Structure-Related Pharmacokinetics of Xanthines after Direct Administration into the Peritoneal Cavity of Rats

Takafumi KUZUYA, Takaaki HASEGAWA, Rika SHIRAKI, and Toshitaka NABESIMA

Department of Hospital Pharmacy, Nagoya University School of Medicine, 65 Tsurumachi-cho, Showa-ku, Nagoya 466, Japan. Received February 21, 1997; accepted July 3, 1997

The pharmacokinetic characteristics, peritoneal permeability and hydrophobicity of three xanthine derivatives, theophylline, enprofylline and 1-methyl-3-propylxanthine (MPX), were investigated in rats. Isotonic saline (30 ml) containing xanthine (2.5, 5 and 10 mg/kg) and blue dextran (0.2%) was administered intraperitoneally. The pharmacokinetic parameters of these xanthines were estimated using concentration-time data obtained from the peritoneal cavity and systemic circulation. Disappearance of these xanthines from the peritoneum declined in almost a monoeponential manner regardless of the dose administered. The volume of distribution (33.9 ml) in the peritoneal cavity was similar to the injection volume, indicating that dialysate was not diluted by the fluid in the peritoneal cavity and the effect of drug adsorption on the peritoneal membrane was minimal. The pharmacokinetics of MPX was dose-dependent, but that of theophylline and enprofylline was not. The fraction of the administered dose absorbed through the peritoneal cavity was 0.71, 0.85, 0.93 for theophylline, enprofylline and MPX, respectively. The peritoneal clearance was significantly different \( (p < 0.05) \) among the three xanthines by two-way analysis of variance, and a strong correlation was noted between their peritoneal clearance and hydrophobicity \( (r = 0.98, p < 0.01) \). These findings suggest that hydrophobicity is an important determinant in the peritoneal permeation of these xanthines.

Key words: xanthines; intraperitoneal administration; peritoneal clearance; hydrophobicity

The peritoneum is commonly identified as an available route for administering medication in animal experiments, and it is used clinically for patients requiring treatment for bacterial peritonitis induced by continuous ambulatory peritoneal dialysis \(^1,2\) and those requiring peritoneal chemotherapy against peritoneal metastasis from gastrointestinal or ovarian cancer. The advantage of being able to directly apply intraperitoneal chemotherapy with such anticancer drugs as 5-fluorouracil, cisplatin or mitomycin C is that there are fewer systemic toxicities and greater effect on the peritoneum. \(^3\) - \(^6\)

Various factors affect the rate or extent of drug absorption following intraperitoneal administration, including molecular weight, the degree of ionization and physicochemical properties of a drug, and the function of the peritoneal membranes involved. Dedrick et al. \(^7\) reported that there is a reverse relationship between the molecular weight and permeability of the peritoneal membranes. Such experimental data suggests that the molecular weight of a drug will determine its transportability through the peritoneal membranes. Our previous paper using 5-fluorouracil and mitomycin C, however, did not support such findings. \(^6\) Our observations suggest that there may be other factors such as hydrophobicity or chemical structures which influence the drug permeability of the peritoneal membranes. Information is limited, however, on the role of the physicochemical properties and chemical structures of drugs in their disposition following peritoneal treatment.

We synthesized various xanthine derivatives to establish more effective bronchodilators that would induce no side effects. Previous studies on the N\(^1\)N\(^3\)-alkylxanthine derivatives demonstrated that their hydrophobicity plays an important role in their biological activities and pharmacokinetics in experimental animals. \(^8\) - \(^10\) These experimental data suggest that these xanthine derivatives are useful model compounds for evaluating the role of physicochemical properties of drugs in the permeability of the peritoneal membranes without the effect of molecular weight. The present study aims to determine the peritoneal permeation characteristics of the three xanthines selected, namely, theophylline, enprofylline and 1-methyl-3-propylxanthine (MPX), as illustrated in Fig. 1, after an intraperitoneal administration in rats and to evaluate the role of their physicochemical properties in intraperitoneal absorption.

MATERIALS AND METHODS

Chemicals The xanthines, MPX and enprofylline, were synthesized in our laboratory and were identical to those used previously. \(^9\) - \(^12\) Theophylline was purchased from Sigma Chemical (St. Louis, MO, U.S.A.). All other reagents were commercially available and of analytical grade. Isotonic saline solution was used as a dialysate.

Fig. 1. Chemical Structures of Theophylline, Enprofylline and 1-Methyl-3-propylxanthine (MPX)

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Animal Experiments Eight-week-old Wistar strain male rats (Japan SLC, Hamamatsu, Japan) weighing 280—320 g (mean: 297.6 g) were used for all experiments. Rats were given free access to food and water. One day before the experiments, the rats were anesthetized with sodium pentobarbital (25 mg/kg), and cannulated in cavity. After fasting overnight with free access to water, dialysate (30 ml) containing each drug (theophylline, enprofylline and MPX) at different doses of 2.5, 5 and 10 mg/kg and 0.2% blue dextran as a volume marker for the dialysate were administered intraperitoneally. The dialysate (0.2 ml) was collected at designated time intervals (just before and 5, 10, 20, 30, 45 and 60 min after the administration). Blood samples of 0.25 ml each were collected at designated intervals after the administration (7.5, 15, 25, 37.5, 52.5, 75 and 105 min for enprofylline and MPX, and thereafter at 135, 165 and 210 min for theophylline). Plasma samples were obtained by centrifugation at 6000 × g for 5 min. The dialysate and plasma samples were stored at −40°C until they could be assayed.

Drug Analysis The HPLC apparatus was a Shimadzu LC-6A system (Shimadzu Co., Kyoto, Japan) consisting of an LC-6A pump, an SPD-6AV UV-VIS spectrophotometric detector and an SII-6A autoinjector. The column was a Cosmosil SC18 packed column (4.6 × 150 mm; Nacalai Tesque, Kyoto, Japan). HPLC conditions and parameters were the same as described previously. Briefly, the mobile phase was a 30 mm phosphate buffer (pH 4.0): methanol (85:15, v/v). The elution was carried out at 40°C, and the effluent column was monitored at 274 nm. Fifty microliter plasma or peritoneal fluid diluted with distilled water and 0.35 ml of the internal standard solution (1-methyl-3-ethylxanthine, 1.0 μg/ml) were centrifuged at 6000 × g for 2 min. The obtained supernatant was then evaporated under a gentle stream of nitrogen at 40°C. The residue was dissolved in 0.2 ml of the mobile phase and the solution was then injected into the column. For calculations, standard curves obtained for plasma and peritoneal fluid were shown to be linear for concentrations ranging from 0.5 to 200 μg/ml for theophylline, from 0.2 to 200 μg/ml for enprofylline and from 0.2 to 200 μg/ml for MPX. The within- and between-day coefficients of variation were less than 5% for all three drugs. The quantitative limit of the assay for each drug was 0.1 μg/ml. No interference peaks from the substances in plasma or dialysate were observed. The concentration of blue dextran in the dialysate was measured by the standard colorimetric method at 620 nm. Drug concentrations in the dialysate were corrected on the basis of decreased percentage.

Data Analysis Assuming that the drug enters the systemic circulation at an apparent first-order rate from the peritoneal cavity following rapid intraperitoneal administration, after which it is metabolized or excreted into the urine through a first-order process, the systemic pharmacokinetic parameters for each xanthine could be calculated using Eq. 1:

\[ C_t = \frac{F \times k_s \times X_0}{V_s \times (k_s - k_e)} (e^{-k_e \cdot t} - e^{-k_s \cdot t}) \]

where \( F \) is the absorption fraction of the administered dose (\( X_0 \)) following the peritoneal administration, \( k_s \) is the apparent first-order absorption rate constant from the peritoneal cavity to the systemic circulation, and \( k_e \) is the apparent first-order elimination rate constant from the systemic circulation. \( V_s \) is the concentration of drug in the systemic circulation at time \( t \). \( V_s \) represents the apparent volume of distribution in the systemic circulation.

The area under the concentration–time curves in the dialysate (\( AUC_s \)) and plasma (\( AUC_p \)) were calculated by the trapezoidal method with extrapolation to infinity. Peritoneal clearance (\( CL_p \)) and systemic clearance (\( CL_s \)) were calculated as \( CL_p = \text{Dose} / AUC_s \) and \( CL_s = \text{Dose} 	imes F / AUC_p \), respectively. The apparent volume of distribution in the peritoneal cavity (\( V_p \)) was calculated by dividing the \( CL_p \) by the apparent first-order elimination rate constant from the peritoneal cavity (\( k_e \)).

All computer analyses were performed by the nonlinear least-squares regression program, MULTI, written by Yamaoka et al. 14

Statistical Analysis Values are expressed as the mean ± S.D. for the indicated number of experiments. Statistical analyses of the pharmacokinetic parameters among the different doses for each drug and xanthine derivative were performed by one-way analysis of variance (ANOVA) and two-way ANOVA, respectively. When statistically significant differences were found, pairwise comparisons were performed by Fisher's test, with \( p < 0.05 \) being the minimum level of significance.

RESULTS

The disappearance curve for peritoneal fluid is shown in Fig. 2. Only approximately 3 ml of the peritoneal fluid was absorbed through the peritoneal cavity in 1 h. Volume changes among the three xanthine derivatives were not significantly different despite the various doses administered (data not shown). Mean semilogarithmic dialysate and plasma concentration–time data for each xanthine are illustrated in Fig. 3. The profiles for theophylline and enprofylline declining from both the dialysate and plasma were dose-independent, and their concentration–time data were adequately described as a monoeponential equation. The disappearance of MPX from plasma at doses of 2.5

![Fig. 2. Volume Changes in the Dialysate of the Rat Peritoneal Cavity](image-url)
and 5 mg/kg declined monoexponentially but the dose of 10 mg/kg of MPX was eliminated via capacity limited process. The pharmacokinetic parameters for each xanthine calculated from dialysate concentration–time data are summarized in Table 1. Dose-dependent differences in the $CL_p$ were observed for MPX. No dose-dependent changes in the $V_p$ were seen for any of the xanthines. The pharmacokinetic parameters of $k_p$ and $CL_p$ were significantly different among the three xanthines. The computer-estimated pharmacokinetic parameters for each xanthine from plasma concentration–time data are shown in Table 2. The parameters $(k_a, k_e$ and $V_s/F)$ were deleted for MPX at a dose of 10 mg/kg because the kinetic model could not satisfactorily fit the plasma concentration–time data. No significant differences in the $k_a$, $k_e$ or $V_s/F$ were observed for these xanthines among the various doses. Dose-dependent differences in the $CL_s/F$ were also observed for MPX at a dose of 10 mg/kg.

**DISCUSSION**

This study aimed to identify the contribution of hydrophobicity to the peritoneal permeability of three selected xanthines administered intraperitoneally. We also tried to estimate their pharmacokinetic behaviors in the peritoneal cavity and systemic circulation. Structure-related differences were observed in the systemic bioavailability of these xanthines. The values of
Table 1. Pharmacokinetic Parameters of Xanthine Derivatives in the Peritoneal Cavity after Intraperitoneal Administration in Rats

| Xanthine | Dose (mg/kg) | \( k_\text{p} \)** | \( V_p \)** | \( CL_p \)** | \( AUC_p \)**
<table>
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<tbody>
<tr>
<td>Theophylline</td>
<td>2.5</td>
<td>1.93 ± 0.13</td>
<td>0.118 ± 0.011</td>
<td>0.225 ± 0.023</td>
<td>11.2 ± 1.2</td>
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<td></td>
<td>5</td>
<td>1.95 ± 0.18</td>
<td>0.113 ± 0.004</td>
<td>0.219 ± 0.024</td>
<td>23.0 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.90 ± 0.32</td>
<td>0.124 ± 0.014</td>
<td>0.238 ± 0.012</td>
<td>42.1 ± 2.1</td>
</tr>
<tr>
<td>Enprofylline</td>
<td>2.5</td>
<td>2.35 ± 0.20</td>
<td>0.105 ± 0.020</td>
<td>0.251 ± 0.041</td>
<td>10.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.42 ± 0.32</td>
<td>0.114 ± 0.015</td>
<td>0.274 ± 0.012</td>
<td>18.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.27 ± 0.21</td>
<td>0.120 ± 0.022</td>
<td>0.272 ± 0.022</td>
<td>37.9 ± 2.9</td>
</tr>
<tr>
<td>MPX</td>
<td>2.5</td>
<td>4.05 ± 0.60</td>
<td>0.118 ± 0.027</td>
<td>0.474 ± 0.063</td>
<td>5.30 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.22 ± 0.42</td>
<td>0.119 ± 0.022</td>
<td>0.503 ± 0.042</td>
<td>9.99 ± 0.79</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4.20 ± 0.96</td>
<td>0.095 ± 0.015</td>
<td>0.402 ± 0.021</td>
<td>24.91 ± 1.20</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D. (n = 4). * Significantly different from 2.5 and 5 mg/kg (p < 0.05). ** Significantly different among the three xanthines by two-way ANOVA (p < 0.05).

Table 2. Pharmacokinetic Parameters of Xanthine Derivatives in the Systemic Circulation after Intraperitoneal Administration in Rats

| Xanthine | Dose (mg/kg) | \( k_{\alpha} \) | \( k_{\beta} \) | \( V/F \) | \( CL_{p,F} \) | \( AUC_{p,F} \)
<table>
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</thead>
<tbody>
<tr>
<td>Theophylline</td>
<td>2.5</td>
<td>1.26 ± 0.04</td>
<td>0.235 ± 0.038</td>
<td>0.858 ± 0.057</td>
<td>0.221 ± 0.039</td>
<td>11.38 ± 1.57</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.59 ± 0.20</td>
<td>0.243 ± 0.046</td>
<td>0.855 ± 0.054</td>
<td>0.196 ± 0.030</td>
<td>25.49 ± 1.15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.28 ± 0.12</td>
<td>0.233 ± 0.036</td>
<td>0.857 ± 0.066</td>
<td>0.206 ± 0.031</td>
<td>48.74 ± 2.36</td>
</tr>
<tr>
<td>Enprofylline</td>
<td>2.5</td>
<td>1.81 ± 0.37</td>
<td>1.91 ± 0.07</td>
<td>0.756 ± 0.084</td>
<td>1.355 ± 0.116</td>
<td>18.4 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.11 ± 0.39</td>
<td>1.95 ± 0.09</td>
<td>0.773 ± 0.102</td>
<td>1.377 ± 0.106</td>
<td>3.63 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.13 ± 0.21</td>
<td>1.90 ± 0.36</td>
<td>0.725 ± 0.091</td>
<td>1.314 ± 0.234</td>
<td>7.70 ± 0.83</td>
</tr>
<tr>
<td>MPX</td>
<td>2.5</td>
<td>4.39 ± 0.32</td>
<td>1.02 ± 0.11</td>
<td>0.292 ± 0.034</td>
<td>0.326 ± 0.031</td>
<td>7.72 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.31 ± 0.62</td>
<td>1.05 ± 0.13</td>
<td>0.284 ± 0.018</td>
<td>0.345 ± 0.032</td>
<td>14.59 ± 2.93</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>0.151 ± 0.044</td>
<td>66.65 ± 3.81</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D. (n = 4). * Significantly different from 2.5 and 5 mg/kg (p < 0.05).

\( AUC_p \) ratio of other routes of administration to intravenous administration are usually used to calculate \( F \). In the present study, the \( F \) values for theophylline, enprofylline and MPX, when administered intraperitoneally to doses of 10, 2.5 and 2.5 mg/kg, respectively, could be recalculated from the mean \( AUC_p \) values (68.41, 2.15 and 8.26 \( \mu \)l/h/ml, respectively) as reported previously, and the resulting values were 0.71, 0.86 and 0.93.

Our previous studies showed that approximately 50% of an administered dose of theophylline is metabolized in the liver and that MPX is almost completely metabolized in the liver, whereas enprofylline is mainly excreted in the urine. From the obtained \( F \) value, the after-effect from the peritoneal cavity to the systemic circulation is negligible in spite of the fact that elimination processes are completely different among the xanthine derivatives as described above.

Molecular weight is considered an important factor influencing drug transportability from the peritoneal cavity into the systemic circulation. Experimental evidence has revealed that the peritoneal absorption of drugs with molecular weights ranging from 18 to 2 million decreases as the molecular weight increases. Dedrick et al. also reported an inverse relationship between the \( CL_p \) and molecular weight. Our recent studies on intraperitoneal chemotherapy indicated that \( CL_p \) for 5-fluorouracil (0.83 l/h/m²) is lower than that for mitomycin C (1.77 l/h/m²), while the molecular weight of 5-fluorouracil (130) is smaller than that of mitomycin C (334). The contribution of hydrophobicity on the rate of drug transport through the peritoneal membranes was confirmed by the results of this current study showing that there is a linear logarithmic relationship between the \( CL_p \) of these xanthines and their apparent partition coefficients (\( r = 0.98, p < 0.01 \)). These results indicate that hydrophobicity plays an important role in absorption rate of xanthines through the peritoneal membranes. Similarly, a significant relationship was also found between log \( P \) and log \( k_{\alpha} \) (\( r = 0.89, p < 0.05 \)).

Most drugs generally cross through biological membranes, such as through the small intestine or peritoneal membranes, by intercellular or intracellular transport. The permeability through any intercellular pores depends on the molecular weight of the drug, while intracellular permeability depends on the hydrophobicity or degree of ionization. The xanthines that we studied had similar molecular weights of 180, 194 and 208 for theophylline, enprofylline and MPX, respectively, although each \( CL_p \) for these xanthines differed from others more than two fold. In addition, the \( CL_p \) was correlated significantly with their hydrophobicity (Fig. 4). These observations indicate that the transcellular transport system plays an important role in the xanthine permeability through the peritoneal cavity.

The mean value of \( V_p \) for these xanthines was obtained (0.114/l/kg, \( n = 36 \)), and could be recalculated to 33.9 ml based on the mean body weight of rats (297.6g, \( n = 36 \)), which was nearly equal to the injection volume. These findings suggest that dialysate was not diluted by the fluid in the peritoneal cavity and that the effect of drug ad-
sorption on the peritoneal membrane is minimal.

Since the elimination of MPX from the systemic circulation mostly depends on hepatic metabolism, the dose-dependent characteristics of MPX in the systemic circulation as observed in this study may have been due to the capacity limitation of the metabolism. On the other hand, dose-dependent decreases in the $CL_p$ of MPX may be caused by dose-dependent elimination from the peritoneal cavity, probably due to the limited permeation capacity through the peritoneal membrane or the reverse transport tendencies from the systemic circulation to the peritoneal cavity as a result of increase in the plasma concentration of MPX by a saturable metabolism.

In summary, the results obtained in this study suggest that the $CL_p$ was significantly different among three xanthines, although they have similar molecular weights (180—208), and that xanthine hydrophobicity plays an important role in their peritoneal permeability. These findings provide useful information for the peritoneal treatment of patients in a clinical setting.

REFERENCES


