Structure-Activity Relationship in N3-Alkyl-Xanthine Derivatives

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Abstract—Structure-activity studies were carried out to compare the relaxant effects of various xanthine derivatives synthesized by substitution of the alkyl groups of the N3 position in the xanthine molecule. We evaluated the relaxant effects and the inhibitory activities on c-AMP phosphodiesterase (PDE) in tracheal smooth muscle isolated from guinea pigs. A comparative study on their pharmacokinetic characteristics was also carried out in rabbits. Dose-dependent relaxant effects were observed, and the relaxant effect of propylxanthine was nearly equal to the effects of butyl- and isobutylxanthines. Based on the estimation of the Ki values for PDE inhibition, it was found that butylxanthine is a potent inhibitor of PDE. There was good correlation between the alkyl chain length and the Ki value of these derivatives. The results showed that the alkyl chain length plays an important role in the inhibition of PDE. There were no significant differences in the volume of distribution, although the half-life showed significant differences. It is likely that the half-lives of these derivatives are affected by their chain lengths. The present study indicated that butylxanthine may be a new candidate as a bronchodilator. However, clinical studies have to be carried out to compare its efficacy and adverse effects with those of existing bronchodilators such as theophylline.

It is well-known that theophylline (1,3-dimethylxanthine) exhibits a strong bronchial smooth muscle relaxant effect and that its effect is much stronger than the effects of other known xanthises, including caffeine and theobromine. Furthermore, theophylline is widely used in the treatment of patients with reversible obstructive airway diseases, and its bronchodilating 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, propylxanthine (enprofylline), was recently synthesized. The information on the pharmacodynamic characteristics of propylxanthine has been published (4-7). In particular, the potency of various xanthine derivatives with different types of substitutions at various positions as adenosine antagonists was determined (5, 6). However, studies on the relationship between the chemical structure and pharmacological actions (relaxant and PDE-inhibitory effects) or pharmacokinetics of N-alkylxanthine derivatives have yet to be fully clarified.

In the present study, we synthesized various xanthine derivatives by substitution of alkyl groups at the N3 position of the xanthine molecule in order to clarify whether or not the chain length of the N-alkyl substituents is important for the bronchodilating effect. Additionally, the pharmacokinetic characteristics of these N3-alkylxanthine derivatives in rabbits were estimated using...
theophylline as the reference compound.

Materials and Methods

Chemicals: The N3-alkyl-substituted xanthine derivatives tested in this study were synthesized according to the methods reported previously (8-11). Their physico-chemical data are listed in Table 1.

Animals: Male Hartley-strain guinea pigs, weighing 230-300 g, and male JW-NIBS rabbits, weighing 2.4-2.5 kg, were used.

In vitro experiments: 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 as previously reported (12). We also estimated the effect of each compound on c-AMP phosphodiesterase (PDE) in homogenates prepared from isolated guinea pig tracheal smooth muscle. The procedure was carried out according to the method of Pöch (13), and the Kᵢ values were estimated by using the method of Dixon (14). The protein concentration of the homogenate was determined by the method of Lowry et al. (15).

In vivo experiments: Experiments were conducted to compare the pharmacokinetic characteristics in three groups of rabbits. The three groups consisted of five male rabbits each. Only one of the compounds was administered to each group. Blood samples of about 1 ml were collected at 0.25, 0.5, 1, 2, 4, 6 and 8 hr after intravenous administration. The plasma was immediately separated by centrifugation and stored at -40°C until analysis.

Analytical method: The concentration of each xanthine derivative was determined by a modification of the method previously reported (16), using 8-chlorotheophylline as the internal standard. Separation was carried out on a Zorbax column with an eluent of 0.01 M sodium acetate (pH 4.0)-acetonitrile (95/5 by vol.). Absorbance was measured at 278 nm. The xanthine derivative concentrations were calculated from their relative peak height ratios based on a standard curve.

Pharmacokinetic analysis: The area under the plasma concentration-time curve (AUC) was calculated by applying the trapezoidal method, and the AUC from the last data point to infinity was extrapolated by dividing the last data point by the apparent first-order elimination rate constant (kₑ) obtained from the slope of the least-squares regression line from the elimination phase of the log plasma concentration-time curve after administration. The total body clearance (CL) and the plasma half-life (t₁/₂) were calculated using the following equations:

\[ CL = \frac{D}{AUC} \]

\[ t_{1/2} = \frac{0.693}{k_e} \]

Statistical analysis: The results were expressed as the mean±S.E.M. Statistical analyses were performed using Student's t-test. Regression lines were obtained by the linear least-squares method.

Results

Figure 1 shows the dose-response curves of the relaxant effect of each xanthine deriva-

| Alkyl group | mp (°C) | Formula | Analysis (%) | MS (m/z) |
|-------------|---------|---------|--------------|----------|
|             | C₇H₈N₄O₂ |         |              |          |
| Ethyl       | >300    | C₇H₈N₄O₂ | 46.67 (46.65) | 180 (M⁺, base) |
| Propyl      | 290-291 | C₈H₁₄N₄O₂ | 49.48 (49.69) | 194 (M⁺) |
| Isopropyl   | 298-299 | C₈H₁₄N₄O₂ | 49.48 (49.69) | 194 (M⁺) |
| Butyl       | 281-283 | C₉H₁₄N₄O₂ | 51.92 (52.01) | 208 (M⁺) |
| Isobutyl    | 296-297 | C₉H₁₄N₄O₂ | 51.92 (52.10) | 208 (M⁺) |
tive on tracheal smooth muscle preparations, and Table 2 lists the EC50 values. In the concentration range from $1 \times 10^{-7}$ to $1 \times 10^{-5}$ M, a dose-dependent relaxant effect was observed in each group. The relaxant effects increased in the order of isobutyl-, propyl-, butyl-, theophylline, ethyl- and isopropylxanthines. The results show that the in vitro potency of the relaxant effect of propylxanthine was nearly equal to the potencies of butyl- and isobutylxanthines. As shown in Fig. 2, a good correlation was found between the alkyl chain length and the $K_i$ value of these xanthine derivatives. The alkyl chain length thus played an important role in the inhibition of PDE.

Figure 3 compares the mean semilogarithmic plots of the plasma concentration-time of three xanthine derivatives (theophylline, propyl- and butylxanthines) after a single intravenous administration to five rabbits. The mean plasma concentration-time curve of theophylline is similar to that of propylxanthine, but butylxanthine exhibited a shorter elimination half-life than theophylline and propylxanthine. Table 3 summarizes the values of the various pharmacokinetic parameters. There were no significant differences in the volume of distribution ($V_d$) among the three derivatives, but they showed significant differences in CL and $t_{1/2}$.

**Discussion**

In the present study, the potencies of five

![Figure 1. Dose-response relationships of effects of xanthine derivatives on resting tone in tracheal smooth muscle preparations isolated from guinea pigs. Symbols of combined compounds: ■ theophylline; ○ ethylxanthine; □ propylxanthine; △ isopropylxanthine; ● butylxanthine; ▲ isobutylxanthine. Each point represents the mean of 8 determinations.](image)

**Table 2. Effects of alkylxanthine derivatives on resting tone in guinea pig tracheal smooth muscle preparations**

| Structure | $R_1$ | $R_2$ | EC50 ($\times 10^{-5}$ M) |
|-----------|-------|-------|-------------------------|
| ![Chemical structure](image) | H     | ethyl | 6.9                     |
|           | H     | propyl | 0.7                     |
|           | H     | isopropyl | 7.2                    |
|           | H     | butyl | 1.0                     |
|           | H     | isobutyl | 0.5                    |
|           | CH$_3$ | methyl (theophylline) | 4.3 |
different alkylxanthine derivatives as bronchodilators were examined in vitro. The results of this study indicated that the potencies of the relaxant effect of two different alkylxanthine derivatives (butyl- and isobutylxanthines) are nearly equal to that of propylxanthine. Propylxanthine has been found to be 4–5 times more potent than theophylline both in vitro and in vivo (4, 17, 18). Our in vitro study using guinea pig tracheal smooth muscle preparations also indicated that propylxanthine is approximately 6-fold more potent than theophylline. On the other hand, the inhibitory effect of butylxanthine on PDE in guinea pig tracheal smooth muscle was much stronger than the effects of propylxanthine and theophylline. Both theophylline and propylxanthine have been indicated to be inhibitors of PDE, and propylxanthine is a more potent inhibitor than theophylline (4, 19). From these observations, it was thought that butylxanthine may also be useful for elucidating the mechanism of the bronchodilatory effect of theophylline.

For many years, it has been believed that
the mechanism of the bronchodilatory effect of theophylline is elevation of the intracellular c-AMP by inhibition of PDE. Recently, a hypothesis that theophylline antagonizes the activity of adenosine has become widely accepted (5). Moreover, it has been found that a new synthetic xanthine, propylxanthine, does not exert theophylline-like antagonism on adenosine receptors (3, 20), although its chemical structure is similar to that of theophylline. Therefore, the bronchodilatory effect of theophylline may not be related to adenosine receptor antagonism. However, the precise mechanism is still not well understood. Further studies on the mechanism of the bronchodilatory effect of theophylline using theophylline analogs must be performed.

The present study demonstrated correlations between the chain length of the alkyl groups and their relaxant and PDE-inhibitory effects. There was a good correlation with the inhibitory effect on PDE, although it was not so clear with the relaxant effect.

It is generally thought that hydrophobicity increases with the length of the alkyl chain. The present study at least indicated that the magnitude of the relaxant and PDE-inhibitory effects of the N3-alkyl-substituents of xanthine are attributed to their hydrophobicity. Our speculation is that the different permeabilities of the alkylxanthine derivatives across the tracheal smooth muscle cell membrane led to the expected order of relaxant and PDE-inhibitory effects. Consequently, it can be considered that either the hydrophobic properties or the chain length of the xanthine derivatives are inevitably related to the magnitude of their effects. However, the relationships between the various physico-chemical properties and their relaxant and PDE-inhibitory effects must be investigated further. Furthermore, the correlation between the relaxant effect and the PDE-inhibitory effect of various xanthine derivatives, including 3-alkylxanthine and 1,3-dialkylxanthine derivatives, will be reported in the next paper of this series.

Regarding the pharmacokinetic properties of xanthine derivatives, approximately 90% of the total body clearance of theophylline is well-known to be due to biotransformation in the liver. On the other hand, approximately 90% of propylxanthine is excreted in the urine as the unchanged drug, and it has a short half-life compared with theophylline (21). The present study in rabbits also indicated that there were significant differences both in the total body clearance and half-life among three different xanthine derivatives, and the half-life of butylxanthine was shorter than those of propylxanthine and theophylline. These results show that the N-alkyl chain length of these xanthine derivatives may play an important role in the biotransformation. For these reason, propylxanthine is most likely to be useful as a bronchodilator as a substitute for theophylline in the treatment of asthmatic patients with a hepatic disorder. We therefore postulate that butylxanthine is also useful for patients with a hepatic disorder because its chemical structure is very similar to that of propylxanthine, although its half-life is shorter than that of propylxanthine. However, in vivo studies have to be carried out to compare its efficacy and adverse effects with those of existing bronchodilators such as propylxanthine and theophylline.

In conclusion, although the action mechanism of theophylline’s bronchodilator effect is still unknown, it is expected that butylxanthine is a new type of antiasthmatic drug which will be useful as a substitute for theophylline because it shows much stronger

| Table 3. Pharmacokinetic parameters of theophylline, propyl- and butylxanthines |
|----------------------------------|--------------|----------------|
|                                | CL (ml/kg/hr) | Vd (l/kg)     | t1/2 (hr)   |
| Theophylline                    | 84.53±4.24a   | 0.388±0.011   | 3.21±0.17a  |
| Propylxanthine                  | 113.28±13.09b | 0.366±0.023   | 2.13±0.20b  |
| Butylxanthine                   | 256.71±22.19b | 0.444±0.032   | 1.21±0.06a  |

Each value represents the mean±S.E.M. of five rabbits. Plasma clearance, CL, was calculated as dose/AUC. a and b represent statistical significances compared with propylxanthine and theophylline at P<0.01, respectively.
relaxant and PDE-inhibitory effects in the present study.

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