.75 hours following IV doses. For patients with end-stage renal disease, blood samples were also collected every half-hour during the 3-hour hemodialysis session. For these patients, dialysate samples were also collected for 1-minute intervals every half-hour during hemodialysis. All blood samples, which were protected from light, were placed on ice until plasma could be separated. The separated plasma and dialysate samples, also protected from light, were stored at –20°C until shipment to Merck Research Laboratories for analysis for etoricoxib. In the first study, plasma samples were also collected predose to examine in vitro protein binding of etoricoxib in patients with renal impairment (patients with end-stage renal disease in Part I, Periods 1 and 2; moderate to severe renal insufficiency patients in Part II, oral portion only).

All patients had a clinical diagnosis of renal impairment (stable for at least 4 weeks) and met the renal function qualifications described above (moderate, severe, or end stage). All patients were within 40% of ideal body weight. Women of childbearing potential were required to have a negative pregnancy test and to use appropriate barrier contraception during the study. Patients were required to refrain from consumption of grapefruit juice, herbal remedies, and nonapproved study drugs/medications prior to and throughout the study. Patients were excluded if they had major gastrointestinal (GI) abnormalities/hepatitis, a history of cardiac disorder, hepatic disease, or any other clinically important systemic disease that might have confounded the results of the study. Patients were also excluded if they had a history of serious adverse experiences related to NSAID use; had documented drug allergies or were allergic or intolerant to aspirin, ibuprofen, or other NSAIDs or COX-2 inhibitors; or had a history of asthma and nasal polyps associated with aspirin or NSAIDs. The patients provided written informed consent and agreed to follow study procedures. The study was conducted at multiple clinical sites, including the Orlando Clinical Research Center (Orlando, FL), PPD Development (Austin, TX), Total Renal Research (Minneapolis, MN), Radiant Research–Austin (Austin, TX), and Christchurch Clinical Studies Trust (Christchurch, New Zealand). The protocol conformed with the Declaration of Helsinki as written at the time of study initiation and was reviewed and approved by the Orlando Regional Healthcare System In-
stutional Review Board (Orlando, FL), Research Consultants Review Committee (Austin, TX), Human Subjects Research Committee (Minneapolis, MN), Research Consultants Review Committee (Austin, TX), and Canterbury Ethics Committee (Christchurch, New Zealand)/Standing Committee on Therapeutic Trials (Wellington, New Zealand), respectively.

Analytical Methods

Plasma samples collected following each etoricoxib dose in both studies and dialysate samples collected only in the first study (patients with end-stage renal disease in Part I, Periods 1 and 2) were assayed for etoricoxib by an analytical method involving solid-phase extraction for analyte isolation followed by reverse-phase high-performance liquid chromatography (HPLC) with postcolumn photochemical derivatization and fluorescence detection. The assay for etoricoxib in dialysate is essentially identical to another assay that was previously developed to measure etoricoxib concentrations in urine. The assay for etoricoxib in plasma is similar to that for dialysate, except that a protein precipitation step was needed prior to extraction. The standard curve analyzed daily with clinical samples ranged from 5 to 500 ng/mL. Intraday accuracy determined by replicate (n = 5) analysis of standards ranged from 94% to 113% for etoricoxib in plasma and from 96% to 107% for etoricoxib in dialysate. Intraday precision (expressed as percent coefficient of variation) was < 8% for etoricoxib in plasma and < 10% for etoricoxib in dialysate, as assessed by replicate analysis of standards. As assessed by coefficients of variation of low-, medium-, and high-quality control samples, interday precision was < 14% for etoricoxib in plasma and < 8% for etoricoxib in dialysate. The limit of reliable quantification for etoricoxib in plasma and dialysate was 5 ng/mL using 1 mL of sample.

Plasma samples collected predose in the first study (patients with end-stage renal disease in Part I, Periods 1 and 2; moderate to severe renal insufficiency patients in Part II, oral portion only) were also analyzed for in vitro binding of etoricoxib to plasma proteins using the ultrafiltration method. Briefly, predose patient plasma samples were incubated with [14C]etoricoxib at concentrations of 50, 500, and 5000 ng/mL. Following incubation at 37°C for 30 minutes, samples were subjected to centrifugation in an ultrafiltration device. Radioactivity in the plasma samples prior to filtration and in the ultrafiltrates was determined by liquid scintillation counting.

Pharmacokinetic Methods

Plasma and dialysate etoricoxib concentrations and actual sampling and dialysis times relative to the dose were used to estimate pharmacokinetic parameters for each treatment in each patient. The maximum concentration observed in plasma (Cmax) and its time of occurrence (tmax) following oral doses were obtained by inspection of the concentration-time data. The terminal half-life (t1/2) was estimated from the best-fit parameters of a single exponential to the log-linear portion of the plasma concentration-time curve. The best-fit parameters were obtained using unweighted nonlinear regression by the Simplex algorithm. Apparent terminal t1/2 values for the 48 hours postdose hemodialysis treatment were typically estimated using time points after dialysis, but time points during dialysis were also included for cases in which hemodialysis did not appear to affect the concentration-time profiles. The area under the plasma concentration-time curve (AUC) and the area under the first moment curve (AUMC) (following IV administration) were calculated using the linear trapezoidal method up to the last measured concentration, as well as the additional area estimated from that concentration and the value of the terminal t1/2 estimated for that administration. Dialysis clearance was calculated as the mean of the point clearances during hemodialysis, where point clearances were estimated as the ratio of the amount of etoricoxib recovered in 1 minute in dialysate (dialysate etoricoxib concentration × dialysate volume) to the corresponding plasma AUC of etoricoxib (plasma etoricoxib concentration × 1 min) for each plasma/dialysate sampling time during hemodialysis. The recovery of etoricoxib in dialysate, expressed as a percentage of the dose, was calculated from the product of the mean rate of recovery of etoricoxib in dialysate (dialysate etoricoxib concentration × dialysate volume collected in 1 min) and the dialysis time. Plasma clearance (CL) was calculated from IV data as the ratio of dose to AUC. For each individual, the actual IV dose administered was used, where actual dose = weight of dosing solution administered + formulation density × assayed potency of the dosing solution administered. The steady-state volume of distribution (Vdss) was calculated from IV data as

\[
V_{dss} = CL \cdot MRT = \frac{Dose_{IV}}{AUC_{\infty}} \left( \frac{AUMC_{\infty} - t_{inf}}{AUC_{\infty}} \cdot \frac{rinf}{2} \right)
\]

where MRT is the mean residence time and tinf is the infusion time (0.25 h). The absolute bioavailability of etoricoxib was assessed by dose-adjusted AUC ratios.
ETORICOXIB PHARMACOKINETICS IN RENAL IMPAIRMENT

(oral/IV), where the actual IV and oral doses administered, adjusted for assayed potencies of the formulations, were used. The assumption inherent in this calculation is that clearance is independent of dose. This assumption is valid since the kinetics of etoricoxib have been found to be linear following administration of single oral doses.\textsuperscript{10,11}

The presence of a measurable etoricoxib concentration in the predose plasma sample of 1 patient (IV treatment period) indicated that the 19-day washout period was insufficient for this patient. For this patient, the contributions to the observed plasma concentrations from the previous dose were estimated assuming exponential decay of the concentration observed at \( t = 0 \) using the terminal \( t_{1/2} \) estimated for that administration. Prior to determination of AUC\(_{\infty}\), the plasma profile of etoricoxib was corrected by subtracting the estimated contribution of the previous dose.

Statistical Methods

Historical Healthy Composite Control Group

Data from patients with renal insufficiency were compared to those from a healthy control group obtained from a composite analysis of pharmacokinetic data across all appropriate Merck Phase I pharmacokinetic studies in which single oral or IV doses of etoricoxib were administered to healthy subjects. In some of those studies, the subjects received several different single doses of etoricoxib. To obtain one observation per subject, the average of the pharmacokinetic measurements across doses (after potency adjusting and dose adjusting, if appropriate) was computed prior to comparisons to the renal insufficiency patients in this study. This is valid because dose proportionality has been well established for etoricoxib.\textsuperscript{10,11}

Rationale for Clinically Meaningful Bounds

To help evaluate the effect of renal insufficiency on the pharmacokinetics of etoricoxib, the safety and efficacy profiles of etoricoxib were used to define bounds within which 90\% confidence intervals (CIs) for geometric mean ratios (GMRs) of appropriate pharmacokinetic parameters (AUC\(_{\infty}\), C\(_{\text{max}}\)) should fall to conclude a lack of a clinically meaningful effect. The lower bound was determined based on the efficacy profile of etoricoxib. Since results from a Phase IIIB study\textsuperscript{14} in osteoarthritis show that a significant degree of efficacy is maintained at half the clinical dose (i.e., 30 mg as opposed to the clinical dose of 60 mg), a lower bound of 0.5 is justified. The upper bound was determined based on safety experience and the clinical dose range. The highest chronic dose of etoricoxib tested in clinical studies is 150 mg. This dose was found to be well tolerated and is 1.67-fold greater than the highest anticipated chronic clinical dose of 90 mg. Therefore, an upper bound of 1.67 is justified based on the safety profile of etoricoxib. However, it should be mentioned that this upper bound, though justified by existing data, is conservative since the 150-mg dose was generally well tolerated in healthy elderly volunteers. Thus, the safety and efficacy profile of etoricoxib supports the bounds of (0.5, 1.67) to designate a lack of a clinically meaningful effect.

Effect of Renal Impairment on Etoricoxib Pharmacokinetics

The pharmacokinetic parameters following a single 120-mg oral dose of etoricoxib in patients with renal dysfunction were compared to those of an appropriate combined historical database of healthy control subjects, as described above, using an analysis of covariance (ANCOVA) model. The ANCOVA model contained the factors population (healthy subjects; end-stage, severe, and moderate renal insufficiency patients), age, weight, and height. The data for moderate renal insufficiency patients were pooled across both studies. A log transformation was applied to the AUC\(_{\infty}\) and C\(_{\text{max}}\) data, a rank transformation was applied to the \( t_{\text{max}} \) data, and an inverse transformation was applied to the apparent terminal \( t_{1/2} \) data. The normality assumption was tested using the Shapiro-Wilk statistic, and the homogeneity of variance assumption was tested using Levene’s test on the absolute value of the residuals from the ANCOVA model.

AUC\(_{\infty}\), and C\(_{\text{max}}\) values for patients with end-stage renal disease were analyzed with the ANCOVA model described above and compared to the composite analysis of pharmacokinetics in healthy subjects. A 90\% CI ratio of least squares means (back-transformed from the log scale) from the above ANCOVA model between the patients with end-stage renal disease and healthy control subjects was computed for AUC\(_{\infty}\) and C\(_{\text{max}}\). The same analysis was used to compare severe and moderate renal insufficiency patients to the healthy subjects. Summary statistics were provided for t\(_{\text{max}}\) and apparent terminal \( t_{1/2} \) for patients with renal insufficiency and healthy subjects.

The relationship between AUC\(_{\infty}\) and creatinine clearance for patients with renal insufficiency and healthy subjects was assessed. All patients with end-stage renal disease were assumed to have a creatinine clearance of less than 5 mL/min/1.73 m\(^2\). Creatinine...
clearance for the healthy subjects was estimated at the prestudy screening visit using the Cockcroft-Gault equation.\textsuperscript{15}

**Effect of Hemodialysis on Etoricoxib Pharmacokinetics**

To assess the effect of hemodialysis on etoricoxib pharmacokinetics, patients with end-stage renal disease were given single doses of etoricoxib both 48 hours prior to and 4 hours prior to their normally scheduled 3-hour hemodialysis session. The pharmacokinetic parameters (AUC, C\text{max}, t\text{max}, and apparent terminal t\text{1/2}) from each treatment period were computed and compared using an analysis of variance (ANOVA) model with the factors subject and part (equivalent to a paired t-test); in addition, a 90% CI was also provided for the AUC and C\text{max} GMR (hemodialysis initiated 4 h postdose/hemodialysis initiated 48 h postdose). Summary statistics for dialysis clearance and recovery of etoricoxib in dialysate were also calculated.

One patient with end-stage renal disease was excluded from all statistical analyses of pharmacokinetic data because this patient was found to have pharmacokinetic parameters in one period that were markedly different from those of all the other patients with end-stage renal disease. Furthermore, after repeat administration of etoricoxib in a second period, the pharmacokinetic parameters for this patient were similar to those of all other patients with end-stage renal disease (Period 1 AUC = 121.09 µg•h/mL; Period 2 AUC = 35.60 µg•h/mL). The unusual pharmacokinetic data in the first period for this patient were not attributable to a dosing error, changes in concomitant medications, or any other readily identifiable cause. In any case, inclusion of the pharmacokinetic data for this patient would not have altered the conclusions regarding the lack of effect of renal insufficiency on the pharmacokinetics of etoricoxib; indeed, inclusion of the data for this patient would have further reduced the small differences observed in etoricoxib pharmacokinetics between patients with end-stage renal disease and healthy control subjects.

**Single-Dose IV Pharmacokinetics and Absolute Bioavailability of Etoricoxib in Moderate to Severe Renal Insufficiency**

AUC values following a single 120-mg oral dose and a single 25-mg IV dose in patients with moderate to severe renal insufficiency were compared using an ANOVA model appropriate for a two-period crossover design. The ANOVA model contained the factors sequence, subject within sequence, period, and route of administration (IV, oral). The absolute bioavailability of etoricoxib was estimated by a 90% CI on the dose-adjusted GMR (oral/IV) of AUC. A log transformation was applied to AUC∞.

Summary statistics were provided for AUC, t\text{1/2}, CL, and V\text{dss} following administration of the IV dose of etoricoxib. An ANCOVA model with the terms population (moderate to severe renal insufficiency patients, healthy subjects), gender, race, age, weight, and height was used to assess the between-population difference.

**Assessments of Safety and Tolerability**

Vital signs, physical examination, routine hematology, blood chemistry, urinalysis, and ECGs were monitored before, during, and after each treatment. Adverse experiences were evaluated by the investigator as to their intensity, seriousness, and relationship to study drug.

**RESULTS**

**Pharmacokinetics**

**Effect of Renal Impairment on Etoricoxib Pharmacokinetics**

The mean plasma concentration-time profiles of etoricoxib following oral administration of single 120-mg doses to patients with varying degrees of renal impairment and healthy control subjects are shown in Figure 1. A summary statistical comparison of pharmacokinetic parameters in patients with renal impairment and healthy control subjects accounting for the factors age, weight, and height is presented in Table I. Pharmacokinetic parameters for the healthy control group were obtained from a composite analysis of pharmacokinetic data across all appropriate Phase I studies, as described in Statistical Methods. The data for the healthy control subjects in Figure 1 encompass a subset of the data used for the composite statistical analysis; this mean profile is representative of the combined healthy control group.

The AUC∞ of etoricoxib was somewhat lower in patients with end-stage renal disease compared to healthy subjects, but no consistent trend was observed between AUC∞ and any other degree of renal impairment. The adjusted AUC∞ GMRs (90% CI) (renal insufficiency patients/healthy subjects) were 0.68 (0.51, 0.90), 0.93 (0.71, 1.22), and 1.04 (0.86, 1.25) for patients with end-stage, severe, and moderate renal impairment, respectively, and thus were contained within the bounds of (0.50, 1.67), indicating the lack of a clinically meaningful effect. C\text{max} values tended to be slightly
lower for patients with renal insufficiency than healthy subjects. Nevertheless, the adjusted $C_{\text{max}}$ GMRs (90% CI) (renal insufficiency patients/healthy subjects) were 0.79 (0.66, 0.95), 0.76 (0.64, 0.91), and 0.87 (0.76, 0.99) for patients with end-stage, severe, and moderate renal impairment, respectively, which were also contained within the bounds of (0.50, 1.67). The $t_{\text{max}}$ and apparent terminal $t_1/2$ values were similar between patients with renal impairment and healthy subjects.

To further examine the effect of renal impairment on etoricoxib pharmacokinetics, individual AUC∞ values were plotted as a function of creatinine clearance for all renal insufficiency patients and healthy subjects. As shown in Figure 2, no apparent relationship exists between AUC∞ and creatinine clearance.

### Table I  Comparison of Pharmacokinetic Parameters following Administration of Single Oral Doses of Etoricoxib to Patients with Different Degrees of Renal Impairment and Healthy Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population</th>
<th>$n$</th>
<th>Adjusted Mean</th>
<th>GMR</th>
<th>(90% Confidence Interval)</th>
<th>p-Value vs. Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{AUC}_\infty$ ($\mu g\cdot h/mL$), dose adjusted to 120 mg</td>
<td>End-stage renal$^c$</td>
<td>5</td>
<td>22.87</td>
<td>0.68</td>
<td>(0.51, 0.90)</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>Severe renal</td>
<td>5</td>
<td>31.36</td>
<td>0.93</td>
<td>(0.71, 1.22)</td>
<td>0.671</td>
</tr>
<tr>
<td></td>
<td>Moderate renal$^d$</td>
<td>12</td>
<td>34.71</td>
<td>1.04</td>
<td>(0.86, 1.25)</td>
<td>0.758</td>
</tr>
<tr>
<td></td>
<td>Healthy subjects$^e$</td>
<td>152</td>
<td>33.49$^f$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL), dose adjusted to 120 mg</td>
<td>End-stage renal$^c$</td>
<td>5</td>
<td>1752</td>
<td>0.79</td>
<td>(0.66, 0.95)</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>Severe renal</td>
<td>5</td>
<td>1698</td>
<td>0.76</td>
<td>(0.64, 0.91)</td>
<td>0.014</td>
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<td>12</td>
<td>1917</td>
<td>0.87</td>
<td>(0.76, 0.99)</td>
<td>0.069</td>
</tr>
<tr>
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<td>Healthy subjects$^e$</td>
<td>152</td>
<td>2209$^f$</td>
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<td>—</td>
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<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>End-stage renal$^c$</td>
<td>5</td>
<td>1.0$^g$</td>
<td>—</td>
<td>—</td>
<td>0.958</td>
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<tr>
<td></td>
<td>Severe renal</td>
<td>5</td>
<td>1.0$^g$</td>
<td>—</td>
<td>—</td>
<td>0.687</td>
</tr>
<tr>
<td></td>
<td>Moderate renal$^d$</td>
<td>12</td>
<td>1.1$^g$</td>
<td>—</td>
<td>—</td>
<td>0.360</td>
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<tr>
<td></td>
<td>Healthy subjects$^e$</td>
<td>152</td>
<td>1.0$^g$</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Apparent $t_{1/2}$ (h)</td>
<td>End-stage renal$^c$</td>
<td>5</td>
<td>19.3$^h$</td>
<td>—</td>
<td>—</td>
<td>0.596</td>
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<td></td>
<td>Severe renal</td>
<td>5</td>
<td>24.8$^h$</td>
<td>—</td>
<td>—</td>
<td>0.778</td>
</tr>
<tr>
<td></td>
<td>Moderate renal$^d$</td>
<td>12</td>
<td>21.0$^h$</td>
<td>—</td>
<td>—</td>
<td>0.673</td>
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<tr>
<td></td>
<td>Healthy subjects$^e$</td>
<td>152</td>
<td>20.9$^{1h}$</td>
<td>—</td>
<td>—</td>
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</tr>
</tbody>
</table>

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a. Back-transformed least squares mean from the log-scale ANCOVA model.
b. GMR = geometric mean ratio (renal insufficiency/healthy subjects).
c. Hemodialysis initiated 48 hours postdose.
d. Pooled moderate renal insufficiency patients (3 from first study, 9 from second).
e. Healthy composite control group obtained from all relevant studies, as described in Statistical Methods.
f. The adjusted mean for healthy subjects varies slightly depending on the model and population in question (hemodialysis or severe or moderate renal patients), and this value was obtained from the ANCOVA model for pooled moderate renal insufficiency patients.
g. Median
h. Harmonic mean.

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**Figure 1.** Mean concentration-time profiles of etoricoxib in plasma following administration of single 120-mg oral doses to patients with different degrees of renal impairment and healthy subjects.
hemodialysis, then, was assessed by comparing the pharmacokinetics of etoricoxib in the same patients with end-stage renal disease undergoing their normally scheduled 3-hour hemodialysis session at 4 and 48 hours postdose in two different treatment periods. The mean plasma concentration-time profiles of etoricoxib following administration of single 120-mg oral doses in the two treatment periods are shown in Figure 3. As illustrated in Figure 3, the mean etoricoxib concentrations increase transiently after the start of the 3-hour hemodialysis session. This increase is most likely due to etoricoxib becoming concentrated in the plasma as excess body water is removed during hemodialysis.

Examination of the mean plasma concentration-time profiles (Figure 3) and the mean pharmacokinetic parameter values (Table II) shows that although the $C_{\text{max}}$ values were somewhat lower (~18%) for the 4 hours postdose hemodialysis treatment, the $AUC_{\infty}$, $t_{\text{max}}$, and apparent terminal $t_{1/2}$ values were similar between treatments. The lower $C_{\text{max}}$ values for the 4 hours postdose hemodialysis treatment period may be attributable to a larger volume of total body water during this treatment period than during the 48 hours postdose hemodialysis treatment period (because of the differences in timing of the dose relative to the last hemodialysis treatment). The recovery of etoricoxib in the dialysate was low (< 6% of dose), also supporting the conclusion that hemodialysis has little effect on the plasma pharmacokinetics of etoricoxib. The mean dialysis clearance values appear to be slightly different between the two treatments (50 mL/min for the 4 hours postdose hemodialysis treatment vs. 18 mL/min for the 48 hours postdose hemodialysis treatment). However, this difference is most likely an artifact; the mean dialysis clearance value for the 48 hours postdose hemodialysis treatment is underestimated because the concentrations of etoricoxib in dialysate were below analytical detection limits for 2 of the 5 subjects.

**Single-Dose IV Pharmacokinetics and Absolute Bioavailability of Etoricoxib in Moderate to Severe Renal Insufficiency**

Patients with moderate to severe renal insufficiency in the first study were also administered a single IV dose of 25 mg to assess the effect of renal insufficiency on the disposition of etoricoxib and to estimate the absolute bioavailability of etoricoxib in these patients. A summary statistical comparison of IV pharmacokinetic parameters for renal insufficiency patients and healthy control subjects, accounting for the factors age, weight, and height, is presented in Table III. All pharmacokinetic parameters were found to be comparable between these two populations.

The absolute bioavailability of etoricoxib in patients with moderate to severe renal insufficiency was estimated to average 100%, with a corresponding 90% CI of (82%, 121%), which is essentially identical to that in healthy subjects (100%).

**In Vitro Protein Binding of Etoricoxib in Renal Impairment**

The effect of renal impairment on the binding of etoricoxib to plasma proteins in predose samples was also examined. The average in vitro protein binding was 87.4% for patients with moderate to severe renal insufficiency, 86.5% for patients with end-stage renal
disease with hemodialysis initiated 4 hours postdose, and 87.6% for patients with end-stage renal disease with hemodialysis initiated 48 hours postdose. These results are not clinically meaningfully different from those determined for healthy subjects (91.9%).

**Table II** Comparison of Pharmacokinetic Parameters between Patients with End-Stage Renal Disease (n = 5) Administered 120-mg Single Oral Doses 4 and 48 Hours before the Start of a 3-Hour Dialysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Timing of Hemodialysis Relative to Etoricoxib Administration (h)</th>
<th>Geometric Mean</th>
<th>GMR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>(90% Confidence Interval)</th>
<th>Between-Period p-Value</th>
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<tbody>
<tr>
<td>AUC&lt;sub&gt;∞&lt;/sub&gt; (µg•h/mL)</td>
<td>4</td>
<td>20.23</td>
<td>0.92</td>
<td>(0.49, 1.74)</td>
<td>0.814</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>21.96</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>4</td>
<td>1327</td>
<td>0.82</td>
<td>(0.64, 1.05)</td>
<td>0.172</td>
</tr>
<tr>
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<td>48</td>
<td>1620</td>
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<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>4</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>—</td>
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<tr>
<td></td>
<td>48</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Apparent t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>4</td>
<td>18.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td>48</td>
<td>19.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
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<tr>
<td>Dialysis clearance (mL/min)</td>
<td>4</td>
<td>50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
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<td></td>
<td>48</td>
<td>18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>Recovery in dialysate (% of dose)</td>
<td>4</td>
<td>5.1&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>—</td>
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<tr>
<td></td>
<td>48</td>
<td>0.6&lt;sup&gt;d&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup> GMR = geometric mean ratio (hemodialysis initiated 4 h postdose/hemodialysis initiated 48 h postdose).
<sup>b</sup> Median.
<sup>c</sup> Harmonic mean.
<sup>d</sup> Arithmetic mean.

**Table III** Comparison of Pharmacokinetic Parameters following IV Administration of Etoricoxib to Moderate to Severe Renal Insufficiency Patients and Healthy Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population</th>
<th>n</th>
<th>Mean&lt;sup&gt;a&lt;/sup&gt;</th>
<th>GMR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>(90% Confidence Interval)</th>
<th>p-Value vs. Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;∞&lt;/sub&gt; (µg•h/mL), dose</td>
<td>Moderate to severe renal&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8</td>
<td>8.18</td>
<td>1.10</td>
<td>(0.81, 1.48)</td>
<td>0.598</td>
</tr>
<tr>
<td></td>
<td>Healthy subjects&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24</td>
<td>7.45</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>Moderate to severe renal&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8</td>
<td>51</td>
<td>—</td>
<td>—</td>
<td>0.602</td>
</tr>
<tr>
<td></td>
<td>Healthy subjects&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24</td>
<td>56</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>V&lt;sub&gt;ss&lt;/sub&gt; (L)</td>
<td>Moderate to severe Renal&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8</td>
<td>132</td>
<td>—</td>
<td>—</td>
<td>0.456</td>
</tr>
<tr>
<td></td>
<td>Healthy subjects&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24</td>
<td>124</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>Moderate to severe renal&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8</td>
<td>29.6</td>
<td>—</td>
<td>—</td>
<td>0.661</td>
</tr>
<tr>
<td></td>
<td>Healthy subjects&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24</td>
<td>27.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> Back-transformed least squares mean ANCOVA model.
<sup>b</sup> GMR = geometric mean ratio (renal insufficiency/healthy subjects).
<sup>c</sup> Renal insufficiency patients (3 moderate, 5 severe) from the first study.
<sup>d</sup> Healthy composite control group obtained from all relevant studies, as described in Statistical Methods.

**Safety and Tolerability**

Single doses of etoricoxib were generally well tolerated by patients with renal impairment in both studies. In the first study, 5 patients reported a total of 11 clinical
adverse experiences, 1 of which was serious but did not result in discontinuation. The serious clinical adverse experience that occurred for 1 patient was a soft tissue infection of the right foot that required hospitalization. This patient had the great toe amputated approximately 3 months prior to study initiation, and at the screening visit, her physical examination showed a toe healing properly without obvious infection. She enrolled in the study and received a single 120-mg oral dose of etoricoxib in Period 1. On Day 6 of this period, she visited her personal physician for a regular checkup, at which time debridement of the great toe was performed, excessive bleeding occurred, and a low hematocrit value (18.4%) was noted after the procedure. She was then hospitalized for low hematocrit, and a severe soft tissue infection of the foot was diagnosed. After 3 days without improvement, a below-knee amputation was performed. The clinical adverse experience (soft tissue infection) lasted for 6 days, was severe in intensity, and was considered definitely not drug related by the investigator. After recovery from her hospitalization and amputation, the patient completed Period 2 without problems. Two patients had clinical adverse experiences (injection site pain and injection site burning) that were considered possibly drug related by the investigator and were mild in intensity.

In the second study, 4 patients reported a total of five clinical adverse experiences, none of which was serious or resulted in discontinuation. Two of these adverse experiences were considered possibly drug related by the investigator (both dysgeusia, bad taste sensation) and were mild in intensity. Hypoglycemia (55 mg/dL [normal range: 70-110 mg/dL] and 49 mg/dL [normal range: 65-115 mg/dL]) was reported as a laboratory adverse experience in 2 patients. These adverse experiences occurred predose on Day 1 and were determined to be definitely not drug related by the investigator. Subsequent glucose laboratory values for those patients were within the normal ranges.

DISCUSSION

The influence of renal insufficiency and hemodialysis on the pharmacokinetics of etoricoxib was investigated in two studies involving 23 patients with varying degrees of renal impairment (12 moderate, 5 severe, 6 with end-stage renal disease requiring hemodialysis) who received single 120-mg oral doses of etoricoxib.

Together, the results from both studies clearly demonstrate that renal impairment has no clinically meaningful effect on the pharmacokinetics of etoricoxib. The AUC∞ values from patients with renal insufficiency were similar to or slightly less than those from healthy control subjects. Importantly, no relationship was found between AUC∞ and creatinine clearance, further supporting the conclusion that renal impairment has no clinically important effect on the pharmacokinetics of etoricoxib. Cmax values tended to be slightly but not clinically meaningfully lower in patients with renal impairment compared with values in healthy subjects. The tmax and apparent terminal t1/2 values for renal insufficiency patients were similar to those from healthy subjects. Because plasma protein binding of a drug can be altered in patients with impaired renal function, in vitro binding experiments were conducted in uremic plasma from the patients in the first study. The binding of etoricoxib to plasma proteins in renal insufficiency patients (86%-88%) was not clinically meaningfully different from that in healthy subjects (91.9%).

A subset of patients with moderate and severe renal insufficiency was also administered a single IV dose of 25 mg to assess the effect of renal insufficiency on the disposition of etoricoxib and to estimate the absolute bioavailability of etoricoxib in these patients. The pharmacokinetics of intravenously administered etoricoxib were found not to be altered to a clinically meaningful extent in these patients compared to healthy historical control subjects. The absolute bioavailability of etoricoxib in these patients (100%) was also found to be similar to that in healthy historical control subjects (100%).

Hemodialysis was also found to have no significant effect on the pharmacokinetics of etoricoxib in patients with end-stage renal disease. Less than 6% of an administered dose was recovered as etoricoxib in dialysate. This finding is not unexpected because of the large volume of distribution (Vss ~120 L) and moderate protein binding of etoricoxib.

The concentrations of the metabolites of etoricoxib were not measured in these studies because the metabolites are not expected to present a safety concern or contribute to pharmacological activity in patients with renal impairment. Elimination of etoricoxib occurs primarily through metabolism, whereas elimination of the metabolites occurs both renally and through biliary excretion. Thus, even though renal elimination of the metabolites is likely to be affected by renal impairment, the metabolites are not likely to present a safety concern since they are not likely to accumulate substantially since another pathway for elimination of the metabolites exists (via biliary excretion). The metabolites of etoricoxib are also not expected to contribute to
pharmacological activity. The metabolites were not active as inhibitors in the COX-1 whole-blood assay, and only two of the metabolites showed weak activity in the COX-2 assay (~20-fold less than etoricoxib).\textsuperscript{16} In addition, in healthy subjects, the bulk of circulating drug-related material was found to be accounted for by etoricoxib (~74%) rather than by metabolites following administration of single doses.\textsuperscript{9}

Single doses of etoricoxib were generally well tolerated by patients with renal impairment in both studies. The clinical adverse experiences that were considered to be possibly or probably drug related, including injection site pain and burning, dysgeusia, and bad taste sensation, were all mild in intensity and resolved. Since this study involved only single doses of etoricoxib, no conclusions can be made about the safety of multiple doses of etoricoxib from this study.

Recent studies have shown that COX-2 is constitutively expressed in the kidneys and that COX-2 may be involved in prostaglandin-dependent renal homeostatic processes.\textsuperscript{17,18} Therefore, although COX-2 selective inhibitors are expected to be as clinically effective as nonselective NSAIDs (which inhibit both COX-1 and COX-2) but with a much reduced risk of gastrointestinal toxicity, it is unlikely that they will provide any renal safety benefits over nonselective NSAIDs.\textsuperscript{19,20} Because patients with advanced renal disease (creatinine clearance below 30 mL/min/1.73 m\textsuperscript{2}) are very sensitive to further decreases in prostaglandin activity accompanied by detrimental changes in renal function,\textsuperscript{21} a known side effect of all NSAIDs and COX-2 selective inhibitors, and there is no long-term clinical experience in these patients, the use of etoricoxib is not recommended in patients with advanced renal disease. This recommendation is consistent with that for all NSAIDs and COX-2 selective inhibitors.

Thus, based on pharmacokinetic considerations, dosing adjustments are not necessary for patients with renal impairment. Although renal insufficiency has little effect on the pharmacokinetics of etoricoxib, the use of etoricoxib is not recommended in patients with advanced renal disease.

REFERENCES


Pharmacokinetics and Pharmacodynamics of the 
ET$\alpha$-Selective Endothelin Receptor Antagonist 
SPP301 in Healthy Human Subjects

Walter Dieterle, PhD, Jessica Mann, MD, PhD, and Klaus Kutz, MD

Endothelins (ETs) are a family of three 21-amino acid peptides—ET-1, ET-2, and ET-3—that have been shown to be potent and long-lasting vasoactive agents.1 ET-1 is the most important isoform in the cardiovascular system and is released mainly from endothelial cells.2 ET-1 plays a significant role in the pathogenesis of a variety of diseases, including hypertension, heart failure, renal failure, and respiratory diseases.3 ET-1 actions are mediated via two receptor types, ET$\alpha$ and ET$\beta$, which are distinguished by different binding affinities for the endothelins. The ET$\beta$ receptor binds the three isotypes with equal affinity, functioning as a clearance receptor and modulator of ET-1 secretion.4 In contrast, the ET$\alpha$ receptor binds ET-1 with a higher affinity than the other isoforms. It is predominantly located in vascular smooth muscle cells, cardiomyocytes, and fibroblasts and mediates vasoconstricting and proliferative responses.5

SPP301 (Figure 1) is a competitive antagonist of ET-1 with a high selectivity for the ET$\alpha$ receptor. A double-blind, placebo-controlled study was performed to investigate the tolerability, pharmacokinetics, and pharmacodynamics of SPP301 after single oral doses in male healthy subjects; doses of 5, 20, 50, 100, and 200 mg were given to different groups of 4 or 8 subjects each. The effect of food on the pharmacokinetics of SPP301 was assessed for the 50-mg dose according to a sequential design in the same subjects. At regular intervals, blood pressure and pulse rate, plasma levels of ET-1 and of SPP301 and its hydroxymethyl metabolite, and urinary excretion of the parent drug and its metabolite were determined. SPP301 was generally well tolerated. At doses > 20 mg, adverse events that are typical for vasodilating agents—namely, headache, nausea and vomiting, dizziness, and postural hypotension—were observed. Maximum plasma levels of SPP301 were reached within 4.5 hours. $C_{\text{max}}$ and AUC values increased linearly with doses up to 100 mg. The apparent terminal half-life was quite constant over the whole dose range and ranged from 7.5 to 15.2 hours. Urinary excretion of SPP301 was below 0.1% of any dose. $C_{\text{max}}$ and AUC of the metabolite amounted to about 5% of the values for SPP301. Concomitant food intake had no effect on the overall exposure but increased average peak plasma concentrations of SPP301 by around 50%. Plasma ET-1 increased nearly twofold at the 5-mg SPP301 dose, with no further relevant increase at higher doses. In conclusion, SPP301 is an active ET$\alpha$-1 antagonist and is well tolerated. The pharmacokinetics of the drug and its metabolite are linear up to 100 mg. Food does not affect overall exposure of SPP301 but increases $C_{\text{max}}$. Urinary excretion of SPP301 is below 0.1% of the dose administered.

**Keywords:** Endothelin-1; SPP301; ET$\alpha$-selective endothelin receptor antagonist; tolerability; pharmacokinetics; pharmacodynamics

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From Drug Disposition Consultants, Loerrach, Germany (Dr. Dieterle); Clinical Research & Development, Speedel Development AG, Basle, Switzerland (Dr. Mann); and AccelPharm, Basle, Switzerland (Dr. Kutz). Submitted for publication May 31, 2003; revised version accepted October 18, 2003. Address for reprints: Dr. Jessica Mann, Speedel Development AG, Department of Clinical Research & Development, Hirschgässlein 11, CH-4051 Basel, Switzerland. DOI: 10.1177/0091270003261047
licensed SPP301 from Hoffmann-La Roche in 2000 and brought it into man in early 2001.

This report describes the tolerability, pharmacokinetics, and pharmacodynamics after single oral dosing with SPP301 over a wide dose range (5-200 mg SPP301) in healthy subjects, as well as the effect of a fat-rich meal on these parameters when a single oral dose of 50 mg SPP301 was administered.

METHODS

Study Subjects

Thirty-two healthy male Caucasian subjects (mean age, 31 ± 7 years; mean weight, 78 ± 8 kg; body mass index [BMI], 23.7 ± 1.9 kg/m²) participated in this study. All subjects gave written informed consent before prestudy screening. Physical examinations were performed, and adverse events, routine laboratory tests, ECGs, and vital signs were recorded frequently before and after each drug administration. Subjects were not eligible for the study if they had any clinically relevant diseases, particularly cardiovascular diseases; had taken any drug within 2 weeks prior to dosing; or had a positive test result for alcohol, drugs of abuse, hepatitis, or HIV. All subjects were institutionalized at the research center from about 24 hours before drug administration until 48 hours thereafter and were served standardized meals. Subjects were not permitted to engage in any strong physical exercise or strenuous sports activities during the study.

Study Design

This was a phase I, randomized, placebo-controlled, double-blind, single-center, escalating, single-dose study in sequential groups using increasing oral doses of SPP301 in healthy male subjects. The effect of food on the pharmacokinetics of SPP301 was assessed for one study medication under fasted and then under fed conditions after 14 days. The dose range investigated was 5 to 200 mg. Five dose levels (i.e., 5, 20, 50, 100, and 200 mg) were investigated in different groups of 4 or 8 subjects each. At the two lowest dose levels, each group consisted of 4 subjects (n = 3 on SPP301 and n = 1 on placebo); all other groups consisted of 8 subjects (n = 6 on SPP301 and n = 2 on placebo).

SPP301 was administered as a capsule formulation. The study medication was administered to sitting subjects between 8:15 and 9:40 a.m. after an overnight fast of at least 10 hours. The same subjects in the 50-mg dose group received the same study medication under fasted and fed conditions. When given under fed conditions, the study medication was administered to sitting subjects 30 minutes after the start of a standardized fat-rich breakfast.

Safety was evaluated through adverse event monitoring, clinical laboratory testing, and physical examination as well as through measurement of supine and standing systolic and diastolic blood pressure and pulse rate (Dinamap, Criticon, Norderstedt, Germany). At specific times during the study, a 12-lead resting ECG was taken after the subjects had been in the supine position for at least 5 minutes. The ECG recorder computed and automatically recorded PR, QT, and QTc intervals; QRS duration; and heart rate.

Drug Assay

Blood samples for the measurement of plasma concentrations of SPP301 and of its hydroxymethyl metabolite Ro 68-5925 were drawn through an indwelling can-
oral clearance (CL/f), and the fraction of dose excreted unchanged or as the hydroxymethyl metabolite in the urine (F\textsubscript{u}).

**PHARMACOKINETICS AND PHARMACODYNAMICS**

Eclipse XDBC18 (5 µm, 2.1 × 150 mm) reversed-phase column and introduced into the mass spectrometer with an ion spray (pneumatically assisted electrospray) interface at a flow rate of 0.3 mL/min without post-column splitting. Deuterated analogs of SPP301 and Ro 68-5925 were used as internal standards for the assay. The limit of quantification was 0.5 ng/mL for SPP301 in plasma and urine and 2.0 ng/mL for Ro 68-5925 in plasma and urine.

The performance of the analytical method was monitored by simultaneous analysis of independently prepared quality control samples at various concentrations. The interassay coefficient of variation for both analytes was always below 9.8% (plasma) and below 6.5% (urine). The inaccuracy for SPP301 was between –0.2% and 1.8% in plasma and between –11.4% and 6.3% in urine and, for Ro 68-5925, between –3.4% and 1.8% in plasma and between –5.0% and 4.5% in urine.

**Pharmacokinetic Evaluation**

Pharmacokinetic evaluation was performed with model-independent methods. The pharmacokinetic parameters calculated were peak plasma concentrations (C\textsubscript{max}), time to reach C\textsubscript{max} (t\textsubscript{max}), area under the curve (AUC\textsubscript{0-inf}), apparent terminal half-life (t\textsubscript{1/2}), apparent oral clearance (CL/f), and the fraction of dose excreted unchanged or as the hydroxymethyl metabolite in the urine (F\textsubscript{u}).

C\textsubscript{max} and t\textsubscript{max} values were taken directly from the observed plasma concentration-time data. The area under the curve from zero to the last measurement point t with a concentration above the limit of quantification, calculated by the trapezoidal rule; C\textsubscript{t} is the concentration measured at t; and β is the apparent elimination rate constant determined by log-linear regression analysis of the terminal phase. The t\textsubscript{1/2} was calculated by division of ln 2 by β. Apparent oral clearance CL/f was estimated by division of the dose by AUC\textsubscript{0-inf}. Cumulative urine excretion F\textsubscript{u}, expressed as the percentage of the administered dose, during the time interval from 0 to 48 hours was calculated from the urinary concentration and the corresponding urine volumes.

**Pharmacodynamic Assessment**

Blood samples for the determination of endothelin (ET-1) were collected in EDTA-containing polypropylene tubes and were centrifuged at 4°C. The plasma samples were stored at –20°C or lower until the assay. The assay employed the quantitative sandwich enzyme immunoassay technique using the ET-1 kit from R&D Systems (Minneapolis, MN). In brief, a monoclonal antibody specific for ET-1 was precoated onto a microplate. Standards, quality controls, and samples were pipetted into the wells; an enzyme-linked monoclonal antibody specific for ET-1 was added to the wells. Following a washing step to remove any unbound antibody enzyme reagent, an enhanced luminal/peroxidase substrate was added to the wells, and light was produced in proportion to the amount of ET-1 bound in the initial step. A microplate luminometer was used to measure the intensity of the light emitted. The limit of quantification of ET-1 in plasma was 0.35 pg/mL.

To assess the effect of SPP301 on plasma ET-1, the following parameters were determined: ET-1 concentration immediately before drug administration (ET-1\textsubscript{t=0}), ET-1 at the maximum concentration (ET-1\textsubscript{max}), time to reach maximum concentration (ET-1\textsubscript{t=0}), and area under the concentration-time curve (ET-1\textsubscript{AUC(0-48h)}).

**RESULTS**

**Pharmacokinetics**

The time course of SPP301 in plasma after the five different doses is given in Figure 2 (mean plots). The estimated pharmacokinetic parameters are summarized in Table I.

Under fasted conditions, peak plasma levels of SPP301 were reached within an average of 4 to 4.8
hours. These peak levels and the area under the curve increased proportionally with the dose, up to 100 mg (Figure 3).

With 200 mg, there was no further increase in C\text{max} and only a small increase in AUC\text{0-}\infty values (Figure 3). The apparent terminal half-life was quite constant over the whole dose range investigated and ranged from 7.5 to 15.2 hours on average. Less than 0.1% of the dose was excreted unchanged in urine.

Concomitant food intake had no effect on the overall exposure but increased the average maximum plasma concentration of SPP301 by approximately 50% (Figure 4).

The estimated pharmacokinetic parameters of the hydroxymethyl metabolite Ro 68-5925 are summarized in Table 2.

Ro 68-5925 appeared rather slowly in plasma under fasted conditions. Maximum concentrations were reached within 11 hours. C\text{max} and AUC values increased linearly up to 100 mg SPP301. From 100 mg to 200 mg SPP301, the increase was less pronounced and amounted to about 50% for both C\text{max} and AUC values. The apparent terminal half-life was quite constant over the whole total dose range investigated and ranged from 7.6 to 11.7 hours on average. The ratio Ro 68-5925/SPP301 on a nanogram basis was rather low for C\text{max} and AUC and amounted to approximately 0.05.

Concomitant food intake had no effect on the overall exposure of Ro 68-5925 but increased its average maximum plasma concentration, however, to a level lower than that of the parent drug.

Table 1  Mean ± SD and Range Values of the Main Pharmacokinetic Parameters of SPP301 in Plasma after Single Oral Administration of Different Doses of SPP301 under Fasted and Fed Conditions (only 50 mg) in Healthy Young Male Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>5 mg (n = 3)</th>
<th>20 mg (n = 3)</th>
<th>50 mg Fasted (n = 6)</th>
<th>100 mg (n = 5)</th>
<th>200 mg (n = 6)</th>
<th>50 mg Fed (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\text{max} (ng/mL)</td>
<td>156 ± 27</td>
<td>649 ± 435</td>
<td>1238 ± 532</td>
<td>2375 ± 1034</td>
<td>2399 ± 419</td>
<td>1811 ± 520</td>
</tr>
<tr>
<td>t\text{max} (h)</td>
<td>4.5 ± 0.0</td>
<td>4.0 ± 0.9</td>
<td>4.5 ± 0.9</td>
<td>4.8 ± 0.7</td>
<td>4.3 ± 1.1</td>
<td>5.3 ± 2.1</td>
</tr>
<tr>
<td>AUC\text{0-}\infty (h•ng/mL)</td>
<td>2311 ± 491</td>
<td>6847 ± 4171</td>
<td>18,880 ± 6982</td>
<td>32,743 ± 4836</td>
<td>43,963 ± 21,704</td>
<td>19,375 ± 7242</td>
</tr>
<tr>
<td>t\text{1/2} (h)</td>
<td>10.3 ± 0.2</td>
<td>7.5 ± 1.6</td>
<td>10.6 ± 4.5</td>
<td>9.8 ± 2.1</td>
<td>15.2 ± 8.9</td>
<td>6.2 ± 1.1</td>
</tr>
<tr>
<td>CL/f (L/h)</td>
<td>10.1-10.5</td>
<td>5.7-8.9</td>
<td>6.4-18.3</td>
<td>7.3-12.4</td>
<td>6.6-28.1</td>
<td>5.5-8.2</td>
</tr>
<tr>
<td>CL/f (L/h)</td>
<td>2.2 ± 0.5</td>
<td>3.6 ± 1.6</td>
<td>3.0 ± 1.1</td>
<td>3.1 ± 0.4</td>
<td>5.5 ± 2.4</td>
<td>2.9 ± 1.0</td>
</tr>
<tr>
<td>Fu (% of SPP301 administered)</td>
<td>0.04</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>&lt; 0.01</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Figure 2  Mean plasma concentration versus time curves of SPP301 after single oral administration of different doses of SPP301 under fasted conditions in healthy young male subjects given on a linear scale (upper part) and on a semi-logarithmical scale (lower part).
Urinary excretion of Ro 68-5925 was low but more than 100-fold higher than that of the parent compound. There was a dose-dependent decrease in the urinary excretion of Ro 68-5925: it decreased from 5.5% (5-mg SPP301 administration) to 1.8% (200-mg SPP301 administration) of the amount of SPP301 administered, probably due to saturation of urinary excretion. Prior intake of a fat-rich meal had no influence on the urinary excretion of Ro 68-5925.

Pharmacodynamics

Figure 5 shows the mean plasma concentration versus time curves of ET-1 for the five different doses of SPP301 and placebo given under fasted conditions on a linear scale. SPP301 significantly increased plasma ET-1, as expressed by ET-1_{max} and ET-1_{AUC(0-48h)} values (Table III). ET-1 levels did not change in the placebo group. At the lowest dose of 5 mg, a maximum increase in ET-1 levels was observed. The maximum ET-1 level

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**Table II**  Mean ± SD and Range Values of the Main Pharmacokinetic Parameters of Ro 68-5925 in Plasma after Single Oral Administration of Different Doses of SPP301 under Fasted and Fed Conditions (only 50 mg) in Healthy Young Male Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>5 mg (n = 3)</th>
<th>20 mg (n = 3)</th>
<th>50 mg Fasted (n = 6)</th>
<th>100 mg (n = 5)</th>
<th>200 mg (n = 6)</th>
<th>50 mg Fed (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (ng/mL)</td>
<td>6.0 ± 1.8</td>
<td>21.2 ± 8.2</td>
<td>50.5 ± 17.8</td>
<td>72.7 ± 32.4</td>
<td>103.1 ± 20.4</td>
<td>67.1 ± 13.0</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>4.6-8.0</td>
<td>15.9-30.6</td>
<td>31.6-74.6</td>
<td>38.9-120.2</td>
<td>84.5-136.4</td>
<td>51.8-90.2</td>
</tr>
<tr>
<td>AUC_{0-∞} (h•ng/mL)</td>
<td>133 ± 16</td>
<td>398 ± 213</td>
<td>1002 ± 357</td>
<td>1634 ± 335</td>
<td>2411 ± 914</td>
<td>1172 ± 439</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>9.0-12.0</td>
<td>4.5-9.0</td>
<td>9.0-12.0</td>
<td>6.0-12.0</td>
<td>6.0-12.0</td>
<td>6.0 ± 12.0</td>
</tr>
<tr>
<td>Fu (% of SPP301 administered)</td>
<td>5.50</td>
<td>4.08</td>
<td>3.00</td>
<td>2.88</td>
<td>1.79</td>
<td>3.46</td>
</tr>
</tbody>
</table>

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was reached, on average, between 12 and 30 hours postdose. Increasing the dose of SPP301 did not change any pharmacodynamic parameters (Table III). Food had no effect on these parameters of ET-1 when 50 mg SPP301 was given as a single oral dose.

**Safety**

All subjects completed the study. No serious or severe adverse events were reported throughout the study. No adverse events (AE) were reported for the 5-mg and 20-mg doses (Table IV). The 50-mg and higher doses showed headache as the main adverse event. There was no increase in the frequency or the severity of headaches when increasing the doses of SPP301 from 50 mg up to 200 mg. Dizziness, postural hypotension, nausea, and vomiting lasted only a few minutes and resolved spontaneously in most cases. Two subjects on SPP301 experienced an episode of brief loss of consciousness, which did not require specific measures beyond rest. Safety laboratory parameters such as hematology, biochemistry, or urinalysis as well as ECG parameters and physical examination showed neither clinically significant deviations from normal ranges nor clinically significant changes during the study in all treatment groups.

**DISCUSSION**

The present study is the first to report on the safety, tolerability, pharmacodynamics, and pharmacokinetics of the orally available ET_{A}-selective endothelin receptor antagonist SPP301 in humans. Our data show that the tolerability of single oral doses of SPP301 was extremely good up to 20 mg, with no adverse events reported. At higher doses, adverse events were observed that are typical for vasodilating agents—namely, headache, nausea and vomiting, dizziness, and postural hypotension. There was no increase in the number of adverse events with the increase in dose from 50 to 200 mg SPP301. These findings indicate two characteristics of the drug. First, the spectrum of adverse events that are typical for vasodilatory compounds shows that SPP301 has clearly ETA antagonistic properties, as shown in animal experiments. Second, the dose-response curve after single oral administration in the range between 5 and 200 mg with regard to clinical symptoms is sigmoid, with no adverse effects up to 20 mg and no further increase in severity of the adverse events between the doses of 50 and 200 mg. In addition, the spectrum of adverse events did not change by increasing the dose of SPP301. Concomitant food intake had no effect on the tolerability of 50 mg SPP301, although maximum plasma concentrations of the parent drug increased by

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**Table III** Mean ± SD and Range Values of the Main Pharmacodynamic Parameters of ET-1 in Plasma after Single Oral Administration of Different Doses of SPP301 or Placebo under Fasted and Fed Conditions (only 50 mg) in Healthy Young Male Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Placebo (n = 8)</th>
<th>5 mg (n = 3)</th>
<th>20 mg (n = 3)</th>
<th>50 mg Fasted (n = 6)</th>
<th>100 mg (n = 6)</th>
<th>200 mg (n = 6)</th>
<th>50 mg Fed (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1_{t=0} (pg/mL)</td>
<td>1.18 ± 0.52</td>
<td>1.27 ± 0.45</td>
<td>1.31 ± 0.38</td>
<td>1.40 ± 0.18</td>
<td>1.08 ± 0.24</td>
<td>1.45 ± 0.95</td>
<td>1.01 ± 0.21</td>
</tr>
<tr>
<td>ET-1_{max} (pg/mL)</td>
<td>0.67 ± 0.34</td>
<td>0.90 ± 1.77</td>
<td>1.05 ± 1.75</td>
<td>1.21 ± 1.72</td>
<td>0.81 ± 1.50</td>
<td>0.58 ± 3.31</td>
<td>0.70 ± 1.33</td>
</tr>
<tr>
<td>ET-1_{tmax} (h)</td>
<td>0.04 ± 0.24</td>
<td>1.71 ± 2.94</td>
<td>1.84 ± 4.09</td>
<td>2.18 ± 2.86</td>
<td>2.16 ± 2.06</td>
<td>1.48 ± 1.47</td>
<td>1.48 ± 1.47</td>
</tr>
<tr>
<td>ET-1_{AUC0-48h} (h•pg/mL)</td>
<td>47.3 ± 8.0</td>
<td>75.1 ± 18.7</td>
<td>86.5 ± 25.1</td>
<td>77.1 ± 16.0</td>
<td>76.5 ± 16.0</td>
<td>82.2 ± 22.7</td>
<td>65.4 ± 14.1</td>
</tr>
</tbody>
</table>

Figure 5. Mean plasma concentration versus time curves of ET-1 after single oral administration of different doses of SPP301 and of placebo under fasted conditions in healthy young male subjects.
There were no clinically relevant effects on hematology, biochemistry, or urinalysis parameters. The evaluation of vital signs, ECG parameters, and physical examinations showed no time or treatment effects.

SPP301 appeared moderately fast under fasting conditions, reaching maximum mean plasma levels within 4 to 5 hours postdose. The pharmacokinetics were linear up to 100 mg. At the 200-mg dose, systemic exposure was lower than predicted from linearity, most likely due to saturable absorption. The half-life of SPP301 ranged between 7.5 and 15.2 hours, allowing once-daily administration. The formation of the hydroxymethyl metabolite Ro 68-5925 was slow, as is evident from its maximum plasma concentrations observed at 11 hours postdose. Its half-life was comparable to that of the parent drug. The metabolic pathway leading to the hydroxymethyl metabolite might not be of relevance for the clearance of the drug since $C_{\text{max}}$ and AUC values for Ro 68-5925 were only about 5% of those of SPP301. However, this conclusion does not consider a possibly very different distribution pattern of the two compounds. On the other hand, urinary excretion of the metabolite was also low but was more than 100-fold higher than that of the parent drug. Thus, further studies are necessary to clarify the importance of this metabolic pathway in man.

Because only extremely low amounts of SPP301 and low amounts of the hydroxymethyl metabolite were found in urine, hepatic clearance by metabolism and biliary excretion of SPP301 or its metabolites can be assumed as the major pathway(s) of excretion.

Concomitant food intake increased the maximum concentrations of SPP301 in plasma but had no effect on its systemic exposure. Thus, food was not associated with a relevant effect on the absorption of SPP301.

SPP301 significantly increased plasma ET-1 levels at the lowest dose of 5 mg. Plasma ET-1 measurements are useful because plasma concentrations have been found to correlate well with the severity of certain diseases such as primary pulmonary hypertension, cirrhosis, and heart failure. The observation that circulations of ET-1 are increased by the $E_{\text{T}}A$ receptor selective antagonist SPP301 in this study is in line with recent observations by others. Previously, $E_{\text{T}}B$ receptors have been implicated in the clearance of endogenous endothelin.

In conclusion, the study has shown that single oral doses of SPP301 are quite well tolerated. Adverse events that might occur are predictable and typical for potent vasodilating agents and generally resolved spontaneously. The pharmacokinetics of SPP301 and its hydroxymethyl metabolite are linear up to 100 mg. There is a slight food effect, resulting in an increase of $C_{\text{max}}$ but not in the overall exposure. Urinary excretion of SPP301 is below 0.1% of the administered dose. ET-1 plasma concentrations increased nearly twofold after a single dose of 5 mg.

We thank Mrs. Monja Hammer for her assistance in preparing the manuscript.

REFERENCES


The Pharmacokinetics of Nebulized Nanocrystal Budesonide Suspension in Healthy Volunteers

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Nanocrystal budesonide (nanobudesonide) is a suspension for nebulization in patients with steroid-responsive pulmonary diseases such as asthma. The pharmacokinetics and safety of the product were compared to those of Pulmicort Respules. Sixteen healthy volunteers were administered nanobudesonide 0.5 and 1.0 mg, Pulmicort Respules 0.5 mg, and placebo in a four-way, randomized crossover design. All nebulized formulations were well tolerated, with no evidence of bronchospasm. Nebulization times were significantly shorter for nanobudesonide compared to Pulmicort Respules. Because of a low oral bioavailability, plasma concentration of budesonide is a good marker of lung-delivered dose. The pharmacokinetics of nanobudesonide 0.5 and 1.0 mg were approximately dose proportional with respect to $C_{\text{max}}$, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$. Nanobudesonide 0.5 mg and Pulmicort Respules 0.5 mg exhibited similar AUCs, suggesting a similar extent of pulmonary absorption. A higher $C_{\text{max}}$ was noted with nanobudesonide 0.5 mg, and the $t_{\text{max}}$ was significantly different, suggesting a more rapid rate of drug delivery of nanobudesonide 0.5 mg than Pulmicort Respules. In conclusion, nebulized nanobudesonide 0.5 mg was safe in healthy volunteers, with a similar extent of absorption as Pulmicort Respules.

Keywords: Nanobudesonide; Pulmicort Respules; asthma; nebulization; budesonide absorption; pharmacokinetics

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Inhaled anti-inflammatory corticosteroid therapy is an important component of the treatment regimen for chronic asthma. Practice guidelines recommend their use as first-line therapy for all cases of asthma, except mild intermittent asthma. The use of inhaled steroids early in the course of the disease is associated with improved outcomes compared to treatment with beta 2–adrenergic agonists. Indeed, the early use of inhaled corticosteroids may alter the natural history of the disease and possibly reduce the irreversible airway obstruction associated with chronic inflammation of the lungs.

Budesonide is a potent anti-inflammatory corticosteroid with low oral bioavailability. It forms reversible intracellular fatty acid esters in airway and lung tissue, which may prolong lung retention. Budesonide is available as a dry-powder inhaler (DPI) and as Pulmicort Respules (AstraZeneca, Wilmington, DE), a suspension for inhalation with a jet-air tabletop nebulizer. Pulmicort Respules is the only corticosteroid suspension for inhalation available in the United States. The DPI form of inhalation therapy is often limited by difficulty with coordination of inspiration, especially in pediatric patients. In addition, the DPIs require adequate inspiratory flow for drug delivery. For these reasons, budesonide delivered by a tabletop nebulizer has found increasing use as the best alternative for delivery of corticosteroids to the lungs in children.

Nanobudesonide is a new formulation of budesonide that has been developed for use in a tabletop nebulizer. Suspended drug particles (the disperse phase) are in the 75- to 300-nm range in this formulation. This compares to particle size of ~4400 nm for Pulmicort Respules. The small crystal size of budesonide in the...
nanocrystal formulation gives the suspension solution-like characteristics, which may shorten delivery time and improve intrapulmonary distribution compared to the existing preparation. In this study, the safety, delivery, and pharmacokinetics of nebulized nanobudesonide were compared to Pulmicort Respules.

METHODS

Subjects

Sixteen healthy volunteers (13 males and 3 females) with a mean age of 32.5 (range: 25-40), mean height of 171.0 ± 8.4 cm, and mean weight 76.8 ± 14.5 kg participated in this study. The study was approved by the institutional review board of Thomas Jefferson University in Philadelphia. All subjects gave written informed consent to participate prior to undergoing any study procedures. No study subject used a topical or systemic corticosteroid or ingested a cytochrome P4503A inhibitor within 4 weeks of study initiation. Subjects with a history of bronchospasm or a screening FEV1/FVC of 80% of predicted were excluded from participation.

Study Design

This was a randomized, double-blind, four-arm, single-dose crossover study that compared the pharmacokinetics and safety of nanobudesonide and Pulmicort Respules. The four study arms were (1) placebo, (2) 0.5 mg of Pulmicort Respules, (3) 0.5 mg of nanobudesonide, and (4) 1.0 mg of nanobudesonide. Healthy volunteers were admitted to the Clinical Pharmacology Research Unit of Thomas Jefferson Hospital the day before each treatment. Following an overnight fast, they received one of the four randomized treatments. The study protocol specified that each subject was to receive treatments not less than 5 or more than 10 days apart.

Spirometry, oximetry, clinical laboratory tests, ECG, and vital signs were performed at baseline and at specified intervals. The spirometer was calibrated on a daily basis. Adverse events were monitored throughout the study. Twenty-four hours after each treatment, subjects were reevaluated and discharged from the unit.

Test Drugs and Administration

Nanobudesonide and placebo were manufactured by Automated Liquid Packaging (Woodstock, IL) using plastic blow/fill/seal ampules. Packaging and labeling were performed by PCI Services—Clinical Services (Philadelphia, PA). The nanobudesonide placebo consisted of the same ingredients as nanobudesonide without the budesonide. Pulmicort Respules were purchased from a commercial source. Nebulizers were filled by an unblinded investigational pharmacist. The fill volume for all treatments was 4.0 mL. For the 0.5-mg nanobudesonide treatment arm, 2.0 mL of placebo was added to 2.0 mL of active drug.

All treatments were administered by nebulization with a Pari LC Jet nebulizer and a Pari LC Ultra compressor (PARI Respiratory Equipment, Inc., Monterey, CA). A new nebulizer was used for each treatment, and each subject had all four treatments administered with the same compressor. Compressors were checked with a calibration nebulizer each day of use. Research personnel reviewed the inhalation procedure with each subject prior to each treatment using a training nebulizer. Subjects were in a sitting position and trained to inhale slowly and steadily through a mouthpiece with a target respiratory rate of 14 to 20 breaths per minute. Nose clips were used to avoid inspiration/expiration through the nose. Nebulization was continued until 1 minute after sputtering was heard. The duration of nebulization and the residual volumes were measured.

Pharmacokinetics

Blood was drawn for budesonide levels predose; at 5, 10, 15, 20, 25, 30, 40, and 60 minutes; and at 2, 3, 4, 6, 8, and 24 hours postdose. Time zero was the beginning of nebulization. Samples were collected in tubes containing sodium heparin, immediately placed on ice, and spun at 2200 g for 10 minutes.

Analysis of plasma samples was performed by Analytical BioChemistry Laboratories (Columbia, MO) using a validated HPLC-MS/MS method. Using nominal standard concentrations of 60.1 pg/mL and 802 pg/mL, precision for this assay was 12.9% and 4.7% CV, respectively, while accuracy was −8.6 to −2.8% RE of nominal standard concentrations. Plasma was assayed for budesonide in 1.0-mL aliquots. Samples were extracted on a 96-well C-18 SPE plates on a Tomtec Quadra 96 model 320. Desonide (11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione) 30 to 1000 pg/mL was used as an internal standard. During validation, the correlation coefficient (r) was ≥ 0.9976.

Statistical Analyses

Statistical analyses were performed using SAS Version 6.12 (SAS Institute, Cary, NC). Safety variables of FEV1,
adverse events, and vital signs were analyzed with an analysis of covariance at each time point. Clinical laboratory measurements and oximetry were evaluated in each subject. The pharmacokinetic analyses were performed with SAS using the sorting procedure, PROC GLM, PROC MEANS, and G-PLOT. The slope of the terminal phase was used to estimate AUC(0-∞). Analysis of variance (ANOVA) was performed on untransformed and log-transformed AUC and Cmax parameters. The model included subject, period, and treatment. For the log-transformed analysis, adjusted means and confidence intervals were calculated for AUC(0-t), AUC(0-∞), tmax, and Cmax and back-transformed to the arithmetic scale. For untransformed data, treatments were compared for the 95% confidence intervals for the difference between means. For transformed data, a 95% confidence interval was calculated for the ratio of two means. Subjects were evaluated on an intent-to-treat basis, and those receiving at least one dose of the trial medication were included in analyses.

RESULTS

Demographics

Sixteen subjects were enrolled, and all completed the study. One subject exceeded the maximum 10-day window permitted between treatment arms 3 and 4 by 4 days. This protocol variance did not jeopardize study integrity, and this subject was included in the analysis.

Pharmacokinetics

This study compared the pharmacokinetics of Pulmicort and nanobudesonide delivered by inhalation (Table I). Because of the rapid absorption and the limited number of time points during the first 10 minutes after the initiation of inhalation, tmax values obtained are considered estimates. Nevertheless, pairwise comparisons between Pulmicort Respules and nanobudesonide 0.5 mg revealed significant tmax (p = 0.001) differences between treatments. Indeed, maximum concentrations were achieved sooner in the nanobudesonide 0.5-mg treatment compared to Pulmicort. In addition, pairwise comparisons of the log-transformed data revealed that the Cmax for nanobudesonide 0.5 mg was significantly (p < 0.001) higher than for a comparable dose of Pulmicort Respules. The extent of absorption of Pulmicort Respules 0.5 mg and nanobudesonide 0.5 mg, estimated by AUC(0-1) and AUC(0-∞), was comparable, though formal bioequivalence testing was not performed. The pharmacokinetics of nanobudesonide 0.5 mg and 1.0 mg were approximately dose proportional with respect to Cmax, AUC(0-1), and AUC(0-∞).

Nebulization Times and Residual Volumes

Nebulization was continued until 1 minute after sputtering, which was not detected in 1 subject receiving Pulmicort Respules and 1 receiving 0.5 mg of nanobudesonide (Table II). The nebulization times for these subjects were 15 and 16 minutes, respectively. Nebulization times ranged from 4.0 to 16.0 minutes across all treatments, and mean nebulization times were 8.7 minutes for Pulmicort Respules, 7.1 minutes for nanobudesonide 0.5 mg, and 7.0 minutes for nanobudesonide 1.0 mg. The mean nebulization time for the subjects receiving Pulmicort Respules was significantly longer compared to that of the nanobudesonide 0.5-mg group (p = 0.027) and the nanobudesonide 1.0-mg group (p = 0.029). There were 5, 3, and 2 subjects with nebulization times greater than 10 minutes in the Pulmicort Respules group, nanobudesonide 0.5-mg group, and nanobudesonide 1.0-mg group, respectively.

Eight subjects had residual volumes greater than 1.0 mL: 4 in the Pulmicort Respules group, 2 in the nanobudesonide 0.5-mg group, 1 in the nanobudesonide 1.0-mg group, and 1 in the placebo group. There

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Pulmicort (0.5 mg)</th>
<th>Nanobudesonide (0.5 mg)</th>
<th>Nanobudesonide (1.0 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tmax (min)</td>
<td>14.4 (11.4-18.0)</td>
<td>8.4 (5.4-10.8)</td>
<td>11.4 (6.0-16.2)</td>
</tr>
<tr>
<td>Cmax (pg/mL)</td>
<td>662 (416-908)</td>
<td>1212 (751-1674)</td>
<td>2484 (1236-3733)</td>
</tr>
<tr>
<td>AUC(0-t) (pg*h/mL)</td>
<td>1518 (1071-1966)</td>
<td>1472 (1171-1773)</td>
<td>2725 (1981-3469)</td>
</tr>
<tr>
<td>AUC(0-∞) (pg*h/mL)</td>
<td>1631 (1125-2137)</td>
<td>1658 (1284-2031)</td>
<td>2893 (2118-3668)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>5.42</td>
<td>6.62</td>
<td>5.46</td>
</tr>
</tbody>
</table>
was no clear relationship between higher residual volumes and longer nebulization times. Three subjects had residual volumes greater than 1.0 mL in more than one crossover treatment period.

Safety

There were no significant adverse effects associated with the administration of the test drugs. Four subjects reported a total of eight adverse events after dosing with placebo. Two subjects reported a total of four adverse events (nausea, dizziness, paresthesia, and rhinitis) after dosing with Pulmicort Respules. One adverse event was reported with each of the nanobudesonide groups. These included rhinitis (nanobudesonide 0.5 mg) and edema at a venipuncture site (nanobudesonide 1.0 mg). All adverse effects were classified as mild. There was no evidence of bronchospasm in any of the treatment arms. For FEV₁, the overall treatment effect was not significant at any evaluation (p ≥ 0.111).

In addition, there were no clinically significant abnormalities in measured vital signs, pulse oximetry, electrocardiograms, or clinical laboratory tests.

DISCUSSION

Inhaled steroids are the preferred anti-inflammatory therapy in the treatment of asthma. Budesonide delivered by nebulization is effective in controlling pediatric asthma, as measured using multiple clinical endpoints. The efficacy of inhaled budesonide is predominantly mediated by local action, with systemic absorption contributing little, if anything, to the control of inflammation in the lungs. Budesonide has an excellent safety profile in children. Pediatric patients administered chronic inhaled steroids grow to a normal adult height. However, safety concerns of a transient, decreased growth velocity in children have resulted in underutilization of inhaled corticosteroids in this age group. The goal of the new nanocrystal formulation budesonide is to maximize efficiency of pulmonary delivery of the drug, which may serve to obviate some of these safety concerns.

The delivery of budesonide by nebulization is a complex process that is affected by the characteristics of the nebulizer and compressor, droplet size, properties of the formulation, breathing pattern of the patient, and respiratory tree anatomy. A small component of the orally deposited drug may be absorbed through the buccal mucosa. This was minimized in the present study by the use of a mouthwash after nebulization. There is little or no metabolism of budesonide in the lung. There is extensive hepatic first-pass metabolism of swallowed budesonide, with an oral bioavailability of ~11%. Pharmacokinetic evaluation of drug absorption from the lungs, therefore, provides an accurate and reproducible method for comparing the dose delivered to the lung by different inhaler systems or different formulations from the same inhaler system. A Medline search for published reports of the pharmacokinetics of budesonide suspension delivered by a tabletop nebulizer yielded a single report from a study in 10 children. However, neither the budesonide particle size in the suspension nor the manufacturer of the suspension is specified in this European study. Data on file with AstraZeneca report that in children with asthma, the Pari LC Jet Plus nebulizer with the Pari Master compressor delivered approximately 25% of labeled budesonide to the patient. This delivered dose was comparable to that in healthy adults.

In this study of healthy volunteers, the 0.5-mg nanobudesonide and the 0.5-mg Pulmicort Respules...

Table II  Mean Pulmicort and Nanobudesonide Nebulization Times

<table>
<thead>
<tr>
<th></th>
<th>Duration (min)</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>6.56</td>
<td>1.75</td>
<td>5-10</td>
</tr>
<tr>
<td>Pulmicort Respules (0.5 mg)</td>
<td>8.70</td>
<td>3.38</td>
<td>5-16</td>
</tr>
<tr>
<td>Nanobudesonide (0.5 mg)</td>
<td>7.06</td>
<td>3.11</td>
<td>5-16</td>
</tr>
<tr>
<td>Nanobudesonide (1.0 mg)</td>
<td>6.95</td>
<td>2.48</td>
<td>4-13</td>
</tr>
</tbody>
</table>
formulations produce a comparable budesonide AUC in plasma, indicating a similar extent of absorption. Because the oral bioavailability of budesonide is relatively low, it is likely that this observation reflects comparable pulmonary deposition for each formulation. The nanobudesonide formulation exhibited approximate linear pharmacokinetics between the 0.5- and 1.0-mg formulations. The nebulization times of nanobudesonide 0.5 and 1.0 mg were significantly shorter than those observed with Pulmicort Respules. There were no significant adverse effects associated with the delivery of the nanobudesonide formulation.

The comparable AUC, higher $C_{\text{max}}$, and lower $t_{\text{max}}$ of 0.5 mg nanobudesonide compared to 0.5 mg Pulmicort Respules suggest more rapid drug delivery or more rapid absorption. If these differences are due to more rapid absorption, two mechanisms are possible. Nanobudesonide could have more rapid dissolution of the drug in the airways, or a more distal delivery of the drug may facilitate rapid absorption. However, the pharmacokinetic data presented do not allow discrimination between these possibilities. Drug distribution studies will be required to determine the degree and kinetics of pulmonary deposition. While some observations suggest that improved anti-inflammatory drug distribution to small airways might result in increased clinical efficacy, there is no clinical proof that this is the case. Finally, results reported here in healthy adults should be extrapolated to other populations with caution. Budesonide clearance and lung deposition differ in pediatric and adult populations. In addition, drug delivery to the lungs will differ in asthmatic and healthy lungs. The subjects in this study were not balanced with regard to gender, and no subgroup analysis was performed owing to the small number of females participating. However, no gender differences are present in the pharmacokinetic parameters of Pulmicort Respules. The potential for differential regional lung delivery of inhaled nanobudesonide based on gender is unknown.

In conclusion, nanobudesonide exhibited pharmacokinetics that were dose proportional, and nanobudesonide 0.5 mg yielded budesonide absorption that was comparable to Pulmicort Respules. The higher $C_{\text{max}}$ and lower $t_{\text{max}}$ of 0.5 mg nanobudesonide suggest a more rapid rate of either drug delivery or absorption compared to Pulmicort Respules. In addition, nanobudesonide administration demonstrated a safety profile that was comparable to Pulmicort Respules. The significantly shorter nebulization time with nanobudesonide compared to Pulmicort Respules should be advantageous with respect to patient compliance.

**REFERENCES**


Pharmacokinetic and Pharmacodynamic Evaluation of a Novel Proton Pump Inhibitor, YH1885, in Healthy Volunteers

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To evaluate the pharmacokinetic and pharmacodynamic characteristics of YH1885, a novel proton pump inhibitor, a single-blind, randomized, placebo-controlled, dose-rising, parallel-group study was conducted in 46 healthy volunteers. The volunteers were randomly allocated to single dose groups of 60 mg, 100 mg, 150 mg, 200 mg, and 300 mg (6 subjects per dose, including 2 placebos) or to multiple-dose groups of 150 mg and 300 mg (once-daily dosing for 7 days; 8 subjects per dose, including 2 placebos). The multiple-dose study was conducted separately after the single-dose study. YH1885 was administered orally after overnight fasting. Serial blood samples, urine samples, and pharmacodynamic measurements were taken. Drug concentrations in plasma and urine were determined by liquid chromatography/mass spectrometry (LC/MS). Pharmacodynamic changes were evaluated by ambulatory intragastric pH monitoring and by serial measurements of serum gastrin concentrations. Assessments of safety and tolerability also were made. Plasma concentrations of YH1885 reached peak levels 1.3 to 2.5 hours after single-dose administration and then declined monoexponentially with a terminal half-life (t_{1/2}) of 2.2 to 2.4 hours in dosage groups up to 200 mg in the single-dose study. YH1885 showed linear pharmacokinetic characteristics, and little accumulation occurred after multiple administrations. The parent drug was not detected in urine. Dose-related pharmacological effects were obvious for dose groups of 150 mg and higher in the single-dose study. The mean intragastric pH and the percentage of time at pH > 4 were significantly increased. The onset of drug effect was rapid, and maximal effects were observed on the first day of administration during multiple dosing. Serum gastrin levels also showed rapid increases during dosing but with a weak dose-effect relationship. Neither serious nor dose-limiting adverse effects were observed. YH1885 was found to be safe and well tolerated and effectively inhibited acid secretion with dose-dependent increases in intragastric pH. The acid-suppressing efficacy of YH1885 needs to be further evaluated in patients with gastric acid-related diseases.

Keywords: YH1885; proton pump inhibitors; gastric acid-related diseases; pharmacokinetics

From the Department of Pharmacology, Seoul National University College of Medicine (Dr. Yu, Dr. Cho, Ms. Yi, Dr. Chung, Dr. Jang, Dr. Shin); Clinical Pharmacology Unit and Clinical Trial Center/Clinical Research Institute, Seoul National University Hospital (Dr. Lim, Dr. Jang, Dr. Shin); Asan Medical Center, Seoul (Dr. Bae); Department of Pharmacology, Inje University College of Medicine, Busan (Dr. Shon); and Yuhan Corp., Korea (Mr. Song, Dr. Moon). This study was sponsored by Yuhan Corp. Submitted for DOI: 10.1177/0091270003261321

Proton pump inhibitors (PPIs) have emerged as the treatment of choice for acid-related diseases, including gastroesophageal reflux disease (GERD) and peptic ulcer disease, because of their greater and longer-lasting acid-suppressive potentials than H₂ receptor antagonists. PPIs are membrane-permeable weak bases that accumulate in the acid spaces of active gastric parietal cells, where they undergo an acid-catalyzed conversion to active sulfenamide derivatives. These derivatives covalently bind via disulfide bridges to their target, H⁺/K⁺-ATPase, and inhibit acid production by the bound cell. Proton pump inhibitors effectively block the acid production of 70% or more of active pumps.

Existing PPIs, such as omeprazole, lansoprazole, pantoprazole, rabeprazole, and esomeprazole, all share a common structure (substituted benzimidazoles) and mode of action, but they differ somewhat
in terms of clinical pharmacology. YH1885, 5,6-dimethyl-2-(4-fluorophenylamino)-4-((1-methyl-1,2,3,4-tetrahydroisoquinoline-2-yl) pyrimidine hydrochloride, is a novel reversible proton pump inhibitor that is currently under clinical investigation (Figure 1). It has been shown to have potent acid-suppressive properties and favorable safety profiles compared to omeprazole in preclinical studies. The present study was conducted to investigate the pharmacokinetic and pharmacodynamic characteristics of YH1885 after single and multiple oral administrations to healthy volunteers.

METHODS

Subjects

Thirty healthy male volunteers, ages 20 to 36 years (mean ± SD: 25.5 ± 3.7 years) and weighing 55 to 79 kg (66.9 ± 6.2 kg), participated in the single-dose study, and 16 healthy male volunteers, ages 18 to 30 years (24.3 ± 3.0 years) and weighing 55 to 85 kg (68.4 ± 8.0 kg), were enrolled for the multiple-dose study. The subjects were assessed as being healthy by medical history, physical examination, vital signs, 12-lead electrocardiography (ECG), and routine clinical laboratory tests performed within 3 weeks prior to the study. Subjects were excluded for use of any drugs within 7 days prior to the study, regular heavy drinking, smoking more than 20 cigarettes per day, and a body weight beyond 85% to 115% of their ideal body weight. The study protocol was approved by the institutional review board of Seoul National University Hospital (SNUH), and the study was conducted at the SNUH Clinical Trial Center. All procedures were performed in accordance with the recommendations of the Declaration of Helsinki on biomedical research involving human subjects, as well as with the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use—Good Clinical Practice (ICH-GCP) guidelines. All subjects gave written informed consent before being enrolled.

Study Design

This was a single-blind, randomized, placebo-controlled, dose-rising, parallel-group study. In the single-dose study, subjects were randomly assigned to one of five dose groups of 60, 100, 150, 200, and 300 mg; each group included 6 subjects, 2 of which received placebo. Subjects were admitted on the night before baseline evaluation and were fasted overnight beginning at 10 p.m. On the day of the baseline evaluation (day 0), all subjects were given a single dose of placebo with 240 mL of water at approximately 9 a.m. On the following day (day 1), subjects were given a single dose of YH1885 or placebo with 240 mL of water at the same time as the previous morning. Subjects were kept in the fasting state until 4 hours after drug administration on day 0 and day 1, except for 200 mL of water 2 and 4 hours after dosing on each day. The subjects were discharged on the morning of day 3 and returned for a poststudy visit on day 8.

The multiple-dose study was conducted separately after the single-dose study. Subjects were randomly assigned to one of two dose groups of 150 and 300 mg; each group included 8 subjects, 2 of which received placebo. During the first 2 days of the multiple-dose study (days 0 and 1), the subjects underwent a schedule identical to that of the single-dose study. During days 2 to 6, the subjects were given a dose of YH1885 or placebo once daily at the same time as day 1, after an overnight fasting that was continued until 2 hours after drug administration. On day 7, after overnight fasting, the subjects were given a single dose of YH1885 or placebo with 240 mL of water at the same time as day 1. Subjects were maintained in the fasting state for 4
hours after drug administration on day 7, except for 200 mL of water 2 and 4 hours after dosing. The subjects were discharged on the morning of day 9 and returned for a poststudy visit on day 14.

In both the single- and multiple-dose studies, alcohol, soft drinks, and beverages containing caffeine were prohibited beginning 2 days prior to admission for the entire study duration, which ended with the poststudy visit.

**Pharmacokinetic Evaluation**

Blood samples (8 mL) were taken via an indwelling cannula inserted into a forearm vein at 0 (predose), 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours after drug administration on day 1 (and also on day 7 during the multiple-dose study). All blood samples were obtained in the semirecumbent position. The samples obtained were centrifuged at 1200 g for 10 minutes at 4°C, and plasma was immediately stored in polypropylene tubes at −20°C or less until required for analysis.

Urine was collected at intervals of 0 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 hours after dosing on day 1 in the single-dose study. During the multiple-dose study, urine was collected from 0 to 6, 6 to 12, and 12 to 24 hours on days 1 and 7. The volume of each collection was recorded, and aliquots were stored in polypropylene tubes at less than −20°C until required for analysis.

Plasma and urine concentrations of YH1885 were determined by liquid chromatography/mass spectrometry (LC/MS).15 In brief, 100 µL of plasma sample was mixed with 200 µL of acetonitrile, which included an internal standard (YH439, Yuhan Co., 1.0 µg/mL). After vortexing for 30 seconds and centrifuging for 10 minutes, 20 µL of the supernatant was injected into an LC/MS system (HP 1100 series, Agilent Technologies, Palo Alto, CA), using a MeCN/acetate buffer (80/20, v/v) mobile phase at a flow rate of 1.0 mL/min and a C8 column (Cosmosil 5C8, 250 × 4.6 mm i.d., Nacalai, Japan). For urine samples, the procedures used were identical, except that 300 µL of the urine sample was mixed with 600 µL of acetonitrile containing the internal standard. The lower limit of YH1885 quantification was 10 ng/mL, and calibration curves were linear over the concentration range 10 to 2500 ng/mL (r > 0.98). The accuracy of this assay was within the range 91.4% to 107.6%, and the interbatch coefficient of variation was less than 13.6% over the calibrated range.

**Pharmacodynamic Evaluation**

Intragastric pH values were recorded continuously for 24 hours on day 0 (baseline) and day 1 (postdose), using an ambulatory intragastric pH monitoring system. During the multiple-dose study, intragastric pH was also monitored on day 7. Before dosing, a bipolar glass pH electrode catheter (Medtronic Synectics AB, Stockholm, Sweden) was calibrated, inserted into the stomach via the nose (anaesthetized with 1% lidocaine spray), and fixed at approximately 5 cm below the lower esophageal sphincter, as determined by an abrupt pH change.16 The catheter was connected to a portable digital data recorder (MicroDigitrapper 4Mb, Medtronic Synectics AB), and the intragastric pH was sampled every 8 seconds. After finishing the recording, the electrode was removed and recalibrated. The recorded data were uploaded onto a personal computer where they were further analyzed. The mean pH during each day and the percentage of time at pH > 4 were calculated.17

During pH monitoring, the subjects were requested to maintain an identical time schedule each day, for drinking water, eating (standardized meals 4 and 9 h after dosing), smoking (less than 20 cigarettes per day), and body position. The subjects were not allowed to lie in the supine position before nighttime. Baseline plasma gastrin levels were measured 0 (predose), 6, 12, and 24 hours after placebo administration on day 0, and postdose levels were measured 6, 12, 24, 48, and 168 (poststudy visit) hours after drug administration on day 1 in the single-dose study. During the multiple-dose study, predose gastrin levels were measured on days 1, 2, 4, 7, 8, and 14 (poststudy visit).

**Safety and Tolerability Assessment**

Physical examinations, vital signs, 12-lead ECG, and routine clinical laboratory tests were repeated at prede-
fined time points. Inquiries as to the presence of any adverse events were made at each evaluation period by asking general questions. Additional safety assessments were made at the discretion of the investigator.

**Statistical Analysis**

Linear regression and ANOVA (analysis of variance) on dose-normalized values were used to determine the dose linearity of C<sub>max</sub> and AUC. ANOVA was also used to evaluate any differences in the terminal half-lives and oral clearances of the dose groups. Wilcoxon signed rank tests were used to determine any differences in pharmacokinetic parameters between day 1 and day 7.

Repeated-measures ANOVA (SAS® 6.12, proc GLM) was used for within-subject analysis of pharmacodynamic parameters during the multiple-dose study. ANOVA was used to evaluate any differences in the changes of gastric pH, time at pH > 4, and serum gastrin levels between dose groups. Wilcoxon rank sum tests were used to compare pharmacodynamic parameters between placebo and other dose groups; p-values lower than 0.05 were considered statistically significant.

**RESULTS**

**Pharmacokinetics**

Plasma concentrations of YH1885 peaked 1.3 to 2.5 hours (range of dose group means) after a single-dose administration and then declined monoexponentially, with a terminal half-life (t<sub>1/2</sub>) of 2.2 to 2.4 hours (Figure 2) in dosage groups up to 200 mg in the single-dose study. After 300-mg dosing, the concentrations showed a biphasic decay pattern with an initial half-life similar to that of the lower dose groups but a terminal half-life of 9.8 hours (Table I). The C<sub>max</sub> and AUC of YH1885 increased linearly with dose (r = 0.99 and 0.98 for mean C<sub>max</sub> and mean AUC, respectively; p > 0.05 for ANOVA on dose-normalized values). Similarly, no significant oral clearance differences were found between the dose groups.

In the multiple-dose study, concentration-time profiles were similar on days 1 and 7 for once-daily oral dosing (Figure 3). Pharmacokinetic parameters showed similar individual values for C<sub>max</sub>, t<sub>max</sub>, and oral clearance for days 1 and 7. However, the terminal half-

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**Table I** Pharmacokinetic Parameters following a Single Oral Administration of YH1885

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;∞&lt;/sub&gt; (ng•h/mL)</th>
<th>CL/F (L/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>78.1 ± 34.5</td>
<td>2.3 ± 0.9</td>
<td>2.4 ± 0.8</td>
<td>363.9 ± 155.1</td>
<td>200.6 ± 115.8</td>
</tr>
<tr>
<td>100</td>
<td>168.7 ± 86.3</td>
<td>1.5 ± 0.4</td>
<td>2.4 ± 0.3</td>
<td>667.8 ± 423.1</td>
<td>202.0 ± 116.6</td>
</tr>
<tr>
<td>150</td>
<td>266.7 ± 43.3</td>
<td>2.5 ± 0.6</td>
<td>2.2 ± 1.0</td>
<td>1175.4 ± 286.9</td>
<td>133.6 ± 32.8</td>
</tr>
<tr>
<td>200</td>
<td>361.4 ± 124.1</td>
<td>2.1 ± 1.3</td>
<td>2.4 ± 0.2</td>
<td>1343.1 ± 365.9</td>
<td>157.6 ± 43.3</td>
</tr>
<tr>
<td>300</td>
<td>722.4 ± 239.2</td>
<td>1.3 ± 0.3</td>
<td>9.8 ± 4.7</td>
<td>2817.5 ± 806.0</td>
<td>116.1 ± 45.1</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation. C<sub>max</sub>, maximum plasma concentration; t<sub>max</sub>, time to maximum concentration; t<sub>1/2</sub>, terminal half-life; AUC<sub>∞</sub>, area under the time-concentration curve extrapolated to infinity; CL/F, oral clearance.
life was greater on day 7 in both dose groups, and the accumulation index was 1.5 ± 0.4 for the 150-mg dose group (Table II).

Regarding the urine samples, no parent drug was detected in any subject.

Pharmacodynamics

During the baseline study period (day 0), intragastric pH fluctuated within a range of approximately 1 to 6. Sharp increases in pH were seen after meals at 4 and 9 hours, which were followed by gradual decreases (Figure 4, dotted lines). After administering YH1885 in the single-dose study, elevations in intragastric pH were noted, especially in the higher dose groups (Figure 4, solid lines). Subjects in the placebo group showed little change. Values obtained beyond 16 hours after dosing were highly variable. In the multiple-dose study, changes in the pH-time profiles for day 1 were similar to those of the single-dose study, and the pH-time profile for day 7 was similar to that of day 1 (Figure 5). This was confirmed by the daily mean pH and the percentage of time at pH > 4 (Table III).

Differences in mean gastric pH and the percentage of time at pH > 4 increased in a dose-dependent manner, with significant values seen in dose groups of 150 mg and higher in the single-dose study (Figure 6). This pattern was more evident when only the daytime values were compared. In the multiple-dose study, increases in mean gastric pH and the percentage of time at pH > 4 were also dose dependent; values for day 7 were similar or slightly higher than those for day 1 (Figure 7).

Serum gastrin levels showed intraday variation during the baseline period (Figure 8). Taking this variation into consideration,

Table II  Pharmacokinetic Parameters following Once-Daily Oral Administration of YH1885 for 7 Days

<table>
<thead>
<tr>
<th></th>
<th>150 mg</th>
<th>300 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 7</td>
</tr>
<tr>
<td>Cₘₐₓ (ng/mL)</td>
<td>215.6 ± 91.3</td>
<td>279.3 ± 108.8</td>
</tr>
<tr>
<td>tₘₐₓ (h)</td>
<td>1.5 ± 0.4</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>4.1 ± 3.4</td>
<td>14.8 ± 13.1</td>
</tr>
<tr>
<td>AUC₀ (ng*h/mL)</td>
<td>940.3 ± 519.9</td>
<td></td>
</tr>
<tr>
<td>AUC₀ (ng*h/mL)</td>
<td>936.4 ± 418.1</td>
<td>1391.7 ± 711.8*</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>206.2 ± 112.5</td>
<td>148.4 ± 105.8</td>
</tr>
<tr>
<td>Accumulation index</td>
<td>1.5 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation. Cₘₐₓ, maximum plasma concentration; tₘₐₓ, time to maximum concentration; t₁/₂, terminal half-life; AUC₀, area under the time-concentration curve extrapolated to infinity; AUC₀, interval AUC (0-24 h); CL/F, oral clearance.

a. CL ss/F (steady-state oral clearance) for day 7.
b. AUC (day 7)/AUC (day 1).
c. p < 0.05 compared to day 1.
into account, serum gastrin levels increased transiently after YH1885 administration in a weakly dose-dependent fashion in the single-dose study. During multiple-dose administration, daily predose gastrin levels increased until day 7, when the final dose was administered (Figure 9). Levels then returned to baseline.

Relationships between pharmacokinetic and pharmacodynamic parameters were explored using AUCs after single-dose administration and (1) changes in mean gastric pH and (2) changes in the percentage of time at pH > 4. When fitted to a simple $E_{\text{max}}$ model [$E = E_{\text{max}} \cdot \frac{AUC}{EC_{50} + AUC}$], the parameter values were $E_{\text{max}} = 1.98$ and $EC_{50} = 1265$ ng•h/mL for the increase in the mean gastric pH, and $E_{\text{max}} = 56$ (%) and $EC_{50} = 1672$ ng•h/mL for the increase in the percentage of time at pH > 4. Regarding the relationship between concentration and effect, pharmacokinetic-pharmacodynamic modeling would be needed since individual time-dependent relationships and the influence of covariates are not considered in correlation analyses. This would provide another topic for investigation.

Safety and Tolerability

No clinically significant changes were observed by physical examination or in terms of vital signs, ECGs, or routine clinical laboratory tests in any subject. Some instances of gastrointestinal disturbances occurred as follows, but all were mild and transient. Diarrhea or loose stools was the most frequent adverse event, with
5 subjects in the single-dose study (1 each in the 60-mg, 200-mg, and 300-mg dose groups; 2 in the placebo group) and 3 subjects in the multiple-dose study (1 in the 300-mg dose group; 2 in the placebo group). Constipation was noted in 2 subjects during the multiple-dose study (1 in the 150-mg dose group; another in the placebo group). Indigestion was reported in 2 subjects (1 each in the 150-mg single-dose group and 300-mg multiple-dose group).

Other adverse events reported were headaches (2 subjects in the 300-mg single-dose group; 1 each in the single- and multiple-dose placebo groups), rhinorrhea (4 subjects in the single-dose groups), and throat discomfort due to the nasogastric probe (3 subjects in the single-dose groups; 1 in the placebo group). These events were also mild and transient.

**DISCUSSION**

The single-dose pharmacokinetic analysis results suggest that orally administered YH1885 was rapidly absorbed and eliminated. The terminal half-life was variable, with a second decay phase emerging in the highest dose group. This finding is thought to be related to the plasma concentration assay detection limit (i.e., the second phase was not observable in the lower dose groups due to the lower concentrations involved). However, the contribution of the second phase to the overall pharmacokinetic profile was considered minimal, and this phenomenon did not compromise dose linearity, as evidenced by the linear relationships between the AUCs and oral clearances by dose.

In the multiple-dose study, the terminal half-life was 4 to 6 times longer on day 7 than on day 1. This could be explained by the fact that the second decay phase
started approximately 12 to 24 hours after dosing, rendering exact characterization of the elimination phase for day 1 difficult during once-daily dosing. The accumulation index was greater in the 150-mg dose group compared to the 300-mg dose group (1.5 ± 0.4 vs. 1.1 ± 0.2; p > 0.05). However, considering that the number of subjects is small, the variance is large, and the fact that the 300-mg dose group showed values near unity, over-

Figure 8. Mean changes (percent changes compared to –24 h for baseline [day 0] values or 0 h for postdose values) in serum gastrin levels after a single oral administration of YH1885 or placebo (upper). Mean (± SD) differences in the percent changes of serum gastrin levels at 6, 12, and 24 hours between baseline (day 0) and after YH1885 or placebo (lower). *p < 0.05 compared with placebo.
all drug accumulation was thought to be minimal. Urinary excretion of the drug was negligible. Dose-related pharmacological effects were clearly demonstrated, especially in dosage groups of 150 mg and higher. Pharmacodynamic parameters, such as the mean intragastric pH and the percentage of time at pH > 4, increased in a dose-dependent fashion. During the multiple-dose study, it was shown that these effects were maximal even with the first dose, and parameter values for day 7 were similar to those of day 1. This finding differs from those of omeprazole or lansoprazole, in which maximal effect was not reached on the first day of multiple once-daily dosing.18,19 Exact comparisons with other studies of PPIs are difficult due to differences in study design (especially the number and timing of meals and snacks, which lead to differences in baseline pH profiles), but the effects of YH1885 seem to be generally comparable to those of other PPIs. In one report involving the once-daily administration of omeprazole or lansoprazole to healthy volunteers, the percentage of time at pH > 4 was 65% after 30 mg of lansoprazole treatment for 5 days and 50% after 15 mg of lansoprazole or 20 mg of omeprazole for 5 days.19 During 300 mg of YH1885 administration, this value was 62% on the first day and 66% on day 7. In another study using rabeprazole in healthy volunteers, the mean gastric pH increased by 1.2 after a single 40-mg dose of rabeprazole,20 while a single 300-mg dose of YH1885 raised the mean pH by 1.4 (Table III).

In this study, smokers were not prohibited from smoking. It has been reported that smoking cigarettes does not influence the median intragastric pH over 24 hours in healthy persons and ulcer patients.21 High variability in nocturnal pH values is attributable to two factors: one is due to irregular changes in body position during sleep that affect the probe position, and the other is a phenomenon called nocturnal acid breakthrough. During PPI therapy given once daily before breakfast, it has been reported that a drop in pH begins at around 11 p.m. and that this occurs in 80% of patients and healthy controls.22

The increased serum gastrin concentration after YH1885 administration is a response to the suppression of gastric acid secretion via a feedback mechanism.23 Although serum gastrin levels do not correspond well to gastric pH,24 early changes in serum gastrin values suggest the early onset of pharmacological actions. Moreover, the rapid normalization of gastrin levels after the last dose suggests the early termination of the pharmacologic effects.25

In summary, YH1885 was found safe and well tolerated and to have linear pharmacokinetic characteristics with little accumulation during multiple once-daily dosing. YH1885 effectively inhibited acid secretion, with dose-dependent increases in intragastric pH, in these healthy volunteers. Although the volunteers were not screened for Helicobacter pylori infection, the prevalence rate is assumed to be less than 60% for this age group in Korea.26-28 In the presence of H. pylori, the effect of proton pump inhibitors on intragastric pH is much increased.29 Therefore, the efficacy of YH1885
needs to be evaluated in patients with gastric acid-related diseases.

REFERENCES

Prostacyclin (PGI2) is an endothelium-derived prostaglandin with potent pulmonary and systemic vasodilatory and antiplatelet aggregation properties.1-6 It is administered by continuous intravenous infusion as epoprostenol (Flolan® for Injection, GlaxoSmithKline, Research Triangle Park, NC) to patients with pulmonary hypertension. Prostacyclin improves survival and hemodynamics in these patients; however, the treatment is limited by a very short half-life (1-2 min) and chemical instability requiring continuous intravenous infusion and refrigeration during administration. Sepsis, thrombosis, paradoxical embolism, and treatment interruptions due to occlusion, perforation, and dislodgment of the catheter and/or pump malfunction have all been reported with intravenous epoprostenol administration, and interruption of intravenous epoprostenol has been associated with syncope and acute pulmonary hypertensive crisis.1,6

Treprostinil (Remodulin® Injection, United Therapeutics Corporation, Research Triangle Park, NC) is a long-acting, stable tricyclic benzindene analog of prostacyclin with similar pharmacological actions and acute hemodynamic effects to those observed with epoprostenol.7,8 Furthermore, treprostinil is chemically stable at room temperature with neutral pH and a longer half-life upon chronic administration (3 h; see companion manuscript9). Given these properties, treprostinil was developed as a less invasive subcuta-
neous infusion for pulmonary arterial hypertension (PAH). In a worldwide 12-week double-blind, placebo-controlled study in 470 patients with PAH, continuous subcutaneous treprostinil infusion (at a mean dose of 9.3 ng/kg/min) resulted in consistent improvements in these patients without the potentially life-threatening events associated with continuous intravenous prostacyclin dosing.11,12

Treprostinil sodium is currently approved in the United States and Canada as a continuous subcutaneous infusion for the treatment of PAH in patients with New York Heart Association (NYHA) Class II-IV symptoms,13 as well as in Israel for the treatment of primary pulmonary arterial hypertension and PAH associated with four connective tissue disorders. Marketing applications for treprostinil use in pulmonary hypertension are also under review in several other countries, and treprostinil is currently being developed as a potential treatment for other vascular diseases.

This study was conducted to evaluate the absolute bioavailability and acute pharmacokinetics of treprostinil administered via short-term continuous subcutaneous infusion in normal healthy subjects.

SUBJECTS AND METHODS

Study Design

This was a single-center, open-label, two-period, single-dose, nonrandomized study conducted at the PPD Development Clinical Research Unit in Austin, Texas, between June 4 and 28, 1999. Eligible volunteers received treprostinil sodium by intravenous infusion (Period 1), followed by a 5- to 7-day washout, and a subsequent subcutaneous infusion at the same rate as that administered intravenously (Period 2). Administering the same dose via the intravenous and subcutaneous routes to each subject provided a reference standard for absolute bioavailability.

All subjects gave written informed consent prior to any study-related assessments. The study was approved by a local institutional review board, the Research Consultants Review Committee, and was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and in conformance with the U.S. Code of Federal Regulations (Title 21 CFR, Parts 50 and 56).

Subject Population

Eligible subjects were healthy males and nonpregnant, nonlactating females who were between 18 and 50 years of age; in general good health, as confirmed by physical examination, medical history, and clinical laboratory evaluations; and without known symptomatic postural hypotension or a history of cardiovascular, pulmonary, or blood-clotting disorders. Prescription and over-the-counter medications were prohibited within 14 days and 72 hours of study entry, respectively.

A total of 15 healthy volunteers were enrolled, including 8 males and 7 females (8 Caucasian, 3 Black, and 4 Hispanic) who were 18 to 49 years of age and 50 to 85 kg in weight.

Treprostinil Dosing

Treprostinil sodium was provided as a sterile, pyrogen-free, isotonic solution in 20-mL multidose vials. A single lot number (800506) was used for all treprostinil dosing in this study. Each milliliter contained 1.0 mg treprostinil sodium, 6.3 mg sodium citrate, 3.0 mg metacresol (preservative), 0.24 mg sodium hydroxide, and 5.3 mg sodium chloride. The formulation was buffered with a citric acid/sodium citrate buffer. Hydrochloric acid or sodium hydroxide was used to adjust the pH to 6.5. Prior to i.v. dosing only, the treprostinil sodium was diluted in D5W (5% dextrose) to an appropriate strength.

On the morning of Day 1, Period 1, each subject received treprostinil sodium by intravenous infusion at a fixed rate of 15 ng/kg/min over 150 minutes (2.5 h). The infusion was delivered by positive-pressure infusion pump (Baxter Syringe Pump Model AS-4A0) via a dedicated intravenous line to an arm vein. This infusion was followed by a 5- to 7-day washout period (at least seven half-lives) for each subject.

On the morning of Day 1, Period 2, each subject received treprostinil sodium by a subcutaneous infusion at the same rate used for the intravenous infusion—that is, a fixed rate of 15 ng/kg/min over 150 minutes (2.5 h). The infusion was delivered via a subcutaneous catheter placed in the abdominal wall using a micro-infusion, positive-pressure infusion pump designed for subcutaneous drug delivery (MiniMed, Model 506). Both infusions were delivered with subjects in a supine position.

The dose chosen for the study was selected because it is clinically relevant and was expected to produce measurable plasma levels for a sufficient portion of the terminal elimination phase.

Pharmacokinetic Sampling

Nineteen pharmacokinetic blood samples (7 mL each) were collected from each subject during Period 1 (in-
travenous dosing) and Period 2 (subcutaneous dosing) for determination of plasma treprostinil concentrations. Blood samples were drawn from an arm vein in the contralateral limb to the one used for the intravenous infusion or from a vein in either arm during the subcutaneous infusion. The samples were drawn preinfusion (Time 0); during the infusion at 0.25, 0.5, 1, 1.5, 2, and 2.5 hours from infusion start; and post-infusion at 5, 10, and 15 minutes and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 8 hours. A saline lock was inserted into an arm vein to facilitate blood sampling, if needed.

All blood samples were drawn into Vacutainers® containing K3EDTA as an anticoagulant. The plasma for each blood sample was separated from the red cells by centrifugation using a refrigerated centrifuge and then transferred into prechilled, labeled, cryogenic tubes and immediately frozen. The frozen plasma samples were stored at −20°C until analysis.

**Assay Methodology**

Plasma samples were analyzed for treprostinil sodium concentrations by Alta Analytical Laboratory (El Dorado Hills, CA). A validated liquid chromatography atmospheric pressure ionization tandem mass spectrometry (LC/MS/MS) assay with a lower limit of quantitation (LLOQ) of 25 pg/mL for a 1-mL aliquot of plasma was used to analyze plasma samples anticoagulated with K3EDTA. The mean percent accuracy values of quality control samples for the method were 101.3%, 100.0%, and 99.4% of theoretical values, with a precision (expressed as coefficient of variation [CV]) of 2.5%, 1.7%, and 1.3% at 0.075, 4.0, and 8.0 ng/mL of treprostinil, respectively. The CV was 8.9% at the LLOQ of 0.25 ng/mL. A dimethylene homologue of treprostinil (LRXA-97 J02; Cardinal Pharmaceutical Development, Morrisville, NC) was used as an internal standard. Pooled control human K3EDTA plasma (Biochemed Pharmacologics, Winchester, VA, and Bioreclamation, Inc., Hicksville, NY) was used to prepare calibration standards.

Plasma (1.0 mL) was extracted using a 30:70 (v:v) ethyl acetate/hexane mixture. Aqueous phase extracts were evaporated to dryness under nitrogen, reconstituted in a 50:50 methanol/Mobile Phase A, and refrigerated until analyzed. Extracts were analyzed using a 100 × 2 mm reversed-phase C18 analytical column (Betasil C18, Keystone Scientific, State College, PA) at a flow rate of 0.3 mL/min. The mobile phase was isocratic (A/B = 35:65). Mobile Phase A was 95:5 water/100 mM NH₄COOH with 0.1% formic acid. Mobile Phase B was 95:5 ACN/NH₄COOH with 0.1% formic acid. Retention times for treprostinil and its internal standard were approximately 2.5 and 3.5 minutes, respectively.

Detection was by tandem mass spectrometry (PE-SCIEX API III or PE-SCIEX API 365). An ionspray atmospheric pressure ionization inlet connected the high-pressure liquid chromatography (HPLC) system to the mass spectrometer. Analysis was by negative ionization using the [M-H] molecular ions as precursors. Peak areas were integrated using PE-SCIEX MacQuan software. Calibration curves were derived from peak area ratios (analyte/internal standard) using a least squares regression of the ratio versus the nominal concentration of the standards. Reliability of the procedure was evaluated after analysis of duplicate standards at seven concentrations (0.025, 0.05, 0.100, 0.500, 1.000, 5.000, and 10.000 ng/mL). The LLOQ using a 25-mcL injection volume was 0.025 ng/mL.

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**Table I** Treprostinil Sodium Pharmacokinetics Parameters (N = 15)

<table>
<thead>
<tr>
<th></th>
<th>Cmax (ng/mL)</th>
<th>tmax (h)</th>
<th>t1/2 (h)</th>
<th>AUC∞ (ng•h/mL)</th>
<th>Cl (mL/kg/h)</th>
<th>Vz (mL/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(15 ng/kg/min for 150 min)</td>
<td>1.57 (0.31)</td>
<td>2.13 (0.34)</td>
<td>0.87 (0.43)</td>
<td>3.52 (0.71)</td>
<td>663.0 (132.2)</td>
<td>806.1 (669.6)</td>
</tr>
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<td>(Min, Max)</td>
<td>(1.14, 2.18)</td>
<td>(1.50, 2.50)</td>
<td>(0.27, 2.04)</td>
<td>(2.48, 4.81)</td>
<td>(467.4, 907.6)</td>
<td>(334.5, 2029.7)</td>
</tr>
<tr>
<td>Coefficient of variation (CV, %)</td>
<td>19.8</td>
<td>16.1</td>
<td>49.5</td>
<td>20.0</td>
<td>19.9</td>
<td>50.9</td>
</tr>
<tr>
<td>Subcutaneous infusion</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>(15 ng/kg/min for 150 min)</td>
<td>1.47 (0.20)</td>
<td>2.51 (0.22)</td>
<td>1.38 (0.66)</td>
<td>3.97 (0.76)</td>
<td>589.4 (129.6)</td>
<td>1113.6 (453.0)</td>
</tr>
<tr>
<td>(Min, Max)</td>
<td>(1.18, 1.88)</td>
<td>(2.00, 2.77)</td>
<td>(0.63, 2.52)</td>
<td>(2.37, 5.46)</td>
<td>(412.2, 947.8)</td>
<td>(537.6, 2049.5)</td>
</tr>
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<td>CV (%)</td>
<td>13.6</td>
<td>8.8</td>
<td>47.8</td>
<td>19.0</td>
<td>22.0</td>
<td>40.7</td>
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</table>
Pharmacokinetic Parameters and Analyses

Pharmacokinetic parameters were determined from each subject’s plasma treprostinil concentration versus time (actual sampling times) data for both routes of administration. Various pertinent pharmacokinetic parameters were determined using noncompartmental methods. Peak plasma concentration ($C_{\text{max}}$) and the corresponding peak time ($t_{\text{max}}$) for each profile were observed values. The elimination rate constant ($\lambda_e$) was obtained by log-linear regression analysis of the terminal phase of the plasma treprostinil concentration versus time decay curve. The apparent elimination half-life ($t_{1/2}$) was determined by taking the ratio of the natural log of 2 and $\lambda_e$.

Area under the curve from time 0 to infinity ($AUC_{\infty}$) was obtained through summation of area under the curve from time 0 to the last measurable concentration ($AUC_{\text{last}}$), calculated by the linear trapezoidal rule, and the extrapolated area ($AUC_{\text{ext}}$), estimated by taking the ratio of the last measurable concentration and $\lambda_e$. Plasma clearance (CL) for intravenous dosing and plasma clearance divided by absolute availability (CL/F) for subcutaneous dosing were determined by taking the ratio between dose and $AUC_{\text{last}}$. The volume of distribution (Vd) for intravenous dosing and the volume of distribution divided by absolute bioavailability (Vz/F) for subcutaneous dosing were obtained by taking the ratio between the clearance value and $\lambda_e$. The absolute bioavailability of the subcutaneous administration (F) was determined by taking the ratio of the $AUC_{\text{last}}$ values between the subcutaneous and intravenous administrations.

The pharmacokinetic analyses were performed by CPKD Solutions, LLC (Research Triangle Park, NC) using the noncompartmental routine in WinNonlin Version 1.1. Pharmacokinetic parameter values were summarized by route of administration using descriptive statistics (mean, median, standard deviation, coefficient of variation, maximum, and minimum).

RESULTS

All 15 subjects were successfully administered treprostinil via intravenous infusion and subcutaneous infusion at the protocol-specified dose (i.e., 15 ng/kg/ min for 150 min). All 15 subjects also provided plasma treprostinil concentration data sets for pharmacokinetic analysis of both administration routes. Thirty adverse events, all nonserious, were reported in 11 subjects. Of these events, 90% were mild, and the remainder were moderate; most were previously well-characterized events related to treprostinil’s pharmacologic activity. The adverse events included 8 and 4 reports of headache with i.v. dosing and s.c. dosing, respectively; 3 and 1 reports of nausea with i.v. and s.c. dosing, respectively; and 5 reports of dizziness, all with i.v./s.c. dosing. The remaining 9 adverse events included 2 reports of s.c. infusion site pain and single reports of vomiting, s.c. infusion site edema, somnolence, myalgia, rash, vasodilatation, and urination impairment. None of the events required clinical intervention.

Figure 1 shows the mean plasma treprostinil concentration profiles for both intravenous and subcutaneous administration ($N = 15$). The mean plasma treprostinil concentration during intravenous infusion climbed steeply from zero concentration and achieved a $C_{\text{max}}$ of 1.5 ng/mL in a $t_{\text{max}}$ of 150 minutes, which was the time point for the end of infusion. Decline from $C_{\text{max}}$ in individual patients was biphasic. Although serial plasma samples were collected for up to 8 hours postinfusion, the majority of subjects had plasma treprostinil concentrations below 25 pg/mL (the LLOQ of the LC/MS/MS assay) by 3 hours postinfusion.

The mean plasma treprostinil concentration during subcutaneous infusion climbed less steeply from the origin compared to that observed for the intravenous infusion and did not achieve $C_{\text{max}}$ until a few minutes after the infusion ended. Furthermore, mean $C_{\text{max}}$ was lower for the subcutaneous infusion compared to the intravenous infusion (Figure 1). Decline from $C_{\text{max}}$ was also biphasic for the subcutaneous infusion, but both the distributive and elimination phases were less steep when compared to the decline from $C_{\text{max}}$ observed for intravenous dosing. As a result, the majority of subjects had quantifiable plasma treprostinil concentrations up to 5 to 6 hours after termination of the subcutaneous infusions.

Mean values for the pharmacokinetic parameters observed with intravenous and subcutaneous dosing are summarized in Table I. Mean $C_{\text{max}}$ was slightly higher following intravenous dosing (1.57 ng/mL) compared to subcutaneous dosing (1.47 ng/mL), and mean $t_{\text{max}}$ was lower following intravenous dosing (2.13 h) compared to subcutaneous dosing (2.51 h). Mean $AUC_{\text{last}}$ values for intravenous and subcutaneous dosing were 3.52 and 3.97 ng·h/mL, respectively, resulting in a mean (SD) absolute bioavailability of 113.1% (10.0%) (CV = 8.9%; min, max = 95.8%, 131.8%) for the subcutaneous infusion.

Mean Vz for intravenous dosing was 806.1 mL/kg, and Vz/F for subcutaneous dosing was 1113.6 mL/kg. The intersubject variability, as reflected by the CV for...
all parameters, except t1/2 and Vz, was low at approximately 20% or less. The CV for t1/2 and Vz terms was 40% to 50%.

Mean CL for intravenous dosing was 663.0 mL/kg/h, and mean CL/F for subcutaneous dosing was 589.4 mL/kg/h. The apparent mean t1/2 was shorter following intravenous infusion (0.87 h) compared to the subcutaneous infusion (1.38 h).

DISCUSSION

This was the first study to evaluate treprostinil administered by both short-term, continuous intravenous and subcutaneous administration in normal subjects. The resulting pharmacokinetic profiles showed that the decline from Cmax was rapid for both types of infusion; however, there was a slight delay in tmax for the subcutaneous infusion. Specifically, mean plasma treprostinil concentration during intravenous dosing climbed steeply from the origin, with a Cmax of 1.57 ng/mL and a tmax at the end of the infusion. In contrast, mean plasma treprostinil concentration during subcutaneous dosing climbed less steeply from the origin, with a Cmax of 1.47 ng/mL and a tmax a few minutes after the infusion end. This small delay in tmax was most likely due to the extra step required for absorption from the injection site into the capillaries during the subcutaneous infusion.

The subcutaneous half-life of treprostinil was found to be longer than the i.v. half-life (1.38 h vs. 0.87 h), although this finding should be interpreted with caution given that the CV for t1/2 was more than 40%. In any case, treprostinil was readily and completely absorbed after subcutaneous infusion. The absolute bioavailability for the subcutaneous administration was estimated to be 113%, which is slightly greater than unity. The AUC∞ for the intravenous administration may have been underestimated due to the potency of this agent and limitations of the LC/MS/MS assay. The Cmax values observed in this study were less than 2 ng/mL, and despite best efforts to develop a more sensitive LC/MS/MS assay for treprostinil, the LLOQ was limited to 25 pg/mL. Therefore, the decline from Cmax following acute intravenous administration could be monitored for 2 to 3 hours only, which is less than five times the intravenous half-lives of treprostinil (estimated to be 0.87 h). As a result, a small percentage of the intravenous AUC∞ may have been truncated due to the limitation of the LC/MS/MS assay. Alternatively, the finding could be explained by enterohepatic recirculation of subcutaneously administered Remodulin®; however, this explanation seems less likely since both routes involve delivery into the venous system, and no secondary peak representing the reabsorption of Remodulin® from the gastrointestinal tract was observed in the elimination curve by either route.

Values for the other pertinent pharmacokinetic parameters were similar for the two routes of administration once the absolute bioavailability value of the subcutaneous administration was taken into consideration. Specifically, mean Cmax, tmax, CL, and Vz values during intravenous dosing were 1.57 ng/mL, 2.13 hours, 633.0 mL/kg/h, and 806.1 mL/kg, respectively; during subcutaneous dosing, these values were 1.47 ng/mL, 2.51 hours, 589.4 mL/kg/h, and 1113.6 mL/kg, respectively. Intersubject variability for these parameters was small, as reflected by the CV of < 25%. The exception was the apparent volume of distribution, with intersubject variability (CV) between 40% and 51%. This higher variability was probably observed because this parameter is calculated from the ratio of plasma clearance to the elimination rate constant, and the accuracy of the elimination rate constant was uncertain due to below-LLOQ drug levels in the terminal elimination phase. One reason that the terminal elimination phase appears different for the two routes may be a flip-flop phenomenon, with the rate of absorption being slower than elimination for the subcutaneous route. It is also possible that the curve observed with i.v. administration may be better explained by a two-compartment model.

The Vz/F term for subcutaneous dosing was 1113.6 mL/kg, indicating that this agent was not widely distributed outside the highly perfused organs. This find-
ing is in agreement with the results of a tissue distribution study performed in rats (data on file, United Therapeutics Corp.). The rodents were administered [14C]treprostinil via continuous subcutaneous infusion for 6 hours. Distribution of [14C]treprostinil radioactivity was found in all collected tissues, with the highest concentrations in the plasma, red blood cells, liver, small intestine, nonpigmented skin, kidneys, pigmented skin, and large intestine. [14C]treprostinil was also found in the heart, lungs, abdominal aorta, mesenteric lymph nodes, pancreas, spleen, and other organs.

A mass balance and metabolism study in normal volunteers using [14C]-treprostinil has shown that approximately 79% of an administered dose of treprostinil is excreted in the urine as unchanged drug (4%) and as identified metabolites (64%), including the glucuronide conjugate of treprostinil. Approximately 13% of a dose is excreted in the feces (manuscript in preparation). The chronic pharmacokinetics of treprostinil are described in the companion manuscript.10

In summary, treprostinil administered by subchronic subcutaneous infusion is rapidly and completely absorbed into the systemic circulation. Relatively modest differences in pharmacokinetic parameters between subcutaneous and intravenous treprostinil include a slightly longer time to maximum plasma level and a longer apparent plasma half-life following subcutaneous administration.

We thank Lauren Cierpial and Courtney Peters for assistance with the manuscript. This study was funded by United Therapeutics Corporation.

REFERENCES


Effects of St. John’s Wort (Hypericum perforatum) on Tacrolimus Pharmacokinetics in Healthy Volunteers

Mary F. Hebert, PharmD, FCCP, Jeong M. Park, MS, PharmD, Yu-Luan Chen, PhD, Shahzad Akhtar, and Anne M. Larson, MD

Tacrolimus is an immunosuppressant approved for the prevention of rejection following transplantation and is a substrate for CYP3A and P-glycoprotein. A pharmacokinetic interaction between St. John’s wort (antidepressant herbal product and inducer of CYP3A and P-glycoprotein) and tacrolimus was evaluated in 10 healthy volunteers. The pharmacokinetics of tacrolimus were obtained from serial blood samples collected following single oral doses (0.1 mg/kg) prior to and during an 18-day concomitant St. John’s wort dosing phase (300 mg orally three times daily). Coadministration of St. John’s wort significantly decreased tacrolimus AUC (306.9 µg*h/L ± 175.8 µg*h/L vs. 198.7 µg*h/L ± 139.6 µg*h/L; p = 0.004) and increased apparent oral clearance (349.0 mL/h/kg ± 126.0 mL/h/kg vs. 586.4 mL/h/kg ± 274.9 mL/h/kg; p = 0.01) and apparent oral volume of distribution at steady state (11.5 L/kg ± 4.3 L/kg vs. 17.6 L/kg ± 9.6 L/kg; p = 0.04). St. John’s wort appears to induce tacrolimus metabolism, most likely through induction of CYP3A and P-glycoprotein.

Keywords: St. John’s wort; tacrolimus; organ transplantation; depression

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METHODS

The whole-blood concentration-time profiles of single-dose oral tacrolimus (Prograf, Fujisawa USA, Inc., Deerfield, IL) in healthy volunteers, with and without coadministration of oral St. John’s wort (Lichtwer Pharma AG, Berlin, Germany) at steady state, were examined. Pharmacokinetic parameters were estimated using noncompartmental techniques. The St. John’s wort was supplied by Lichtwer Pharma AG. Each 300-mg St. John’s wort tablet from the lot used in this study contained 440.0 µg hypericin, as measured by high-performance liquid chromatography (HPLC). This study was conducted at the University of Washington Clinical Research Center.

Subject Selection

Ten healthy volunteers (8 female, 2 male) participated in this study after giving informed consent. The study was approved by the University of Washington Institutional Review Board.
protocol was approved by the investigational review board at the University of Washington. Eight subjects were Caucasian and 2 were Asian, with ages between 20 and 30 years old, weight between 47 and 90 kg, and heights between 152 and 192 cm. Subjects were considered to be healthy based on physical examination, medical history, and standard blood and urine laboratory tests. Subjects with active medical problems, those taking any medications, those weighing in excess of 20% over their ideal body weight, and smokers were excluded from the study.

Serial blood samples were collected from an indwelling venous catheter at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 14 hours following tacrolimus administration. In addition, blood samples were collected at 24, 36, 48, 72, and 96 hours following tacrolimus administration by separate peripheral vein needle sticks.

Dosing Regimen

During phase I (study days 1-7), subjects received a single oral tacrolimus dose (approximately 0.1 mg/kg, adjusted to commercially available units) on day 1. Pharmacokinetic parameters of tacrolimus were estimated in the absence of St. John’s wort. In phase II of the study (study days 8-26), the pharmacokinetics of tacrolimus were estimated in the presence of the inducer, St. John’s wort. To achieve maximum inductive effects of St. John’s wort before tacrolimus dosing in phase II, administration of St. John’s wort (300 mg orally three times daily with meals for 18 days) began on study day 8. On study day 22, subjects received a second single oral dose of tacrolimus. The first St. John’s wort administration on study day 22 followed tacrolimus administration by 2 hours. The lower weight between the subject’s actual and ideal body weight was used for the calculation of tacrolimus dosing.

Compliance

Subjects were asked to record on a calendar the actual time of St. John’s wort administration during phase II of the study. St. John’s wort pill counts were conducted prior to tacrolimus administration to evaluate compliance.

Diet

Subjects were asked to abstain from ethanol, grapefruit, and caffeine-containing foods and beverages throughout the entire study period. All subjects received the same diet at the same time on both tacrolimus administration days to control for the effects of food on tacrolimus pharmacokinetics.5,6

Analysis of Blood Samples

Whole-blood tacrolimus concentrations were determined by a liquid chromatography tandem mass spectrometry (LC/MS/MS) assay. In brief, 0.25 mL of a whole-blood sample was mixed with 0.5 mL of the internal standard (IS, Ascomycin) working solution (10.0 ng/mL in methanol). A 3-mL solution of 5% ZnSO₄-methanol-acetonitrile (5:3:2, v/v) was added into the tube and vortex-mixed 30 seconds for de-proteinization, then centrifuged at 3500 rpm for 5 minutes. The supernatant was decanted into a Varian Bond Elut C₁₈ cartridge that was conditioned with 1 mL of methanol followed by 1 mL of water. After the sample passed through the cartridge under minimum vacuum, 1 mL of methanol-water (40:60, v/v) followed by 1 mL of hexane was applied to wash the cartridge. The sample was then eluted with 1 mL of methanol. The eluent was evaporated to complete dryness at 40°C under a stream of nitrogen in a Turbo-Vap evaporator. The residue was reconstituted in 100 µL of acetonitrile-water (50:50, v/v). An aliquot of a 10-µL sample was injected into an LC/MS/MS (Applied Biosystems, Sciex API 3000). Separation was achieved on an Alltech Alltima C₁₈ column (5-µm, 150 × 2.1 mm) at 55°C using a mobile phase of methanol–0.1 M ammonium acetate (99:1, v/v) at a flow rate of 0.2 mL/min. Turbo ionspray was operated at positive mode, and the source temperature was 400°C. Tandem mass-spectrometric detection was monitored m/z at 821.3 → 768.3 for tacrolimus and at 809.6 → 756.4 for the IS. Analyst Software 1.2 (Applied Biosystems) was used to collect and analyze data. A calibration curve ranging from 0.1 to 30.0 ng/mL was employed to calculate the concentration of clinical samples. Samples with a concentration of over 30 ng/mL were analyzed by fivefold dilution with a blank matrix. Assays were provided by Fujisawa Research Institute of America.

Data Analysis

The total area under the observed tacrolimus concentration-time curve (AUC) was calculated using the linear trapezoidal rule for the ascending concentrations and the log-trapezoidal rule for descending concentrations. AUC values were extrapolated from the 96-hour concentration to infinity by Cₙ₉₆/kₑₙ₉₆, in which
Clast was the last measurable tacrolimus concentration and kelim was the terminal elimination rate constant, as determined by log-linear regression. Area under the moment curve (AUMC) was calculated by the linear trapezoidal rule for both the ascending and descending portions of the curve and was extrapolated to infinite time. Apparent oral tacrolimus clearance was calculated as CL/F = Dose/AUC. Apparent oral tacrolimus steady-state volume of distribution was determined by Vss/F = CL × (AUMC/AUC). All results are reported as mean values ± standard deviations before and with St. John’s wort. Differences in pharmacokinetic parameters before and with St. John’s wort were analyzed using the paired Student’s t-test and were considered significant at p < 0.05.

### RESULTS

Individual estimated pharmacokinetic parameters of tacrolimus in whole blood are shown before and with the coadministration of St. John’s wort (phases I and II, respectively) in Table I. With coadministration of St. John’s wort, a statistically significant increase in tacrolimus apparent oral clearance (CL/F; 349.0 mL/h/kg ± 126.0 mL/h/kg vs. 586.4 mL/h/kg ± 274.9 mL/h/kg; p = 0.01) and apparent oral volume of distribution at steady state (Vss/F; 11.5 L/kg ± 4.3 L/kg vs. 17.6 L/kg ± 9.6 L/kg; p = 0.04) was observed. A significant decrease in the total area under the observed tacrolimus concentration-time curve (AUC; 306.9 µg•h/L ± 175.8 µg•h/L vs. 198.7 µg•h/L ± 139.6 µg•h/L; p = 0.004) and

### Table I  Estimated Pharmacokinetic Parameters Determined from Whole-Blood Tacrolimus Concentrations after Oral Tacrolimus Administration before (Phase I) and with St. John’s Wort (Phase II)

<table>
<thead>
<tr>
<th>Subject #</th>
<th>AUC (µg•h/L)</th>
<th>CL/F (mL/h/kg)</th>
<th>Vss/F (L/kg)</th>
<th>Half-life (h)</th>
<th>Cmax (ng/mL)</th>
<th>tmax (h)</th>
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AUC, area under the concentration-time curve; CL/F, apparent oral clearance; Vss/F, apparent oral volume of distribution at steady state; Cmax, maximum concentration; tmax, time to reach maximum concentration. Tacrolimus doses before and with St. John’s wort were 0.1 mg/kg orally adjusted to the closest commercially available unit.

a. Significantly different from the phase I (before St. John’s wort) parameter, as determined by the two-tailed paired Student’s t-test (p < 0.05).
maximum tacrolimus concentration (Cmax; 29.0 ng/mL ± 10.1 ng/mL vs. 22.4 ng/mL ± 12.8 ng/mL; p = 0.001) was also seen. No significant changes were seen in tacrolimus half-life or time to reach maximum concentration.

Figure 1 depicts representative (subject 1) oral tacrolimus concentration-time profiles before (phase I) and with (phase II) St. John’s wort administration. As demonstrated by Figure 1, the AUC for oral tacrolimus is substantially higher in phase I as compared to phase II, despite the administration of the same tacrolimus dose in both phases of the study. As with most drug interactions reported with tacrolimus, there is a large amount of variability in effects of St. John’s wort on tacrolimus AUCs. On average, there is a 34% decrease in tacrolimus AUC with St. John’s wort. However, the percent decrease in AUC ranges from 15% to 64% in 9 of the 10 individuals. In 1 volunteer (subject 10), the AUC of tacrolimus increased 31%.

No adverse events were reported with tacrolimus or St. John’s wort in this study either by subject report or as reflected by laboratory tests.

DISCUSSION

St. John’s wort has become a popular alternative medicine in the United States for the self-management of anxiety and depression. In 2002, it ranked seventh in the top-selling herbal supplements sold in food, drug, and mass-market retail outlets, with retail sales more than $24 million.5 This figure includes supermarkets, drug stores, and mass merchandisers (excluding Walmart). One problem with herbal supplements sold in the United States is that there is a great deal of variability in their content. Glisson et al6 analyzed 13 St. John’s wort preparations purchased from health food stores. HPLC analysis revealed that none of the products were within 10% of their label claim for hypericin and that 2 of the products contained no hypericin at all. The variability in St. John’s wort content certainly can affect the potential for drug interactions with this agent. To do what is possible to control for this issue, we obtained the St. John’s wort for this study from a company in Germany, where St. John’s wort is considered a drug rather than a nutritional supplement, as it is in the United States. In addition, we have a confirmed amount of hypericin in the lot of St. John’s wort that we use in this study. It is clear that when a St. John’s wort product contains St. John’s wort, such as that used in this study, there is an interaction with tacrolimus. However, if a different dose were used or if the St. John’s wort purchased did not actually contain any St. John’s wort, then the magnitude of the effect would potentially be different or be nonexistent. Since St. John’s wort is a natural product, the relative amounts of its components (e.g., hypericin and hyperforin) can also vary from product to product. This may also affect the magnitude of the interaction.

There is a growing body of evidence documenting pharmacokinetic interactions with St. John’s wort.3,10-13 In addition to the one case report of an interaction between St. John’s wort and tacrolimus, there have been multiple reports in the literature of an interaction between St. John’s wort and cyclosporine resulting in subtherapeutic concentrations of cyclosporine and rejection following solid organ transplantation.10-13 Tacrolimus, like cyclosporine, is a substrate for both CYP3A and P-glycoprotein.14,15 These interactions are of particular concern because depression is reported in approximately one-third of the patients following solid organ transplantation.12

Modulation of the drug-metabolizing enzyme CYP3A and the drug transporter protein P-glycoprotein has been suggested as the underlying mechanism of these interactions. St. John’s wort is an inducer of P-glycoprotein and both intestinal and hepatic CYP3A4 in humans.16-19 Durr et al16 found in healthy volunteers that the administration of St. John’s wort (300 mg three times daily for 14 days) increased expression of duodenal P-glycoprotein by 37%, which correlated with an 18% decrease of digoxin AUC. In addition, they reported a 48% increase in duodenal

Figure 1. Representative concentration-time curves (whole blood) for oral tacrolimus before St. John’s wort administration (open squares, phase I) and with St. John’s wort administration (open circles, phase II).
CYP3A4 expression and a 44% increase in hepatic CYP3A4 activity as measured by the $^{14}$C-erythromycin breath test following St. John’s wort administration. Studies using in vivo phenotypical probes of CYP isozyms and P-glycoprotein also support this proposed mechanism.17-20

The whole-blood tacrolimus pharmacokinetic parameters estimated during the pre–St. John’s wort phase of this study (phase I) are similar to those previously reported in healthy volunteers.21-22 Tacrolimus pharmacokinetics have a fair amount of intra- and intersubject variability. Given the narrow therapeutic range for tacrolimus, greater than a 25% change in pharmacokinetic parameters is considered to be clinically significant. Tacrolimus metabolism and transport are known to be induced by multiple agents.21,23,24 In comparison to rifampin, a very strong and well-established inducer of tacrolimus metabolism, St. John’s wort (for the product and dose used in this study) appears to be a more moderate inducer. Like rifampin, St. John’s wort has been shown to induce CYP3A and P-glycoprotein in humans.16,25,26 Previously published work has shown that concomitant administration of rifampin with tacrolimus results in significant decreases in oral tacrolimus area under the concentration-time curve in healthy volunteers. Average decreases in tacrolimus AUCs were approximately 67% with rifampin but ranged from 58% to 74%. This is in contrast to the average decrease in oral tacrolimus AUCs with St. John’s wort of 34%.

St. John’s wort contains multiple substances, only a few of which have been investigated for their CYP3A and P-glycoprotein modulating activity. An in vitro study demonstrated that long-term exposure to hypericin strongly induced P-glycoprotein expression in LS-180 intestinal adenocarcinoma cells in a dose-dependent fashion, whereas hypericin at high concentrations inhibited P-glycoprotein-mediated rhodamine 123 transport in Caco-2 cell monolayers.27 Hyperforin induced CYP3A expression in vitro through activation of the pregnane X receptor, but hypericin did not appear to cause CYP3A induction.28,29 A temporal relationship between the initiation of St. John’s wort and falling tacrolimus trough concentrations has been described in a single case report.3 In addition, the concomitant administration of St. John’s wort 600 mg orally daily has been reported to significantly decrease tacrolimus area under the concentration-time curve, as well as maximum and minimum tacrolimus concentrations.4 One of the major limitations of their study is that the assay used was the microparticle enzyme immunoassay, which has the potential to cross-react with metabolites. This is in contrast to the specific assay for tacrolimus (LC/MS/MS) used in our study. In addition, pharmacokinetic parameters were not reported in their study.4 Our study confirms their results and describes the effects of St. John’s wort on the estimated pharmacokinetic parameters of tacrolimus. The potential consequences of this interaction for a solid organ transplantation recipient are rejection and graft loss. Since St. John’s wort is available over the counter and consumers generally assume that it is safe, patient education regarding this interaction is essential.

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REFERENCES


Effect of St. John’s Wort on the Pharmacokinetics of Theophylline in Healthy Volunteers

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The extract of St. John’s wort (SJW, Hypericum perforatum) is a popular herbal remedy used for the treatment of depression, anxiety, and sleep disorders. SJW induces cytochrome P450(CYP)3A4 and P-glycoprotein (Pgp)1-3 and thereby alters the pharmacokinetics of coadministered drugs such as cyclosporine, tacrolimus, midazolam, indinavir, simvastatin, and digoxin. Since theophylline is metabolized mainly by CYP1A2 and partly by CYP2E1 and CYP3A4, the effect of SJW on CYPs may alter the pharmacokinetics of theophylline. Several review papers have mentioned theophylline as one of the drugs that interacts with SJW. However, the report cited by those review papers is a case in which an increase in plasma theophylline concentrations was observed after discontinuation of SJW in a smoker taking 11 other drugs. Thus, the interaction of SJW with theophylline has not been fully evaluated yet. The objective of the present study was to investigate the effect of SJW on the pharmacokinetics of theophylline in healthy volunteers.

METHODS

Subjects

Twelve healthy Japanese male volunteers (ages 25.0 ± 6.4 years, height 171.9 ± 4.6 cm, weight 63.3 ± 8.6 kg, mean ± SD) participated in the present study. Subjects were considered to be in good health on the basis of medical history, physical examination, electrocardiogram, urinalysis, and clinical laboratory testing. They were nonsmokers and not receiving any other medication. The protocol was approved by the institutional review board of Oita Medical University. All subjects gave written informed consent.
Study Design

This was a randomized, open-labeled, crossover study. Each subject was administered a single oral dose of theophylline 400 mg (two tablets of THEODUR®; Nikken Chemicals Co., Tokyo, Japan) on two occasions (with and without SJW) over a 15-day period. According to the predetermined schedule, one of the two doses of theophylline was given on day 14 out of 15 days of treatment with SJW, and the other was without SJW.

To minimize intraproduct variation in phytochemical content, a single lot of SJW extract (TruNature®, Leiner Health Products, Carson, CA; bulk no. 1EA0316), labeled as containing 0.3% hypericin, was used throughout the study. The subjects took the usual dose of SJW 300 mg three times a day for 15 days. The brand and the dose of SJW in this study were chosen on the basis of a previous report. Kizu et al with minor modifications. First, 300 µL of water.

Procedures

The subjects were asked to refrain from consuming grapefruit juice and SJW from 2 weeks before the first trial until the end of the second trial. Alcoholic drinks and caffeine-containing foods were not allowed for 4 days before and during each trial.

At 6:30 a.m. on the study day, the subjects entered the Clinical Pharmacology Center at Oita Medical University. They took a standardized breakfast at 7:00 a.m. and received theophylline at 8:00 a.m. with 150 mL of water. Theophylline was given on day 14 out of 15 days before and during each trial. Alcoholic drinks were not allowed for 4 days. The subjects were asked to refrain from consuming grapefruit juice and SJW 300 mg three times a day for 15 days. The brand and the dose of SJW in this study were chosen on the basis of a previous report. Kizu et al with minor modifications. First, 300 µL of water. and received theophylline at 8:00 a.m. with 150 mL of water. Theophylline was given on day 14 out of 15 days before and during each trial. Alcohol.

Venous blood samples were collected before administration and at the following times after theophylline administration: 2, 4, 6, 8, 10, 12, 14, 16, 24, 36, and 48 hours. Blood samples were obtained with a catheter placed in a forearm vein that was kept patent with heparinized saline. Subjects remained in the room until 8:30 a.m. the next day, and blood samplings at 36 and 48 hours after administration were performed on an outpatient basis. Urine samples were collected for 48 hours. Blood was centrifuged at 3000 g for 15 minutes to separate the plasma. Plasma and urine samples were stored in polypropylene tubes and frozen at −20°C until the time of assay.

HPLC Determination of Theophylline in Plasma

The concentration of unchanged theophylline in plasma was measured based on the method reported by MORIMOTO ET AL

Kizu et al with minor modifications. First, 300 µL of acetonitrile, containing 0.3 µg of β-hydroxyethyl-theophylline as an internal standard (IS), was added to 100 µL of a plasma sample. After vigorous mixing, the sample was centrifuged at 5000 g for 5 minutes, and 300 µL of supernatant was transferred into a new tube and evaporated to dryness with a gentle stream of nitrogen gas. The residue was dissolved in 300 µL of mobile phase, and 50 µL of the solution was injected into a high-performance liquid chromatography (HPLC) column (TSK-GEL® ODS-80TM, 250 × 4.6 mm i.d., 5 µm, Tosoh Co., Tokyo, Japan). The mobile phase consisted of 20 mM sodium acetate buffer (pH 4.8), acetonitrile, and methanol (900:35:65, v/v/v). The flow rate was 1 mL/min. The column was operated at 40°C. Peaks were quantified by a UV absorbance detector (2487 Dual λ UV/VIS Detector Waters, Milford, MA) at 275 nm. The standard curve for theophylline was linear over the range from 0.1 to 20 µg/mL. The coefficient of correlation of the standard curve was > 0.999. The coefficient of variation of intra-assay variations (n = 10) was 4.8% (0.1 µg/mL) and 1.5% (10 µg/mL), respectively. Interassay variations (n = 6) were 1.6% (0.1 µg/mL) and 1.5% (10 µg/mL), respectively. The analysis was performed in duplicate.

HPLC Determination of Theophylline and the Metabolites in Urine

We measured the concentrations of urinary unchanged theophylline, 1,3-dimethyluric acid (13U), 1-methyluric acid (1U), and 3-methylxanthine (3X) using the method reported by Tsutsumi et al with minor modifications. After a 100-µL urine sample was extracted twice with 3 mL of chlororform/isopropanol (95:5, v/v), the organic layer was evaporated to dryness. The residue was reconstituted to 1 mL in 0.05% acetic acid, and the sample (100 µL) was injected into an Ultrasphere ODS column (250 × 4.6 mm i.d., 5 µm, Beckman Coulter, Fullerton, CA) operated at room temperature. The mobile phase consisted of 1% acetic acid/methanol (90:10, v/v). The flow rate was 1 mL/min. Peaks were quantified by a UV absorbance detector (UV-8000 Detector Tosoh Co., Tokyo, Japan) at 280 nm. The standard curves for unchanged theophylline and the three metabolites were linear over the range from 2 to 100 µg/mL. The coefficient of correlation of each standard curve was > 0.999. The coefficients of intra-assay variations (n = 10) at 2 µg/mL were 5.8%, 3.1%, 12.9%, and 11.9% for unchanged theophylline, 13U, 1U, and 3X, respectively; at 100 µg/mL, they were 5.6%, 9.6%, 11.9%, and 8.4% for unchanged...
theophylline, 13U, 1U, and 3X, respectively. The coefficients of interassay variations \((n = 6)\) at 2 \(\mu\)g/mL were 6.3%, 7.2%, 9.1%, and 8.1% for unchanged theophylline, 13U, 1U, and 3X, respectively; at 100 \(\mu\)g/mL, they were 2.5%, 3.2%, 5.1%, and 3.2% for unchanged theophylline, 13U, 1U, and 3X, respectively. All urine samples were analyzed in duplicate.

All samples extracted from plasma and urine were kept at 4°C in an automatic injector and analyzed within 24 hours. The storage of samples in the automatic injector for 24 hours did not change the theophylline and metabolite concentrations. All samples were free from substances that interfered with the peaks of interest.

**Data Analysis**

The actual data observed were used for peak plasma concentration \((C_{\text{max}})\) and time of peak concentration \((t_{\text{max}})\). The elimination constant \(k_e\) was calculated by log-linear regression in the terminal elimination phase. The terminal half-life \((t_{1/2})\) was calculated by \(\ln 2/k_e\). The area under the plasma concentration-time curve \((AUC)\) and the area under the first moment curve \((AUMC)\) were determined by the linear trapezoidal method. These moments were extrapolated to infinity using \(k_e\) \((AUC_{0-\infty})\) and \((AUMC_{0-\infty})\).

The mean residence time \((\text{MRT})\), total clearance \((\text{CL/F})\), and volume of distribution \((\text{Vd/F})\) were calculated as follows:

\[
\text{MRT} = \frac{\text{AUMC}_{0-\infty}}{\text{AUC}_{0-\infty}}, \\
\text{CL/F} = \frac{\text{Dose}}{\text{AUC}_{0-\infty}}, \\
\text{Vd/F} = \frac{\text{Dose} \times \text{MRT}}{\text{AUC}_{0-\infty}}.
\]

The urinary excretion ratio was calculated on a molar basis using the total recovery of unchanged theophylline and metabolites in urine for 48 hours. The equations are as follows:

\[
\text{Total urinary excretion ratio} = \frac{\text{unchanged theophylline} + 13U + 1U + 3X}{\text{Dose of theophylline}},
\]

\[
\text{Urinary unchanged theophylline or metabolite ratio} = \frac{\text{unchanged theophylline or metabolite}}{\text{unchanged theophylline} + 13U + 1U + 3X}.
\]

All numeric data were given as mean ± standard deviation. Pharmacokinetic data for plasma theophylline concentrations and urine volume were analyzed by the Student \(t\)-test (two-tailed). The Wilcoxon signed rank test was used for \(t_{\text{max}},\) total urinary excretion ratio, and urinary metabolite ratio. All statistics were calculated with StatView (version 5.0, 1998, SAS Institute, Cary, NC); \(p < 0.05\) was considered statistically significant.

We also presented 95% confidence intervals (CIs) on differences in the means of parameters where appropriate.

**RESULTS**

All enrolled subjects completed the study protocol. Both theophylline and SJW were well tolerated with no adverse events. The actual washout periods between two trials were from 19 to 35 days.

The treatment with SJW produced no significant difference in the pharmacokinetics of theophylline in plasma (Figure 1, Table I). The total urinary excretion ratio was not significantly affected by SJW \((74.8\% ± 15.5\% \text{ without SJW vs. } 72.3\% ± 11.3\% \text{ with SJW, } p = 0.31).\) The treatment with SJW tended to increase the urinary metabolite ratio of 1U \((p = 0.06, \text{ Table II}). No significant change with SJW was found in the urinary unchanged theophylline ratio or the urinary metabolite ratio of 13U and 3X.

### Table I  The Effects of St. John’s Wort (SJW) on the Pharmacokinetic Parameters of Theophylline in Plasma

<table>
<thead>
<tr>
<th></th>
<th>Without SJW</th>
<th>With SJW</th>
<th>95% CI</th>
<th>(p)-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(t_{\text{max}}) (h)</td>
<td>9.0 (6.0-14.0)a</td>
<td>11.0 (6.0-12.0)a</td>
<td>NC</td>
<td>0.21</td>
</tr>
<tr>
<td>(C_{\text{max}}) ((\mu)g/mL)</td>
<td>7.5 ± 1.0</td>
<td>7.4 ± 1.1</td>
<td>(–0.7, 0.9)</td>
<td>0.78</td>
</tr>
<tr>
<td>(AUC_{0-\infty}) ((\mu)g h/mL)</td>
<td>176.9 ± 34.0</td>
<td>171.1 ± 25.7</td>
<td>(–9.3, 23)</td>
<td>0.41</td>
</tr>
<tr>
<td>(\text{CL/F}) (L/h/kg)</td>
<td>0.037 ± 0.009</td>
<td>0.038 ± 0.009</td>
<td>(–0.05, 0.002)</td>
<td>0.53</td>
</tr>
<tr>
<td>(\text{Vd/F}) (L/kg)</td>
<td>0.74 ± 0.11</td>
<td>0.73 ± 0.14</td>
<td>(–0.06, 0.06)</td>
<td>0.94</td>
</tr>
<tr>
<td>(t_{1/2}) (h)</td>
<td>9.7 ± 1.4</td>
<td>9.1 ± 1.3</td>
<td>(–2.9, 1.4)</td>
<td>0.18</td>
</tr>
<tr>
<td>(\text{MRT}) (h)</td>
<td>20.1 ± 2.2</td>
<td>19.3 ± 1.6</td>
<td>(–0.5, 2.1)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

\(a\) Median (minimum – maximum).

**EFFECT OF ST. JOHN’S WORT ON THEOPHYLLINE**

**DRUG INTERACTIONS**

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Methylxanthines have declined in importance for asthma therapy. The advent of inhaled glucocorticoids, β-adrenergic receptor agonists, and leukotriene-modifying drugs have significantly diminished the extent to which theophylline is used. Nonetheless, in the United States, theophylline has a role as a third-line treatment for patients whose asthma is difficult to control.²⁰

Theophylline is predominantly metabolized by CYP1A2.¹² Figure 2 is a scheme proposed for the biotransformation of theophylline.¹²,²¹,²² CYP2E1 and 3A4 have been thought to have a minor effect on theophylline metabolism.¹²

In the present study, the 15-day treatment with SJW produced no significant changes in the pharmacokinetics of theophylline in plasma. These results indicate that the effect of SJW on CYP1A2, if any, is not sufficient to influence the pharmacokinetics of theophylline in plasma. The results are in agreement with the findings reported by Wang et al,⁷ who showed that a 2-week administration of SJW did not alter the pharmacokinetics of caffeine, which is likewise predominantly metabolized by CYP1A2.

A previous study reported that the fractions of theophylline metabolites in urine (urinary metabolite ratio) were 50.4% ± 2.0%, 17.4% ± 0.3%, and 13.2% ± 1.3% (mean ± SE) for 13U, 1U, and 3X, respectively.²¹ Our results were consistent with these data. The urinary metabolite ratio of 1U tended to increase when SJW was coadministered in the present study. However, the enzymes involved in the biotransformation of theophylline have not been fully defined. The pathway from theophylline to 1U is initially the formation of 1X by CYP1A2,²² followed by the oxidation of 1X by xanthine oxidase.²² A previous study suggests that 1U is also formed by the demethylation of 13U,²² although the enzyme involved has not yet been determined. 13U, the major metabolite of theophylline, is transformed mainly by CYP1A2 and partly by CYP2E1 and 3A4.²² These enzymes may be involved in the increase in the urinary metabolite ratio of 1U produced by SJW. However, the result of 1U observed in the present study is unlikely to be sufficient to conclude that the metabolism of theophylline is affected by SJW. Concerning this issue, further studies are needed.

Gurley et al¹¹ found a modest increase in the paraxanthine/caffeine serum ratio after a 4-week treatment with SJW, indicating that CYP1A2 was induced by SJW. In contrast, SJW for 2 weeks did not alter the pharmacokinetics of caffeine in plasma.⁷ In the present study, we were not able to obtain results that clearly supported an induction of CYP1A2 by SJW. Differences in the duration of treatment may explain this discrepancy. Gurley et al also found an activation of CYP2D6 after a 4-week administration of SJW, as indicated by an

<table>
<thead>
<tr>
<th>Without SJW</th>
<th>With SJW</th>
<th>95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of urine (L)</td>
<td>2.3 ± 0.6</td>
<td>2.5 ± 0.7</td>
<td>(–0.5, 0.2)</td>
</tr>
<tr>
<td>Unchanged theophylline (%)</td>
<td>19.8 ± 6.0</td>
<td>18.3 ± 4.1</td>
<td>NC</td>
</tr>
<tr>
<td>13U</td>
<td>49.0 ± 2.9</td>
<td>48.3 ± 2.5</td>
<td>NC</td>
</tr>
<tr>
<td>1U</td>
<td>14.9 ± 3.1</td>
<td>17.1 ± 3.3</td>
<td>NC</td>
</tr>
<tr>
<td>3X</td>
<td>16.3 ± 3.3</td>
<td>16.2 ± 2.1</td>
<td>NC</td>
</tr>
</tbody>
</table>

Numeric data with and without SJW are given as mean ± standard deviation. 95% CI: 95% confidence interval on differences in the means of parameters. NC, not calculated.
increase in the debrisoquine urinary recovery ratio. However, an 8-day treatment with SJW caused no significant changes in CYP2D6 activity (dextromethorphan probe). On the other hand, an increase in CYP3A4 activity (6-β-hydroxycortisol/cortisol ratios, midazolam probe, and indinavir probe) was clearly observed after SJW treatment for 2 weeks. It appears that the duration necessary to alter enzyme activities varies among isozymes of CYP. Together with findings for CYP1A2 reported by Gurley et al and Wang et al, the results obtained in the present study suggest that the induction of CYP1A2 requires a longer duration of SJW treatment, probably 4 weeks or more.

Another issue to be considered is the duration of SJW treatment required to obtain a plateau level of the plasma constituent of SJW. The duration of SJW treatment in the present study was sufficient to obtain a steady state of hyperforin and hypericin (t1/2 = 9 and 41 h, respectively). However, other potential constituents responsible for the enzyme induction may exist, and their dispositions in the body are still to be determined.

The SJW caplet used in the present study contained 0.81 ± 0.002 mg of hypericin (the labeled content was 0.9 mg). This was similar to the content of hypericin reported by Wang et al (0.84 mg). Although the constituents responsible for the enzyme-inducing effect of SJW have not been studied systematically, in vitro studies suggest that hyperforin and/or hypericin may contribute to the enzyme induction. Commercial versions of SJW show significant differences in their quantitative composition. Ingredient variability among brands, as well as its role in the effectiveness of the product, is an issue to be studied further in an attempt to understand herb-drug interactions.

The induction of CYP3A4 with SJW appears to occur mainly at the gut wall and, to a lesser extent, in the liver. The intestine shows intraintestinal variations in CYP3A4 activity, which declines from the jejunum to the ileum. Since THEODUR® tablets used are sustained-release preparations, there is a possibility that theophylline absorption in the present study occurred mostly at the distal site of the intestine where enzyme induction with SJW is insignificant. However, since theophylline undergoes little presystemic extraction, it is unlikely that CYP3A4 activity at the absorption site of the intestine contributes to the results obtained in this study.

The washout period between two trials in the present study was designed to be over 14 days. Little data
are available concerning the sufficient period for the offset of the effect of SJW on CYPs. Piscitelli et al\(^1\) reported that a reduction in AUC of sanquinavir by garlic returned only to 60% to 70% of the baseline value 10 days after discontinuing garlic. The findings of their study indicate that washout periods should be carefully determined when conducting a food-drug interaction study with crossover design. In the present study, an additional statistical analysis indicated that AUC of theophylline in the baseline (without SJW) did not significantly differ between two groups (185.3 ± 39.8 vs. 168.4 ± 27.9 µg•h/mL [mean ± SD], 95% CI = −27.3, 3, 61.2). In addition, the actual washout periods in the present study were from 19 to 35 days. It is not likely that the preceding treatment with SJW affects the second trial.

In summary, the 15-day treatment with SJW produced no significant changes in the pharmacokinetics of theophylline in plasma. Although the treatment with SJW showed a tendency to increase the urinary metabolite ratio of theophylline in plasma. Although the treatment with SJW showed a tendency to increase the urinary metabolite ratio of 1U in the present study, further studies are necessary to elucidate the effect of SJW on the metabolism of theophylline.

We thank all the participating volunteers and Sato Pharmaceutical Co. for measuring the content of hypericin.

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through activation of the pregnane X receptor. *Proc Natl Acad Sci USA* 2000;97:7500-7502.


