Enhanced Elimination of Theophylline, Phenobarbital and Strychnine from the Bodies of Rats and Mice by Squalane Treatment

Hidetoshi KAMIMURA,* Nobuyuki KOGA,** Kazuta OGURI,*** and Hidetoshi YOSHIMURA**

Faculty of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

(Received October 29, 1991)

Our previous study suggested that squalane would be a good candidate for an antidote to reduce the toxicity of drug ingested accidentally at a high dose by enhancing the drug elimination from the body. In the present study, we investigated whether squalane given orally could enhance the elimination of theophylline, phenobarbital and strychnine which were administered parenterally to rats or mice. Squalane increased the fecal excretion of theophylline and reduced the serum level of the drug in rats. Squalane accelerated the fecal excretion of strychnine in mice. These results suggest that squalane may stimulate more the elimination of neutral (theophylline) or basic (strychnine) drugs which should be present in unionized form in intestinal lumen, than that of acidic drugs.

Keywords — squalane; theophylline, phenobarbital; strychnine; enhanced fecal excretion; antidote; rats; mouse; serum level; pharmacokinetic parameter

Introduction

At present, numerous toxic chemicals including drugs, pesticides and industrial substances are available even at home, so that there are apprehensions of occurring accidents by misuse of these chemicals. In the case of acute chemical intoxication, it is important to remove the causal agent from the body as rapidly as possible. Since most of the chemicals and their metabolites are excreted into urine and/or feces, a substance which can stimulate the excretion of the toxic compounds, has been desired for treatment of the toxicity.

Previously, we found that squalane (2,6,10,15,19,23-hexamethyldotetracosane) stimulated the fecal excretion of 2,3,4,7,8-pentachlorodibenzofuran (PenCDF), the most important causal agent of Yusho disease, and improved its toxic signs (fatty liver and thymus atrophy) in rats.1,2) PenCDF is considered to be eliminated by exsorption through the intestinal wall into the lumen,3) and squalane is known to be absorbed little from the gastrointestinal tract in a single4) and a long-term treatment.5) Therefore, it is considered that the stimulating effect of squalane on fecal excretion of PenCDF is due to inhibition of the re-absorption of PenCDF, which is eliminated into the intestinal lumen.6)

Besides PenCDF, some drugs such as theophylline,7,8) and phenobarbital7) have been reported to be transported to the intestinal lumen from blood after intravenous administration in rats. In addition, we recently demonstrated that a part of strychnine given subcutaneously is excreted into the feces of rats.8)

Theophylline and phenobarbital have been used as antiasthmatic and antiepileptic drugs, respectively. However, their therapeutic dose ranges are narrow and their adverse effects are severe. Strychnine is a potent central nervous stimulant, and this alkaloid has been recently tried for children with nonketotic hyperglycinemia.9) In addition to these therapeutic applications, strychnine is also used as a poison for killing rodents because of its high toxicity.

The present study was undertaken to learn whether squalane could enhance the fecal excretion of theophylline, phenobarbital and strychnine, which were injected intravenously to rats.

* Present address: Laboratory of Safety Assessment, Panapharm Laboratories, Co. Ltd., Uto., Kumamoto 869-04, Japan.
** Present address: Department of Food and Nutrition, Nakamura Gakuen College, 5-7-1 Befu, Johnan-ku, Fukuoka, 814-01, Japan.
*** To whom correspondence should be addressed.
and subcutaneously to mice.

**Materials and Methods**

**Chemicals** — Theophylline, phenobarbital-sodium and strychnine nitrate were purchased from Wako Pure Chemical Industries (Osaka, Japan), Yoneyama Chemical Industries Ltd. (Osaka, Japan) and Kanto Chemical Co. (Tokyo, Japan), respectively. Squalane was kindly supplied from Nippon Petrochemicals Co. Ltd. (Tokyo, Japan). Other reagents were of highest purity commercially available.

**Animal Treatments** — Male Wistar rats and male ddY mice (7 weeks old, approximately 250 and 25 g, respectively) were purchased from Kyudo Co. (Tosu, Japan). Eight rats were used for the experiments with theophylline and phenobarbital. Twelve mice were used for the strychnine experiment. Rats were housed in individual metabolism cages which allowed separate collection of urine and feces. Three mice were housed in one metabolism cage. Rats and mice were fed *ad libitum*. The animals were randomly divided into 2 groups, each of which consisted of 4 rats or 6 mice. Theophylline or phenobarbital-sodium were dissolved in saline (20 mg/ml) and were injected intravenously at a dose of 20 mg/kg through the tail vein of rats. Squalane was administered orally at an initial dose of 250 mg/body 2 h after theophylline dosing, and at additional doses of 200 mg/body after 4, 6, 8, 10 h. In the case of phenobarbital, squalane was administered orally at doses of 200 mg/body at 0, 2, 4, 6, 8 h after phenobarbital injection. Blood samples were collected at the times indicated from the tail vein of the rats anesthetized by ether. Strychnine nitrate was dissolved in saline (0.125 mg/ml) and was given at a dose of 0.5 mg/kg subcutaneously in mice. Squalane was administered at an oral dose of 200 mg/body immediately after strychnine dosing. Urine and feces of the animals were collected for 24 h after injection of the test drugs and were stored at −20 °C until the chemical analysis. The feces were dried in a desiccator on silica gel bags and pulverized with an electric coffee mill before extraction.

**Determination of Theophylline** — Theophylline in serum, urine and feces was extracted by the method of Arimori and Nakano and determined by high performance liquid chromatography (HPLC). HPLC was performed by use of a Radial Pak µBondapak C₁₈ column (8 mm × 10 cm, particle size 10 μm, Waters Assoc.) and a mobile phase consisting of 10% (v/v) acetonitrile (CH₃CN) in water with a flow rate of 1.5 ml/min. Theophylline was detected using absorbance at 270 nm. Mean recoveries of theophylline in serum, urine and feces by the method described above, were about 98%.

**Determination of Phenobarbital** — Phenobarbital in serum and urine was extracted with ethylacetate (AcOEt) after addition of an equal volume of 1 N HCl to each sample. The solvent was evaporated *in vacuo* from the extract, and the residues obtained from serum and urine were dissolved in 30% and 20% CH₃CN, respectively. Feces were dried over a desiccator and shook with 1 N HCl and AcOEt for 1 h. After centrifugation, the solvent was evaporated *in vacuo* from the extract. The residue was dissolved in 30% CH₃CN and this solution was then applied to a Sep-Pak C₁₈ cartridge (Waters Assoc.). After washing the cartridge with 0.1 M acetate buffer (pH 4.0), phenobarbital was eluted with 30% CH₃CN and determined by HPLC. HPLC was performed by use of a Nova pak C₁₈ column (8 mm × 10 cm, particle size 4 μm, Waters Assoc.) and a mobile phase consisting of 30% CH₃CN for serum samples or of 20% CH₃CN for urinary and fecal samples with a flow rate of 1.5 ml/min. Phenobarbital was detected using absorbance at 220 nm. Mean recoveries of phenobarbital from serum, urine and feces by the method described above, were 96.1%, 99.5% and 103.7%, respectively. Variance of the determination was within 5%.

**Determination of Strychnine** — Urine was diluted with 3 volumes of 0.5 M ammonium sulfate ((NH₄)₂SO₄) buffer (pH 9.3), and then applied to a Sep-Pak C₁₈ cartridge. The cartridge was washed successively with 5 mM (NH₄)₂SO₄ buffer (pH 9.3), H₂O and 75% methanol (MeOH). Strychnine in the cartridge was eluted with MeOH. To this MeOH eluate was added an equal volume of 0.5 M (NH₄)₂SO₄ buffer. It was then shaked with chloroform (CHCl₃). The
CHCl₃ was evaporated from the extract and the residue was dissolved in the mobile phase of HPLC. Feces were dried over a desiccator and homogenized with MeOH. It was allowed to stand for 1 h and then centrifuged at 2500 r.p.m for 30 min. The MeOH solution was taken and evaporated in vacuo. The residue was dissolved in 0.5 M (NH₄)₂SO₄ buffer−MeOH (7 : 3, v/v). This solution was applied to a Sep-Pak C₁₈ cartridge. After washing the cartridge similarly as that for the urinary sample, strychnine in the cartridge was eluted with 95% MeOH and the eluate was submitted to HPLC. HPLC was performed by use of a Nova pak C₁₈ column and a mobile phase consisting of 30% CH₃CN in 10 mM sodium phosphate buffer (pH 2.5) with flow rates of 1.5 ml/min and 1.0 ml/min for urinary and fecal samples, respectively. Strychnine was detected using absorbance at 254 nm. Mean recoveries of strychnine in both urine and feces by the method described above, were about 80%. Variance of the determination was within 5%.

**Pharmacokinetic Analysis** — The one- and two-compartment models were used for pharmacokinetic analysis of theophylline and phenobarbital, respectively. The Eq. (1) was fitted to the serum theophylline concentrations, using nonlinear least squares program, MULTI,¹¹ on a personal computer, PC8801 mkiISR (Nippon Electric Company Co., Tokyo, Japan):

\[ C = C₀e^{−kₑt} \]  \hspace{1cm} (1)

where \( C \) is the serum theophylline concentration at time \( t \) after dosing, \( C₀ \) is the extrapolated initial serum concentration at the end of the rapid infusion, and \( kₑ \) is the elimination rate constant. The total body clearance (\( Cl \)), the serum half-life \( (t₁/₂) \), and the area under serum concentration-time curve was extrapolated to time infinity \( (AUC) \) were calculated by means of the following Eq. (2—5):

\[ V_d = D/C₀ \]  \hspace{1cm} (2)

\[ t₁/₂ = 0.693/kₑ \]  \hspace{1cm} (3)

\[ Cl = kₑ·V_d \]  \hspace{1cm} (4)

\[ AUC = D/C₀ \]  \hspace{1cm} (5)

where \( V_d \) is the apparent volume of distribution, and \( D \) is the dose.

Bi-exponential Eq. (6) was fitted to the serum phenobarbital concentrations by using MULTI:

\[ C = Ae^{−αt} + Be^{−βt} \]  \hspace{1cm} (6)

where \( C \) is the serum phenobarbital concentration at time \( t \) after dosing, and \( A, B, α, β \) are constants. The pharmacokinetic parameters were calculated by Eq. (7—14).

\[ t₁/₂ = 0.693/β \]  \hspace{1cm} (7)

\[ V₁ = D/(A + B) \]  \hspace{1cm} (8)

\[ k₁₂ = (A·β + B·α)/(A + B) \]  \hspace{1cm} (9)

\[ kₑ = α·β/k₂₁ \]  \hspace{1cm} (10)

\[ k₁₂ = α·β/k₂₁ \]  \hspace{1cm} (11)

\[ Vₚ = V₁(1 + k₁₂/k₂₁) \]  \hspace{1cm} (12)

\[ AUC = A/α + B/β \]  \hspace{1cm} (13)

\[ Cl = D/AUC \]  \hspace{1cm} (14)

where \( t₁/₂ \) is the elimination half-life, \( V₁ \) is the apparent volume of central compartment, \( D \) is the dose, \( Vₚ \) is the apparent volume of distri-

---

**Fig. 1. Effect of Squalane on Serum Theophylline Levels at an Intravenous Dose of 20 mg/kg in Rats**

See Materials and Methods for squalane treatment. ○, control; ●, squalane treatment. Each point represents the mean ± S.E. of 4 rats. a) Significantly different from control \( (p<0.05) \).
Fig. 2. Effect of Squalane on Serum Phenobarbital Levels at an Intravenous Dose of 20 mg/kg in Rats

See Materials and Methods for squalane treatment. Each point represents the mean ± S. E. of 4 rats. ○, control; ●, squalane treatment. a) Significantly different from control (p<0.05).

bution, \( k_{12} \) is the rate constant for phenobarbital transfer from central to tissue compartment, \( k_{21} \) is the rate constant for the drug transfer from tissue to central compartment, and \( k_e \) is the elimination rate constant.

### Table I. Effect of Squalane on Pharmacokinetic Parameters of Theophylline in Rats by 1-Compartment Model

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Squalane</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>5.41 ± 0.34</td>
<td>2.69 ± 0.08</td>
</tr>
<tr>
<td>( V_d ) (ml/kg)</td>
<td>355 ± 31</td>
<td>368 ± 17</td>
</tr>
<tr>
<td>( K_e ) (h(^{-1}))</td>
<td>0.128 ± 0.008</td>
<td>0.258 ± 0.008</td>
</tr>
<tr>
<td>( CL ) (ml/h/kg)</td>
<td>58.3 ± 3.9</td>
<td>95.0 ± 4.4</td>
</tr>
<tr>
<td>( AUC ) (h·μg/ml)</td>
<td>342 ± 51</td>
<td>212 ± 10</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S. E. of 4 rats. a) Significantly different from control (p<0.05). Theophylline was given intravenously to rats at a dose of 20 mg/kg. See Materials and Methods for squalane treatment.

### Table II. Effect of Squalane on Pharmacokinetic Parameters of Phenobarbital in Rats by 2-Compartment Model

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Squalane</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>8.45 ± 1.44</td>
<td>4.65 ± 0.73</td>
</tr>
<tr>
<td>( V_1 ) (ml/kg)</td>
<td>133 ± 5</td>
<td>112 ± 19</td>
</tr>
<tr>
<td>( V_m ) (ml/kg)</td>
<td>590 ± 125</td>
<td>279 ± 33</td>
</tr>
<tr>
<td>( k_{12} ) (h(^{-1}))</td>
<td>0.677 ± 0.065</td>
<td>0.546 ± 0.180</td>
</tr>
<tr>
<td>( k_{21} ) (h(^{-1}))</td>
<td>0.232 ± 0.033</td>
<td>0.307 ± 0.061</td>
</tr>
<tr>
<td>( k_e ) (h(^{-1}))</td>
<td>0.322 ± 0.071</td>
<td>0.600 ± 0.074</td>
</tr>
<tr>
<td>( CL ) (ml/h/kg)</td>
<td>43.1 ± 9.6</td>
<td>67.3 ± 5.1</td>
</tr>
<tr>
<td>( AUC ) (h·μg/ml)</td>
<td>394 ± 44</td>
<td>325 ± 25</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S. E. of 4 rats. a) Significantly different from control (p<0.05). Phenobarbital was given intravenously to rats at a dose of 20 mg/kg. See Materials and Methods for squalane treatment.

### Results

Figures 1 and 2 show the time course of the serum theophylline and phenobarbital level, respectively, after intravenous administration to rats with or without oral squalane treatment. Since the therapeutic serum ranges of theophylline and phenobarbital for humans are from 5 to 20 μg/ml\(^{12}\) and from 10 to 30 μg/ml\(^{13}\), respectively, the serum drug concentration from 0 to 6 h after theophylline dosing (Fig. 1) and from 0 to 2 h after phenobarbital dosing (Fig. 2) reached the toxic levels for humans. In these cases, squalane treatment reduced the serum levels of both theophylline and phenobarbital, although the effect of squalane on phenobarbital was less remarkable than that on theophylline.

Pharmacokinetic parameters are shown in Table I and II for theophylline and phenobarbital, respectively. Squalane shortened the serum half-life (\( t_{1/2} \)) of theophylline from 5.41 to 2.69 h, and also decreased the \( AUC \) by about 62% of the control. In the case of phenobarbital, squa-
TABLE III. Stimulating Effects of Squalane on Urinary and Fecal Excretion of Drugs (% of Dose) for 24 h after Their Administration

<table>
<thead>
<tr>
<th></th>
<th>Phenobarbital</th>
<th>Theophylline</th>
<th>Strychnine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16.90 ± 4.70</td>
<td>11.73 ± 2.27</td>
<td>6.11 ± 1.99</td>
</tr>
<tr>
<td></td>
<td>(1.0)</td>
<td>(1.0)</td>
<td>(1.0)</td>
</tr>
<tr>
<td>Squalane</td>
<td>20.15 ± 3.66</td>
<td>10.13 ± 1.13</td>
<td>14.12 ± 2.22(^a)</td>
</tr>
<tr>
<td></td>
<td>(1.2)</td>
<td>(0.9)</td>
<td>(2.3)</td>
</tr>
<tr>
<td><strong>Feces</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.61 ± 0.38</td>
<td>3.52 ± 0.87</td>
<td>4.18 ± 1.01</td>
</tr>
<tr>
<td></td>
<td>(1.0)</td>
<td>(1.0)</td>
<td>(1.0)</td>
</tr>
<tr>
<td>Squalane</td>
<td>2.26 ± 0.60</td>
<td>9.77 ± 1.60(^a)</td>
<td>14.61 ± 2.72(^a)</td>
</tr>
<tr>
<td></td>
<td>(1.4)</td>
<td>(2.8)</td>
<td>(3.5)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S. E. of 4 rats (phenobarbital and theophylline) or 4 groups of 12 mice (strychnine). Figure in parentheses is a relative vs control value of the drug excreted into urine or feces. Phenobarbital and theophylline were given to rats intravenously at a dose of 20 mg/kg, and strychnine was given to mice subcutaneously at a dose of 0.5 mg/kg. \(^a\) Significantly different from control (p<0.05). See Materials and Methods for squalane treatment.

Squalane showed a tendency to reduce the serum half-life \((t_{1/2})\) and AUC, but not significantly. The total body clearances (Cl) of theophylline and phenobarbital were increased significantly by squalane.

Table III shows the cumulative urinary and fecal excretion of the drugs tested during 24 h after administration. In the present study, it was clear that theophylline and phenobarbital were excreted not only into urine but also into feces. It has been known that these drugs in feces were mainly transported through the intestinal wall by the exosorption and in a lesser amount through the bile.\(^5\)-\(^7\) Strychnine was also excreted into feces of mice as well as of rats.\(^8\) Squalane stimulated the fecal excretion of theophylline and strychnine from 3.5 to 9.8% and from 4.2 to 14.6% of dose, respectively, as compared with the control groups. In addition, the urinary excretion of strychnine was enhanced from 6.1 to 14.1% of dose by squalane treatment.

**Discussion**

In a case of drug intoxication, activated charcoal and cathartics are frequently used for patients to minimize the absorption of and to quicken the excretion of the causal agents in the gastrointestinal tract. However, there is a fear that cathartic-induced diaphoresis results in fluid electrolyte imbalance, particularly in children.\(^14\) Activated charcoal itself has no toxicity but absorbs various materials without a high selectivity. Therefore, we have tried to find a more selective antidote for toxic xenobiotics.

In the present study, squalane stimulated the fecal excretion of theophylline and reduced the serum theophylline level in rats (Fig. 1 and Table III). From the pharmacokinetic analysis (Table I), the half-life and AUC of theophylline was reduced by squalane treatment. This was considered to reflect the stimulating effect of squalane on the fecal excretion of theophylline. On the other hand, the half-life and AUC of phenobarbital were not significantly decreased by squalane treatment. At least two reasons were considered for this difference of the effect. One of the reasons is the different ability between the two drugs to transport into the intestinal lumen. Arimori and Nakano demonstrated, using an in situ single-pass perfusion technique, that the amounts of phenobarbital, exsorbed into the perfusate and excreted into the bile, were 6—6.5% and 0.45—0.5% of dose, respectively, for 2 h after the intravenous administration to rats.\(^7\) They also reported that 12—15% of theophylline dosed intravenously was exorhed into the perfusate and 0.17—0.3% of dose was excreted into the bile of rats.\(^5\),\(^6\) In the present study, the fecal excretion of phenobarbital and theophyll-
line in the control group was 1.6% and 3.5%, respectively (Table III). Thus the amount of phenobarbital transported into the intestinal lumen was not larger than that of theophylline.

Another reason was the difference of forms in the intestinal lumen between the drugs. The $pK_a$ values of phenobarbital and theophylline are 7.41 and 8.75, respectively. At the pH of intestinal fluid, more than half of phenobarbital in the intestine exists as the ionized form, while theophylline is present as almost all of the un-ionized form. Since squalane is a nonpolar substance, this hydrocarbon has a higher affinity for unionized drugs.

Recently, it was reported that the anion exchange resin, cholestyramine, accelerated the elimination of the plasma tenoxicam (4-hydroxy-N-[2-pyridyl]-2-methyl-2H-thieno-[2,3e]-1,2-thiazine-3-carboxamine-1,1-dioxide), a new nonsteroidal anti-inflammatory drug after the intravenous administration in dogs.\(^ {15}\) This report also demonstrated that cholestyramine was more effective in the elimination of tenoxicam than activated charcoal. Since the $pK_a$ value of tenoxicam is 5.3, most of the drug exists as anionic form in the intestine. Therefore, cholestyramine adsorbs effectively the anionic form of tenoxicam exsorbed into the intestinal lumen. Based on this finding, cholestyramine may be valuable as a specific antidote for phenobarbital.

The fecal excretion of strychnine in the control group was 4.2% of dose and this value was highest among the drugs tested in the present study. Since the $pK_a$ value of strychnine is 6.0, most of strychnine may exist as unionized form in the intestinal lumen. Therefore, squalane stimulated effectively the fecal excretion of strychnine as well as theophylline.

The urinary excretion of strychnine was also increased from 6.1 to 14.1% of dose by squalane treatment. Squalane cannot affect directly the urinary excretion of strychnine, because squalane is difficultly absorbable from the rat intestine,\(^ {13,15}\) and the mechanism for this effect of squalane is not clear at present. The present study also suggested that squalane may be a good antidote for treatment of intoxications caused by some drugs such as phenytoin,\(^ {16}\) procainamide, $N$-acetylprocainamide\(^ {17}\) and others,\(^ {18}\) which exsorb into the intestinal lumen or excrete in the feces.

References


