Short Communication

PHARMACOKINETIC STUDIES OF 2-AMINO-9-(3-ACETOXYMETHYL-4-ISOPROPOXOXYCARBONYL-OXYBUT-1-YL)PURINE, AN ORAL PRODRUG FOR THE ANTIVIRAL AGENT PENCICLOVIR

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ABSTRACT:

2-Amino-9-(3-acetoxymethyl-4-isopropanoxycarbonyloxybut-1-yl)purine (SK1899) was tested as an oral prodrug for penciclovir. SK1899 was administered orally to rats and dogs at doses up to 2 and 0.68 mmol/kg, respectively. SK1899 was well absorbed, and the major metabolites detected in plasma and urine were penciclovir, the active antiviral compound, and 6-deoxypenciclovir (M4) in both species. In rats, SK1899 was rapidly and extensively metabolized to penciclovir, which reached the peak plasma concentration (C_{max}) of 39.5 μM at 0.5 h after 0.2-mmol/kg dosing. The area under the plasma concentration-time curve (AUC) for penciclovir was 57.5 μM·h. After an oral dose of 0.034 mmol/kg to dogs, extensive conversion of SK1899 to penciclovir also occurred with slower rate of formation of penciclovir from M4 than in rats. The mean C_{max} and AUC for penciclovir were 4.5 μM at 2.7 h and 28.2 μM·h, respectively. The 0- to 24-h urinary recovery of penciclovir represented 36.1 and 36.3% of dose to rats and dogs, respectively. Radioactivity was found in fetuses following an oral administration of [^{14}C]SK1899 to pregnant rats, but no significant accumulation was observed. Although substantial milk transfer of [^{14}C]SK1899 occurred in rats, the radioactivity in milk was rapidly cleared. The values of C_{max}, AUC, and urinary recovery of penciclovir after dosing with SK1899 to rats and dogs were similar or slightly higher than those from famciclovir. These data indicate that introduction of an isopropoxy carbonate group into one of the two hydroxyl groups of M4 did not significantly alter the oral bioavailability of penciclovir compared with famciclovir.

Penciclovir is a potent and highly selective inhibitor of herpes viruses such as herpes simplex virus types 1 and 2, varicella-zoster virus, Epstein-Barr virus, and also of hepatitis B virus (Boyd et al., 1987, 1988a; Harnden et al., 1987; Sutton and Boyd, 1993; Korba and Boyd, 1996). However, penciclovir showed poor bioavailability when administered orally to humans (Boyd et al., 1988b). To improve this poor oral bioavailability, the diacetate ester of the 6-deoxy derivative of penciclovir, famciclovir, was developed as a prodrug of penciclovir (Harnden et al., 1989; Vere Hodge et al., 1989; Pue and Benet, 1993). After an oral administration of famciclovir to rats (0.125 mmol/kg) and dogs (0.078 mmol/kg), the urinary recoveries of penciclovir represented 36.0 and 36.4% of the dose, respectively (Filer et al., 1995). In humans, approximately 60% of dose was excreted as penciclovir in urine following an oral dose of 500 mg (1.56 mmol) of famciclovir (Pue et al., 1994).

To further improve the oral bioavailability of famciclovir, SK1899 was developed in our laboratory as an oral prodrug of penciclovir. SK1899 showed higher urinary recovery of penciclovir than famciclovir after oral administration to mice, and effective in vivo antiviral activities against herpes simplex virus type 1 and duck hepatitis B virus (Kim et al., 1999). These results led us to undertake pharmacokinetic studies of SK1899 to compare plasma concentration-time profiles and urinary excretions of its active metabolite penciclovir and a major intermediate metabolite M4 in rats and dogs with those of famciclovir. Transfer into the fetus and milk of [^{14}C]SK1899 was also investigated.

Experimental Procedures

Materials and Animals. SK1899 and [^{14}C]SK1899 (specific activity, 16.4 μCi/mg) were synthesized by SK Chemicals (Suwon-Si, Korea). Chemical purity of SK1899 and radiochemical purity of [^{14}C]SK1899 detected by reverse phase HPLC were 99.5 and 99.1%, respectively. Trichloroacetic acid, sodium bicarbonate, sodium azide, and oxytocin were purchased from Sigma Chemical Co. (St. Louis, MO). Sep-Pak cartridges were obtained from Waters Co. (Milford, MA). Methanol and water were of HPLC grade. Male and female rats were purchased from Charles River Japan, Inc. (Yokohama, Japan) and housed in temperature- and humidity-controlled facilities on a 12-h light cycle with free access to food and water. Beagle dogs were supplied by Marshall Farms, Inc. (North Rose, NY).

Pharmacokinetic Study. Male Sprague-Dawley rats (250–300 g) housed separately in metabolic cages were each given a single oral dose of SK1899 (0.2 or 2 mmol/kg) or famciclovir (0.2 mmol/kg) with an intragastric needle. Blood sample was collected from the rat’s tail into heparinized Pasteur pipette. One-hundred fifty microliters of each sample was immediately mixed with the same volume of 16% trichloroacetic acid in a separate tube and centrifuged, and 200 μl of the supernatant was neutralized with 40 μl of saturated solution of sodium bicarbonate. The urine sample was collected in a metabolic cage, which efficiently separated urine and feces. A 5% solution of sodium azide...
(0.4 ml per estimated 100 ml of urine) was added to each urine receptacle before collection to prevent bacterial growth. The samples collected from the animals were stored in −70°C until analysis. Three male beagle dogs (approximately 10 months old; 9–13 kg) were housed individually in labeled metabolic cages. The dogs were dosed once orally by capsule at 0.034 and 0.68 mmol/kg of SK1899 and at 0.034 mmol/kg of famciclovir. Samples of blood (approximately 3 ml) were withdrawn from each animal via a cephalic vein up to 24 h after dosing. After collection, blood samples were processed as described above. Urine sample of each dog was separated from feces and collected in a metabolic cage. The urine and plasma samples from rats and dogs dosed with SK1899 or famciclovir were analyzed by reverse phase HPLC (Waters 2690, Waters Co.) as described previously (Kim et al., 1999).

Placental Transfer. A single oral dose of [14C]SK1899 (0.2 mmol/kg, 30 μCi/kg) was given to three pregnant Sprague-Dawley rats (17th day of gestation, 275–320 g) for each sampling time point. Blood sample was collected from tail into heparinized tube and centrifuged to obtain plasma. After each group of animals was anesthetized with diethyl ether, fetus, placenta, and amniotic fluid were taken from pregnant rats.

Excretion in Milk. Before oral administration of [14C]SK1899 to lactating rats, the pups were removed from their mothers for 5 min from day 4 until day 11 after parturition, and each litter was reduced to six pups at day 6 after parturition. A single oral dose of [14C]SK1899 (0.2 mmol/kg, 30 μCi/kg) was given to three Sprague-Dawley rats (12th day after parturition, 285–350 g) for each sampling time point. The maternal rats were separated from their pups at 1 h before sampling and injected with 20 IU/kg oxytocin intraperitoneally at 15 min before sampling to stimulate milk secretion.

Radioactivity Measurements. The aliquots of urine, amniotic fluid, and milk were mixed with Lumagel Safe (Lumac* LSC, Groningen, The Netherlands) and counted directly for radioactivity. Plasma was solubilized with Soluene-350 (Packard Instrument Co., Meriden, CT) and assayed for radioactivity following the addition of Lumagel Safe and glacial acetic acid to minimize chemiluminescence. Fetus and placenta were air-dried and combusted with a sample oxidizer (Tri-Carb model 307, Packard) without further processing. Feces were homogenized with water and then combusted using aliquots. The resulting 14CO2 was adsorbed on Carbo-sorb E (Packard) and then mixed with Permafluor E scintillation fluid (Packard). The radioactivity of sample was measured using a liquid scintillation analyzer (Tri-Carb 1500, Packard) and converted to equivalents of SK1899 based on the specific radioactivity of the administered [14C]SK1899.

Pharmacokinetic and Statistical Analyses. Pharmacokinetic parameters were analyzed by noncompartmental model using the WinNonlin program (Scientific Consulting Inc., Cary, NC). The experimental results were evaluated by analysis of variance for statistical significance.

Results

Evaluation of SK1899 in Rats. After oral dosing with SK1899 to rats, all of the postulated metabolites (Fig. 1) were observed in plasma (data not shown). Among the detected metabolites of SK1899, 6-deoxy penciclovir (M4) was the major intermediate metabolite found in plasma as observed in famciclovir, which was then oxidized quickly to give an active metabolite, penciclovir. The other metabolites were present at significantly low levels in plasma. The parent compound

![Metabolic pathway showing the expected conversion of SK1899 into penciclovir after oral administration.](image)

* the position of 14C for [14C]SK1899.
SK1899 was not detected at all time points. After 0.2-mmol/kg dosing, the mean $C_{\text{max}}$ values for penciclovir were 39.5 $\mu$M at 0.5 h for SK1899 and 34.0 $\mu$M at 0.5 h for famciclovir, respectively, and the mean AUC values for penciclovir were 57.5 $\mu$M h for SK1899 and 54.5 $\mu$M h for famciclovir, respectively (Table 1). M4 showed higher plasma concentrations than penciclovir up to 0.5 h after dosing of both SK1899 and famciclovir (Fig. 2A), but the concentration of M4 decreased more rapidly than that of penciclovir with a mean elimination half-life ($t_{1/2}$) of 0.3 h. The 10-fold increase in dose of SK1899 from 0.2 to 2 mmol/kg led to only approximately 3- and 6-fold increase in the mean $C_{\text{max}}$ and AUC values for penciclovir, respectively, whereas the corresponding values for M4 increased by approximately 6- and 19-fold, respectively. At a 0.2-mmol/kg dose of SK1899 and famciclovir, penciclovir represented 36.1 and 35.5% of dose in 0- to 24-h urine samples, respectively, whereas M4 represented 33.3 and 24.0% of dose, respectively (Table 1). At a 0.034-mmol/kg dose of SK1899, M2 was the only metabolite detected in dog plasma, except for penciclovir and M4 (data not shown). After dosing with 0.68 mmol/kg, all of the postulated metabolites were detected, but the plasma concentrations of M1, M2, M3, and M5 were significantly lower than those of penciclovir and M4. At both dose levels, M4 was the major intermediate metabolite in plasma, which was then oxidized to penciclovir as shown in the rat (Fig. 2B). The parent compound SK1899 was not detected at all time points. Following oral dosing of 0.034 mmol/kg SK1899 and famciclovir, penciclovir represented 36.3 and 38.0% of dose in 0- to 24-h urine samples, respectively, whereas M4 represented 33.3 and 30.2% of dose, respectively (Table 1). With increase in dose of SK1899 from 0.034 to 0.68 mmol/kg, the mean 24-h urinary recovery of penciclovir decreased to 14.1%, whereas that of M4 increased to 52.8% of dose.

**Placental Transfer.** After oral administration of a single dose of [14C]SK1899 (0.2 mmol/kg) to pregnant rats, concentrations of radioactivity in maternal plasma, fetus, placenta, and amniotic fluid are

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rat</th>
<th>SK1899</th>
<th>Dog</th>
<th>SK1899</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>Penciclovir</td>
<td>0.5 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>M4</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>$C_{\text{max}}$ ($\mu$M)</td>
<td>Penciclovir</td>
<td>34.0 ± 3.8</td>
<td>39.5 ± 5.2</td>
<td>113.1 ± 17.5</td>
</tr>
<tr>
<td></td>
<td>M4</td>
<td>46.8 ± 10.6</td>
<td>49.9 ± 5.4</td>
<td>302.8 ± 73.1</td>
</tr>
<tr>
<td>AUC_{24-h} (µM h)</td>
<td>Penciclovir</td>
<td>54.5 ± 6.2</td>
<td>57.5 ± 7.7</td>
<td>369.3 ± 83.6</td>
</tr>
<tr>
<td></td>
<td>M4</td>
<td>36.7 ± 3.5</td>
<td>36.6 ± 1.9</td>
<td>682.2 ± 154.4</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>Penciclovir</td>
<td>0.9 ± 0.1</td>
<td>0.3 ± 0.0</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>M4</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.0</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Excretion (% dose)</td>
<td>Penciclovir</td>
<td>35.5 ± 0.7</td>
<td>14.1 ± 0.3</td>
<td>35.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>M4</td>
<td>24.0 ± 3.3</td>
<td>26.5 ± 2.4</td>
<td>34.0 ± 2.3</td>
</tr>
</tbody>
</table>

*Urine was collected for 24 h after dosing and analyzed by HPLC.
*SK1899 was administered at 0.2 or 2 mmol/kg to rats and 0.034 or 0.68 mmol/kg to dogs.
*Penciclovir was administered at 0.2 and 0.034 mmol/kg to rats and dogs, respectively.
*Each value represents the mean ± S.D. of four animals for the rat and three animals for the dog.
shown in Fig. 3. The highest concentrations of radioactivity for plasma and amniotic fluid were observed at 0.5 h, with values of 40.9 and 4.6 μg/ml, respectively, and those for fetus and placenta were found at 2 h, with values of 10.4 and 24.8 μg/ml, respectively. The concentration of radioactivity in the fetus exceeded that in plasma at 6 h with fetus-to-plasma concentration ratio of 2.17; however, this ratio had declined to 0.01 at 24 h.

**Excretion in Milk.** After oral administration of a single dose of [14C]SK1899 (0.2 mmol/kg) to lactating rats, concentrations of radioactivity in plasma and milk were determined (Fig. 3). The highest concentrations of radioactivity were observed at 0.5 h in plasma (39.8 μg/ml) and at 2 h in milk (226.8 μg/ml), respectively. Concentrations of radioactivity in milk exceeded those in plasma at all measured time points. However, the milk-to-plasma concentration ratio decreased from 32.7 at 4 h to 5.7 at 24 h with rapid elimination of radioactivity in milk.

**Discussion**

The early onset of the mean C_max for the two major metabolites, penciclovir and M4, and their rate and extent of excretion in the urine indicated that SK1899 was rapidly absorbed from the gastrointestinal tract and extensively converted to its metabolites following oral administration to the rat and dog at doses up to 2 and 0.68 mmol/kg, respectively. However, the parent compound SK1899 was not detected in either plasma or urine, indicating that substantial first-pass metabolism of SK1899 occurred in both rats and dogs.

Following oral administration of SK1899 to both species, the ratios of the mean C_max and AUC values for penciclovir to the corresponding values for M4 in plasma and the ratio of penciclovir to M4 concentrations excreted in urine decreased with increasing dose, suggesting that a dose-dependent decrease in the conversion of SK1899 to penciclovir occurred. In the rat, a 10-fold increase in dose from 0.2 to 2 mmol/kg led to a decrease in the ratios of penciclovir to M4 concentrations in the mean C_max values from 0.15 to 0.05 and in the mean AUC values from 0.43 to 0.11 in plasma, respectively. The ratio of penciclovir to M4 concentrations in urine also decreased from 1.09 to 0.27. These results suggest that the rate-determining step in the conversion of SK1899 to penciclovir was oxidation of the intermediate metabolite M4 at the 6-position, as shown in famciclovir (Vere Hodge et al., 1989).

Recent studies reported that aldehyde oxidase is the major enzyme involved in the oxidation of M4 to penciclovir in the rat (Rashidi et al., 1997). In the dog, however, aldehyde oxidase activity was consistently minimal (Beedham, 1987), which may explain why the efficiency in the conversion of M4 to penciclovir is lower in dogs than in rats. It is possible that xanthine oxidase, which has been found in dog liver at high levels, may play a role in the oxidation of M4 (Beedham, 1987). In humans, aldehyde oxidase rapidly and efficiently catalyzed the metabolic conversion of M4 to penciclovir (Clarke et al., 1995; Rashidi et al., 1997), suggesting that SK1899 will be efficiently converted to the active metabolite penciclovir when orally administered to human.

Penetration of radioactivity from [14C]SK1899 into fetus occurred through the placental barrier in pregnant rats with a delay in T_max in fetus, compared with that in maternal plasma. The concentration of radioactivity in fetus exceeded that in maternal plasma at 6 h and then decreased rapidly, indicating that the rate and extent of transfer to fetus are not so high after oral dose of SK1899 to pregnant rats.

It was evident that a considerable amount of [14C]SK1899 was transferred to the milk of lactating rats. The milk-to-plasma concentration ratio ranged from 2.1 to 32.7 after an oral dose of [14C]SK1899. An active or facilitated transport process might be involved in the excretion of SK1899 in milk as in the case of acyclovir, the nucleoside analog antiviral agent (Lau et al., 1987). To summarize, SK1899 was quickly absorbed and extensively converted to the active metabolite penciclovir after oral administration to both rats and dogs. Placental transfer of [14C]SK1899 was limited, and no fetal accumulation of radioactivity occurred. Although there was substantial transfer of [14C]SK1899 to the milk, the concentration of radioactivity declined rapidly. For comparison, famciclovir was administered orally to both species at equimolar doses of SK1899. The mean C_max values for penciclovir in the rat and the mean AUC values for penciclovir both in the rat and dog after oral dose of SK1899 were slightly higher than those observed after oral dose of famciclovir. However, no statistically significant differences between SK1899 and famciclovir were found, indicating that introduction of an isoproxy carbonate group into one of the two hydroxyl groups of M4 did not significantly alter the oral bioavailability of penciclovir compared with famciclovir in rats and dogs.

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**Fig. 3.** Mean concentrations of radioactivity in maternal plasma, amniotic fluid, fetus, and placenta from pregnant rats and plasma and milk from lactating rats following single oral administration of 0.2 mmol/kg [14C]SK1899 (n = 3).

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0.68 mmol/kg led to a decrease in the ratios of penciclovir to M4 concentrations in the mean C_max values from 0.15 to 0.05 and in the mean AUC values from 0.43 to 0.11 in plasma, respectively. The ratio of penciclovir to M4 concentrations in urine also decreased from 1.09 to 0.27. These results suggest that the rate-determining step in the conversion of SK1899 to penciclovir was oxidation of the intermediate metabolite M4 at the 6-position, as shown in famciclovir (Vere Hodge et al., 1989).

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To summarize, SK1899 was quickly absorbed and extensively converted to the active metabolite penciclovir after oral administration to both rats and dogs. Placental transfer of [14C]SK1899 was limited, and no fetal accumulation of radioactivity occurred. Although there was substantial transfer of [14C]SK1899 to the milk, the concentration of radioactivity declined rapidly. For comparison, famciclovir was administered orally to both species at equimolar doses of SK1899. The mean C_max values for penciclovir in the rat and the mean AUC values for penciclovir both in the rat and dog after oral dose of SK1899 were slightly higher than those observed after oral dose of famciclovir. However, no statistically significant differences between SK1899 and famciclovir were found, indicating that introduction of an isoproxy carbonate group into one of the two hydroxyl groups of M4 did not significantly alter the oral bioavailability of penciclovir compared with famciclovir in rats and dogs.

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