Studies on the Factors Affecting Pulmonary Absorption of Xanthine Derivatives in the Rat

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In an attempt to develop a suitable dosage form of systemically acting drugs for pulmonary administration, the absorption of xanthine derivatives from the lung of rats was studied. The pulmonary absorption of xanthine derivatives was rapid, and these drugs were transferred into the circulation. For example, in the case of theophylline (500 µg/rat), the absorption within 1 min was about 90%, and in the cases of aminophylline (500 µg/rat) and caffeine (1000 µg/rat), 96% and 97%, respectively. The maximum plasma levels in all cases were achieved within 30 s. The pulmonary absorption of these drugs was not affected by body weight or wet weight of lung. When the dose of these drugs was raised 10- to 15-fold, the amount of compound absorbed was directly proportional to the dose, the percentage absorption remaining constant. The pulmonary absorption of these drugs was not significantly different at pH between 6.4 and 8.4, but at pH 9.4, the absorption of theophylline and aminophylline was significantly decreased (p < 0.001), though the absorption of caffeine remained nearly constant. Comparison of the partition coefficients of these drugs indicated that the pulmonary absorption of xanthine derivatives depends on lipid solubility. The pulmonary absorption of theophylline was unaffected by changes of the osmotic pressure of drug solutions. These results suggest that the pulmonary absorption of xanthine derivatives can indeed occur very rapidly, and that the lung may afford a favorable route of administration to obtain a systemic effect of drugs.

Keywords—xanthine derivative; pulmonary absorption; pulmonary administration; pH effect; partition coefficient; theophylline; aminophylline; caffeine; rat

Recent investigations concerning the permeability characteristics of lung mucosa indicated that the lung may provide a useful route of administration for systemically acting drugs such as cyanocobalamin, gentamicin and kanamycin, which are not absorbed or are absorbed very slowly from the gastrointestinal tract. Moreover, Schanker et al. suggested that the lung membrane possesses at least two absorption processes: a nonsaturable process and saturable carrier-type transport. According to his data, drug absorption from the lung is rapid, as had been expected from the histological and physiological characteristics of the lung. However, drug administration to the lung is usually done for topical treatment, but not systemic treatment, because dosage forms suitable for pulmonary administration, except for gaseous drugs, have not been developed adequately.

The present study was designed to obtain basic data for developing an effective dosage form for pulmonary administration of systemically acting drugs by investigating the influence of various factors on the absorption of xanthine derivatives from the rat lung.

Experimental

Materials—Three xanthine derivatives: theophylline (Nakarai Chemicals, Ltd.), aminophylline (Eisai Co., Ltd.) and caffeine (Shizuoka Caffeine Co., Ltd.) were selected. They were purchased from commercial sources and used without further purification.
Preparation of Solutions for Administration and for Determination of Partition Coefficient——Test solutions of xanthine derivatives were prepared with Krebs–Ringer phosphate solution (pH 6.4, 7.4 and 8.4) or isotonic sodium borate buffer solution (pH 8.4 and 9.4), and for the study of osmotic pressure effect on the pulmonary absorption, drug solutions having different osmolalities were prepared by adding sodium chloride.

Determination of Partition Coefficient——Isotonic buffered solutions (pH 6.4—9.4) containing xanthine derivatives (200 and 5000 μg/ml) were prepared, and the partition coefficients between the buffer and chloroform, which has been used to evaluate the lipid solubility of substances, were determined. After shaking with an equal volume of the chloroform at 37° C for 1 h, the drug content in the aqueous phase was determined and the partition coefficient was calculated.

Animal Procedures——Male Wistar rats weighing 170—190 g were used in most experiments. However, for the study of the effect of the body weight or of the lung weight on the pulmonary absorption of theophylline, rats having 170—300 g body weight were used. The animals were fed a standard laboratory diet and given tap water ad libitum prior to experiments.

With the rats under sodium pentobarbital (Nembutal, 40 mg/kg i.p.) anesthesia, the trachea was exposed through a ventral midline incision in the neck. A 2.5-cm length of polyethylene tubing, which served as a tight-fitting tracheal cannula, was inserted through an incision between the fourth and fifth tracheal rings caudal to the thyroid cartilage to a depth of 0.6 cm. The incision in the skin was then closed with a wound clip and the body temperature was maintained at 37±1° C with a 40-W incandescent lamp suspended above the animals.

Procedure of Absorption Experiments——According to the method developed by Enna and Schanker, 0.1 ml of solution was injected into the lungs through polyethylene tubing attached to a calibrated 100-μl syringe (Hamilton Company). The injection tubing was inserted through the tracheal cannula to a point 1 mm above the bifurcation of the trachea, the drug solution was injected within 1 to 2 s, and the tubing was quickly withdrawn. At the end of an absorption period, the blood supply to the lungs was quickly halted with an arterial clamp, and then the lungs and attached trachea were removed. These tissues were assayed for unabsorbed drugs.

Determination of Drug Level in Plasma——The blood was collected with a heparinized syringe from the descending aorta at given times after drug administration. The plasma was separated by centrifuging at 3000 g for 20 min, and drug concentration was determined.

Analytical Methods——(1) Theophylline and Aminophylline: Removed lung tissue and attached trachea were weighed, and placed in a glass homogenizer together with sufficient distilled water to make 5 ml. After homogenization, the lung was extracted with 20:1 chloroform–isopropanol (30 ml), and determined spectrophotometrically at a wavelength of 275 nm using a Hitachi spectrophotometer, model 124, according to the modified method of Vasiliades and Turner. The recovery of xanthine derivatives was always more than 90%, so that the method appeared to be suitable for this study. Essentially the same procedures were applied for the determination of the drug in plasma.

(2) Caffeine: Tissue caffeine and plasma caffeine were analyzed spectrophotometrically at a wavelength of 460 nm using a Hitachi spectrophotometer (model 124) after extraction with chloroform (20 ml).

Student’s t test was used to examine the statistical significance of differences. The 0.05 level of probability was regarded as significant.

Results

The results of the pulmonary absorption of xanthine derivatives in rat are shown in Fig. 1. The semilogarithmic plots of percentage of unabsorbed drugs against time were not linear. The pulmonary absorption curves demonstrated that drug absorption from the lung can occur rapidly. The 1-min absorption value of theophylline (500 μg/rat) at pH 7.4 was about 90%, and the percentages absorbed in 1 min at pH 7.4 in the cases of aminophylline (500 μg/rat) and caffeine (1000 μg/rat) were 96 and 97, respectively.

To examine in more detail the rapid disappearance of these drugs, the plasma levels of xanthine derivatives were measured after administration. In Fig. 2, the time courses of plasma level after pulmonary administration are illustrated. The plasma levels were investigated up to 30 min in the cases of theophylline and aminophylline, and 15 min in the case of caffeine. The plasma level of these drugs reached the maximum within 30 s after pulmonary administration, and almost all of these drugs was transferred to the plasma very rapidly.

In order to study the relation between pulmonary absorption and body weight or lung wet weight of animals, the absorption of theophylline from the lung mucosa of rats weighing 170—300 g was investigated. As shown in Figs. 3 and 4, the pulmonary absorption of
theophylline (500 µg/rat) was not significantly affected by the body weight or lung wet weight, and there was no direct relationship between pulmonary absorption of theophylline and body weight of rats ($Y = 0.038X + 57.6$, $r = 0.355$ ($n=17$, not significant)), or lung wet weight of rats ($Y = -5.9X + 73.1$, $r = 0.16$ ($n=17$, not significant)).

The effect of dose on the pulmonary absorption is shown in Fig. 5. When the dose of theophylline and aminophylline was varied over a 10-fold range (theophylline 50—500 µg/rat, aminophylline 100—1000 µg/rat), and that of caffeine over a 15-fold range (caffeine 100—1500 µg/rat), the amount of drugs absorbed was directly proportional to the dose, and the percentage absorption was almost constant in these studies.

As shown in Fig. 6, the pH value of administered solution was varied between 6.4 and 9.4. The pulmonary absorption of all drugs in 15 s remained constant at pH 6.4, 7.4 and 8.4.
In contrast to these results, at pH 9.4, the pulmonary absorptions of theophylline and aminophylline in 15 s were significantly decreased ($p < 0.001$), but the absorption of caffeine did not change.

The partition coefficients of xanthine derivatives at various pH values are summarized in Table I. When the partition coefficient values of theophylline and aminophylline at pH 9.4 decreased from 0.233 to 0.119 and from 0.185 to 0.078, respectively, the 15-s absorption
values of these drugs at pH 9.4 also decreased to 52.4 ± 1.2% (p < 0.001) and 59.0 ± 0.8% (p < 0.001), respectively. In the case of caffeine, neither the partition coefficient value at pH 9.4 nor the 15-s absorption value at pH 9.4 changed significantly. On the other hand, on comparing the partition coefficients and pulmonary absorption of these three compounds at pH 7.4, caffeine, which has 86- to 101-fold (pH 7.4) greater partition coefficient than the other two drugs, was absorbed more rapidly than the other two drugs. These results suggest that the partition coefficient of the derivatives may affect the pulmonary absorption.

The effect of osmotic pressure of the drug solution administered on the absorption of theophylline is summarized in Table II. The 15-s absorptions from drug solutions in distilled water, 0.9% NaCl soln. and 2.7% NaCl soln. showed no significant difference.

**Table II.** Effect of Osmotic Pressure on Pulmonary Absorption of Theophylline

<table>
<thead>
<tr>
<th>Component of drug solution</th>
<th>No. of rats</th>
<th>Time after administration</th>
<th>% absorbed (Mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5</td>
<td>15 s</td>
<td>62.9 ± 2.2</td>
</tr>
<tr>
<td>0.9% NaCl</td>
<td>5</td>
<td>15 s</td>
<td>65.6 ± 2.4</td>
</tr>
<tr>
<td>2.7% NaCl</td>
<td>5</td>
<td>15 s</td>
<td>65.1 ± 2.1</td>
</tr>
</tbody>
</table>

Dose: theophylline 500 µg/rat.

Discussion

It is well known that the lung possesses a membrane for exchange of gas between blood and air; it is located deep inside the chest and is divided into microscopic subsections (alveoli) at the ends of the many bronchi. Moreover, the alveolar membrane is very thin and the alveoli are surrounded by a dense vessel network. Accordingly, it is expected that drugs will be transferred rapidly from the alveoli into the circulation.

In the present study, it was confirmed that xanthine derivatives (theophylline, amin-
ophylline and caffeine) were absorbed from the rat lung very rapidly, suggesting that absorption of these drugs from the lung is fast compared to absorption from the gastrointestinal tract. For example, as reported by Hogben et al., the 30-min absorption of theophylline from the rat small intestine is about 29%, whereas, as shown in the present study, the 1-min absorption of theophylline was about 90% in the case of rat lung (Fig. 1). Since absorption of drugs from the lung is much more rapid than from the intestinal tract, it may be possible to utilize the lungs as a site of drug administration for therapeutic agents that are poorly absorbed from the gastrointestinal tract.

An interesting feature of the present study is the nonlinear, semilogarithmic plots obtained (Fig. 1). In agreement with these findings, Schanker et al. found that digoxin and digitoxin showed multiexponential absorption kinetics. They suggested that one or more of the following factors may be responsible for the secondary phase of slower absorption: 1) reversible binding of drugs to macromolecules in lung tissue or in the pulmonary tree; 2) absorption rates might be different depending on the region of pulmonary tissue involved; or 3) restricted diffusion of drugs within the pulmonary tree owing to entrapment in fluids with high viscosity. However, these possibilities were not examined in the present study.

To confirm in more detail the fate of these drugs after administration into the lung, we measured plasma levels of the drugs (Fig. 2). As the plasma level of these drugs reached the maximum within 30 s, it is thought that the lung mucosa may afford a useful route of administration for systemically acting drugs, provided that pathologic and histological damage to the lung mucosa does not occur. In particular, the pulmonary route may be suitable for the administration of endogenous substances which have a short biological half-life and are effective at a small dose, for example, calcitonin and prostaglandin E.

The present investigations demonstrated that there was no direct relationship between pulmonary absorption of theophylline and body weight or lung wet weight of rats, and that the percentage absorption remained nearly constant (Figs. 3 and 4). From these findings, it seems possible that the total absorption from the lung may be independent of both the body weight and the lung wet weight of rats, since theophylline may be absorbed rapidly from a part of the lung before diffusing throughout the whole lung. Drug administration via this route might be very favorable in clinical therapy because the absorption rate is independent of factors such as body weight and lung wet weight, because the absorption rates of drugs did not vary from individual to individual, and because effective plasma concentrations of drugs can be maintained easily.

To examine the effect of dose on the rate of absorption, these compounds were administered over a wide range of initial dose (Fig. 5). The percent absorbed was not affected by the dose, suggesting that the amount of drug absorbed would be proportional to the dose.

In order to examine the effect of pH on the pulmonary absorption, the pH value of administered drug solution was varied between 6.4 and 9.4. The absorption rates of theophylline, aminophylline and caffeine were unchanged at pH 6.4, 7.4 and 8.4, but at pH 9.4, the absorption rates of theophylline and aminophylline decreased markedly, though that of caffeine did not (Fig. 6). With respect to theophylline and aminophylline, Trochta et al. have reported that both drugs have a pKₐ value of about 8.8. Thus, the absorption rates of both drugs at pH 9.4 may have decreased significantly because both drugs exist in almost wholly nonionized form at pH 6.4, 7.4 and 8.4, but almost wholly ionized form at pH 9.4. In contrast, caffeine, having pKₐ 0.8 exists in a nonionized form at pH 9.4. Therefore, these results suggest that the lung mucosa may be preferentially permeable to the nonionized form of drugs, according to the pH-partition hypothesis. Thus, the pH value of a dosage form must be carefully adjusted.

To examine the effect of ionic components on the absorption, Krebs–Ringer phosphate solution and isotonic sodium borate solution of pH 8.4 were also studied, and no difference in
absorption was found. From these results, it may be possible to conclude that ionic components did not effect on the absorption of drug through pulmonary mucosa, contrary to our previous findings in the case of intestinal absorption.\textsuperscript{19)}

On comparing the absorption rates with the lipid/water partition coefficients of these drugs, since both theophylline and aminophylline have a $pK_a$ value of about 8.8,\textsuperscript{16)} the absorption rates of both drugs at pH 9.4 decreased together with the decrease of the partition coefficients, while, in the case of caffeine, neither the partition coefficient nor the absorption rate decreased at any of the pH values used in the present study (Table I). Moreover, caffeine, having 86- to 101-fold (pH 7.4) greater partition coefficient than theophylline and aminophylline, was absorbed more rapidly than the latter two drugs. These results indicate that the absorption of xanthine derivatives from the lung may depend on their lipid solubilities.

To clarify the effect of osmolality of the solution administered on pulmonary absorption, hypotonic, isotonic and hypertonic theophylline solutions were administered to the rat lung (Table II). The results obtained suggest that the absorption from the lung mucosa is not affected by the tonicity on the drug solution. Kitazawa et al.\textsuperscript{19)} found that the results obtained for intestinal absorption were quite different from those for pulmonary absorption. Namely, the intestinal drug absorption increased with decreasing tonicity of the perfusate. This difference may be related to the unique physiological function of the lung. However, further investigations are required.

In conclusion, the present study has provided some basic data for the development of dosage forms suitable for pulmonary administration.

References