Studies on Alkyl-Xanthine Derivatives
II. Pharmacokinetic and Pharmacodynamic Studies of a New Bronchodilator, 1-Methyl-3-Propylxanthine (MPX)

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Abstract—A new xanthine derivative bronchodilator, 1-methyl-3-propylxanthine (MPX), and 1-methyl-3-butylxanthine (MBX) were synthesized. We evaluated their relaxant effects on tracheal smooth muscle isolated from guinea pigs and pharmacokinetic characteristics in rats using 1,3-dimethylxanthine (theophylline, TPH) as the reference drug. Dose-dependent relaxant effects were observed in the concentration range of $1 \times 10^{-5}$ to $1 \times 10^{-4}$ M, and both MPX and MBX exert very much stronger relaxant effects than TPH with nearly equal potency. There were significant differences in the pharmacokinetic and physico-chemical properties among these drugs, both MPX and MBX having shorter half-lives, higher plasma protein binding in vivo and stronger hydrophobicity compared to TPH. The present study suggested that the N3-alkyl chain length is significant for increasing the relaxant effect and affecting the pharmacokinetic and physico-chemical properties of these drugs.

It is well-known that 1,3-dimethylxanthine (theophylline, TPH) exhibits a strong bronchial smooth muscle relaxant effect, which is much stronger than those of other known xanthine derivatives such as caffeine and theobromine. Furthermore, TPH is widely used in the treatment of patients with reversible obstructive airway diseases, as its bronchodilatory effect is well established (1, 2).

Recent studies on the relationship between the chemical structure and pharmacological action of xanthine derivatives indicated that alkyl groups of the N1 and N3 positions of the xanthine molecule play an important role in adenosine receptor antagonism and bronchodilatory action, respectively (3). Based on these observations, a new xanthine derivative, 3-propylxanthine (enprofylline), was recently synthesized.

As part of a program of research on bronchodilators, we were interested in synthesizing compounds with stronger relaxant effect by chemical modifications of the xanthine molecule. We reported in the preceding paper the syntheses of five xanthine derivatives by substitution of alkyl groups at the N3 position of the xanthine molecule and a comparative study on their relaxant effects, their inhibitory activities on cyclic AMP phosphodiesterase (PDE) and their pharmacokinetic characteristics. It was clarified that the alkyl chain length at the N3 position of the xanthine molecule plays an important role in the inhibition of PDE in tracheal smooth muscle isolated from guinea pigs, and that the half-lives of these derivatives are likely to be affected by their alkyl chain lengths (4).

In the present study, we synthesized a new xanthine derivative, 1-methyl-3-propylxanthine (MPX), and 1-methyl-3-butylxanthine (MBX) by substitution of an alkyl group at the N3 position of the 1-methylxanthine molecule and the structure-
pharmacokinetic characteristics relationships were studied in rats using TPH as the reference drug.

Materials and Methods

Materials: The N3-alkyl-substituted 1-methylxanthine derivatives, 1-methyl-3-propylxanthine (MPX) and 1-methyl-3-butylxanthine (MBX), were synthesized according to the methods reported previously (5-8). 1,3-Dimethylxanthine (theophylline, TPH) was obtained from Wako Chemical Industries, Ltd., Osaka.

Animals: Male Hartley strain guinea pigs, weighing 230-300 g, and male Wistar strain rats, weighing 250-300 g, were used in this study.

In vitro study: We evaluated the relaxant effects of each compound on the tracheal smooth muscle isolated from guinea pigs. The test was performed using an organ bath method, which was essentially the same as that of Kawanishi et al. (9).

In vivo study: One day before the experiment, rats were cannulated in the right jugular vein for blood sampling and drug administration under light anesthesia with sodium pentobarbital. After overnight fasting with free access to water, each drug at a dose of 5 mg/kg was administered intravenously via the jugular vein. Blood samples of about 600 μl each were collected at 10, 20, 30, 60, 90, 120 and 180 min (240 and 300 min for TPH) after the dosing. The plasma samples were obtained by centrifugation at 11,000 rpm for 5 min. Urine samples were also collected over a period of 24 hr after the dosing. The plasma and urine samples obtained were stored at -40°C until analysis.

High-performance liquid chromatography assay (HPLC): The concentration of TPH was determined by the previously reported method (10). The concentrations of MPX and MBX were determined by a modification of the method for determination of TPH, using phenacetin as the internal standard (I.S.). Separation was carried out on a Zorbax ODS (Du Pont Instruments, U.S.A.) with an eluent of 0.01 M sodium acetate buffer solution (pH 4.0)-acetonitrile (82/18 by vol.). The drug concentrations were calculated from their relative peak height ratios based on a standard curve. The typical chromatogram for MPX and MBX is shown in Fig. 1. The detection limits of MPX and MBX in plasma and urine were 0.2 and 0.1 μg/ml, respectively.

Protein binding study: Binding of MPX and MBX to plasma protein was determined by an ultrafiltration technique using a Minicent-30 (Toso Co., Ltd., Tokyo). The protein leakage and the adsorption of drugs to the device or the membrane were negligible. Drugs bound to plasma protein were filtered and the free (unbound) concentrations were measured by HPLC.

Pharmacokinetic analysis: Pharmacokinetic analysis on plasma concentration-time curves for each drug was estimated on the basis of a one-compartment model using a nonlinear least-squares method program, MULTI, writ-

Fig. 1. Chromatogram of 1-methyl-3-propylxanthine (MPX) and 1-methyl-3-butylxanthine (MBX) extracted from rat plasma, with phenacetin as the internal standard (I.S.).
ten by Yamaoka et al. (11). The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity. Total body clearance (Cltot) was determined by \( \text{Cl}_{\text{tot}} = \text{Dose} / \text{AUC} \). The mean residence time (MRT) was calculated by \( \text{MRT} = \text{AUMC} / \text{AUC} \), where AUMC is the area under the first moment curve. The apparent volume of distribution (Vd) was calculated by \( \text{Vd} = \text{Dose} \times \text{MRT} / \text{AUC} \).

**Apparent partition coefficient:** Each drug was dissolved at a concentration of 10 \( \mu \text{g/ml} \) in pH 7.4 phosphate-buffered saline (PBS) solution. Five ml of the PBS solution was added to an equal volume of chloroform and octanol, respectively, and equilibrated at 25°C by consecutive shaking for 2 hr. The concentration of each drug in the aqueous phase was determined by spectrophotometry at 278 nm. The apparent partition coefficient (PC) of each drug was estimated as the ratio of drug concentration in the organic phase to that in the aqueous phase, and hydrophobicity was expressed as log partition coefficient (log PC).

**Statistical analysis:** The results were expressed as the mean±S.D. Statistical analyses were performed with Student’s \( t \)-test.

**Results**

Figure 2 shows the mean dose-response relaxant effect curves of each xanthine derivative on tracheal smooth muscle preparations, and Table 1 lists the EC50 values. In the concentration range from \( 1 \times 10^{-6} \) to \( 1 \times 10^{-4} \) M, a dose-dependent effect was observed in each group. The results show that the relaxant effect of MPX and MBX in vitro were nearly equal, but were 20–30 times more potent than that of TPH.

The partition coefficients (log PC) of each xanthine derivative in chloroform/PBS and octanol/PBS are shown in Table 2. The order of log PC was MBX>MPX>TPH.

Figure 3 compares the mean semilogarithmic plots of the plasma concentration-time of TPH, MPX and MBX after a single intravenous administration at the dose of 5 mg/kg to rats. The mean plasma concentration-time curve of MPX is similar to that of MBX, but both drugs exhibited significantly different elimination patterns from that of TPH. The mean plasma concentrations at 10 min after injection were 17.02 \( \mu \text{g/ml} \) for MPX, 18.68 \( \mu \text{g/ml} \) for MBX, and 13.88 \( \mu \text{g/ml} \) for TPH.

![Fig. 2. Dose-response relationships of xanthine derivatives on resting tone in tracheal smooth muscle preparations isolated from guinea pigs. Key: \( \triangle \), TPH; ■, MPX; □, MBX. Each point represents the mean of 6 determinations.](image-url)
Table 1. Effects of alkylxanthine derivatives on resting tone in guinea pig tracheal smooth muscle preparations

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name\textsuperscript{a}</th>
<th>(R_1)</th>
<th>(R_2)</th>
<th>EC\textsubscript{50} ((\times 10^{-6}) M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPH</td>
<td>(\text{CH}_3)</td>
<td>(\text{CH}_3)</td>
<td>40.00±2.00</td>
</tr>
<tr>
<td></td>
<td>MPX</td>
<td>(\text{CH}_3)</td>
<td>(\text{C}_3\text{H}_7)</td>
<td>1.73±0.17</td>
</tr>
<tr>
<td></td>
<td>MBX</td>
<td>(\text{CH}_3)</td>
<td>(\text{C}_4\text{H}_9)</td>
<td>1.23±0.15</td>
</tr>
</tbody>
</table>

\textsuperscript{a}TPH: Theophylline, MPX: 1-methyl-3-propylxanthine, MBX: 1-methyl-3-butylxanthine.

Table 2. Partition coefficients of xanthine derivatives

<table>
<thead>
<tr>
<th>Xanthine</th>
<th>\text{log PC\textsubscript{CH}\textsuperscript{a}}</th>
<th>\text{log PC\textsubscript{OC}\textsuperscript{b}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPX</td>
<td>0.603</td>
<td>1.022</td>
</tr>
<tr>
<td>MBX</td>
<td>1.230</td>
<td>1.286</td>
</tr>
<tr>
<td>TPH</td>
<td>-0.456</td>
<td>-0.042</td>
</tr>
</tbody>
</table>

Each value represents the mean of three replications. \textsuperscript{a}\text{log partition coefficient between chloroform and pH 7.4 phosphate-buffered saline. \textsuperscript{b}\text{log partition coefficient between octanol and pH 7.4 phosphate-buffered saline.}}

Fig. 3. Mean semilogarithmic plots of plasma concentration-time of respective xanthine derivatives after a single intravenous administration. Key: Same as the legend of Fig. 2. Each point represents the mean±S.D. of 4–5 rats.
The rate of decline in plasma concentrations of TPH was remarkably slower than those of MPX and MBX.

Table 3 summarizes the values of the various pharmacokinetic parameters of TPH, MPX and MBX. There were significant differences in the parameters such as volume of distribution (Vd) and elimination rate constant (K) among the three derivatives, but there were no significant differences in the total body clearance (Cltot) and the mean residence time (MRT) between MPX and MBX. The MRT of MPX and MBX was found to be one sixth to one fifth that of TPH.

The plasma protein bindings in vivo were approximately 97% for MPX and approximately 98% for MBX, as shown in Fig. 4. On the other hand, that for TPH was shown to be about 60%. It was found that the plasma protein binding of MPX and MBX in vivo was linear within the concentration range observed in this study.

### Discussion

In our earlier study, we compared the bronchial smooth muscle relaxant effect and pharmacokinetic characteristics of various xanthine derivatives synthesized by substitution of the alkyl group at the N3 position in the xanthine molecule. The results showed that the straight alkyl chain length plays an important role in both the inhibition of cyclic-AMP phosphodiesterase (PDE) and the relaxant effects, and that the alkyl chain length is likely to affect the half-lives of these derivatives.

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>MPX</th>
<th>MBX</th>
<th>TPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vd (L/kg)</td>
<td>0.237±0.022</td>
<td>0.205±0.007</td>
<td>0.438±0.046</td>
</tr>
<tr>
<td>K (hr⁻¹)</td>
<td>1.157±0.135</td>
<td>1.449±0.129</td>
<td>0.281±0.001</td>
</tr>
<tr>
<td>Cl (L/kg/hr)</td>
<td>0.281±0.037</td>
<td>0.299±0.028</td>
<td>0.124±0.013</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>0.742±0.101</td>
<td>0.611±0.064</td>
<td>3.516±0.052</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. of 5 animals for MPX and 4 animals for MBX and TPH. Statistical analysis showed significant differences of all parameters at P<0.01 between TPH and MPX or TPH and MBX, and at P<0.05 between MPX and MBX for Vd and K.
xanthine derivatives in rabbits, although there are no significant differences in the volume of distribution (4).

In the present study we synthesized a new xanthine derivative, 1-methyl-3-propylxanthine (MPX), by substitution of the alkyl groups at the N3 position in the 1-methylxanthine molecule. Comparative studies on the relaxant effect in vitro and pharmacokinetic characteristics of MPX in rats with those of 1-methyl-3-butylxanthine (MBX) and 1,3-dimethylxanthine (theophylline, TPH) were carried out. Our in vitro study using guinea pig tracheal smooth muscle preparations indicated that the potency of the relaxant effect of MPX is nearly equal to that of MBX, but more than that of TPH.

A new xanthine derivative, propylxanthine (enprofylline), that we recently synthesized has a relaxant effect which is 4-5 times more potent than that of theophylline, but does not exert theophylline-like antagonism on adenosine receptors, although its chemical structure is similar to that of theophylline (3, 12-15).

Therefore, the bronchodilatory effect of theophylline may not be related to adenosine receptor antagonism. However, the precise mechanism is not yet well understood. On the other hand, it was found that the bronchial smooth muscle relaxant effect of MPX is much stronger than the effects of other xanthines, including 3-propyl-, 3-butylxanthines and TPH, and that its effect of theophylline-like antagonism on adenosine receptors is stronger than those of 3-propylxanthine and TPH (T. Hasegawa et al., unpublished data). Based on these observations, the alkyl group (methyl) in the N1 position of the 3-alkylxanthine molecule has not only an important role in adenosine receptor antagonism, but also exhibits additive bronchodilatory action, which is mainly attributable to the alkyl groups in the N3 position of the 1-methylxanthine molecule.

We also compared the partition coefficients (log PC) of each xanthine derivative since it is generally well-known that hydrophobicity increases with the length of the alkyl chain and that hydrophobicity may be an important determinant of the pharmacokinetic and metabolic properties of drugs. The present result at least indicates that the magnitude of the relaxant effect of the N1, N3-di alkyl substituents of xanthine are attributed to their hydrophobicity because the different permeabilities of the di-alkyl xanthine derivatives across the tracheal smooth muscle cell membrane lead to the expected order of relaxant effects. It may be thought that the hydrophobic property and/or the N3-alkyl chain length of the 1-methylxanthine derivatives are inevitably related to the magnitude of the relaxant effect.

As for the pharmacokinetic characteristics of MPX and MBX in rats, the plasma half-life at the elimination phase (t1/2) of MPX and MBX were approximately one fifth to one fourth of TPH, and total body clearances (Cltot) of MPX and MBX were approximately 2 times larger than TPH. Accordingly, both MPX and MBX are short-acting drugs compared to TPH.

Regarding the metabolic characteristics of xanthine derivatives, it is well-known that approximately 90% of the total body clearance of TPH in man is due to biotransformation in the liver. On the other hand, the biotransformation of TPH in rats is reported to be about 50% (16). In contrast, Borga et al. (17) reported that approximately 90% of propylxanthine is excreted in the urine as the unchanged drug. Based on these results, it is suggested that the N3-alkyl chain length of these xanthine derivatives may play an important role in the biotransformation. For the purpose of preliminary investigation of the hepatic metabolic rate of MPX and MBX as compared with TPH, we measured the concentrations of the unchanged drugs excreted in the urine. The present study showed that the excretion of MPX in the urine was extremely low (about 1%) and that the amount of MBX in urine was too low to be detected by HPLC assay, indicating that both drugs are almost completely metabolized in the liver. From these results, we speculated that the low amount of the unchanged drugs in urine is due to either being not filtered through the glomerulus by the influence of their high plasma protein binding because the protein-bound portion of drugs is not filtered through the glomerulus or that both drugs with strong hydrophobicity are easily me-
tabolized in the liver resulting from high affinities to some cytochrome P-450 iso-
yzemes. Moreover, we proposed that the metabolic rate of 1-methyl-3-alkyl-substi-
tuted xanthine derivatives increases with the length of the alkyl chain at the N3 position in
the 1-methylxanthine molecule because there were remarkable differences in the bio-
transformation between 3-propylxanthine and MPX.

For these reasons, we postulate that MPX as well as 3-propylxanthine is also useful for
clarifying the precise mechanism of the bronchodilatory effect of TPH, which is not yet well understood. Furthermore, it is expected that MPX is a new type of anti-
asthmatic drug which will be useful as a substitute for TPH. However, its toxic effects
in animals must be investigated further.

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