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Dose-dependency in the Exsorption of Theophylline and the Intestinal Dialysis of Theophylline by Oral Activated Charcoal in Rats

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Abstract—The elimination half-life of theophylline in serum after intravenous (i.v.) administration of aminophylline increased with increase in dose. Exsorption of theophylline from blood to the gastrointestinal tract was investigated after i.v. administration of aminophylline (10–50 mg kg^{-1}) to rats by the in-situ single-pass perfusion technique. The exsorption rate of theophylline into the intestinal lumen also increased with increase in dose. When the dose of aminophylline was increased five-fold from 10 to 50 mg kg^{-1}, the amount of theophylline exsorbed in 120 min was proportionally increased from 450 to 2300 μg. The average extent of theophylline exsorbed into the intestinal lumen was 12–15% after doses from 10–50 mg kg^{-1}, while the extent of the drug excreted into the bile varied from 0·17–0·30% after doses from 10–50 mg kg^{-1}. However, intestinal and biliary clearance of theophylline did not change significantly in the range 10 to 50 mg kg^{-1}. Oral administration of multiple doses of activated charcoal reduced the serum theophylline levels after i.v. administration of aminophylline (50 mg kg^{-1}) to rats. The serum half-life and the area under the serum concentration-time curve of theophylline were decreased to 52 and 50% by the charcoal treatment, respectively, while the total body clearance of the drug was increased to 188% compared with the corresponding control experiments. The volume of distribution was not significantly different between treated and control rats. As the total body clearance after a high dose is less than after a low dose, because there is a decreased endogenous clearance (as observed in theophylline overdose), clearance after a high dose may be more enhanced by oral activated charcoal than that after a low dose.

Since theophylline has a narrow therapeutic range and its clearance is influenced by various factors including cirrhosis (Piafsky et al 1977a), acute pulmonary oedema (Piafsky et al 1977b), congestive heart failure (Jenne et al 1977) and smoking (Grygiel & Birkett 1981), caution should be used in its administration.

Studies have shown that the clearance of intravenously (Berlinger et al 1983; Mahutte et al 1983) or orally (Neuvonen & Elonen 1980; True et al 1984) administered drugs is accelerated by oral activated charcoal. We have previously demonstrated that intravenously administered theophylline is transported into the small intestinal lumen to a significant extent and into the bile to a small extent in rats (Arimori & Nakano 1985). The mechanism of the accelerated elimination of intravenously administered drugs by oral activated charcoal might be adsorption of drugs exsorbed into the gastrointestinal (g.i.) tract by the charcoal (Arimori & Nakano 1985). However, more detailed information on the dose-dependent exsorption of the drug into the g.i. tract has been lacking.

Recently, it has been reported that theophylline shows dose-dependent kinetics at plasma concentrations encountered clinically (Massey et al 1984). The present study was undertaken to investigate the effect of dose upon the transport of theophylline into the g.i. lumen, and to evaluate the effect of oral activated charcoal on the clearance of the drug at a high dose (50 mg kg^{-1}) compared with that at a low dose (10 mg kg^{-1}) observed previously in rats (Arimori & Nakano 1986).

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Materials and Methods

Materials

Aminophylline (Neophylline) was purchased from Eisai Co., Tokyo. Activated charcoal was obtained from Inuhinode Seiyaku Co., Osaka and its particle size used in this study was less than 62 μm (250 mesh).

Exsorption study

Intestinal exsorption experiments were performed by the in-situ single pass perfusion technique reported previously (Arimori & Nakano 1985). Male Wistar rats, 240–350 g, were anaesthetized by an intraperitoneal injection of ethyl carbamate (1·2 g kg^{-1}). The small intestine was exposed by a midline abdominal incision. The upper duodenum and the ileocaecal junction were cannulated with a polyethylene tube and the small intestine was washed with 0·9% NaCl maintained at 37°C and was perfused with isotonic phosphate buffer (0·1 M, pH 6·0) at the rate of 1·3 mL min^{-1} from the duodenum through the small intestine to the ileocaecal junction using a glass infusion pump (GM-24, Tokyo Rikakikai, Tokyo). Aminophylline (25 mg mL^{-1}) was injected over about 2–5 min into the right femoral vein at a dose of 10–50 mg kg^{-1}. After the injection, perfusates were collected every 15 min from the ileal outflow in a 25 mL volumetric flask and diluted with the phosphate buffer solution to 25 mL before assay. Exsorption rate was defined as the amount exsorbed into the intestinal lumen in 1 min. The bile was collected separately every 15 min from a cannula introduced into the common bile duct. To determine serum drug concentrations blood samples were taken from a cannula introduced into the left femoral vein, at the midpoint of the perfusate collection period.

In-vivo study

Male Wistar rats, 270–355 g, were fasted overnight with free access to water. Each experiment was carried out using a crossover design, and an interval of more than two weeks was allowed between experiments. Aminophylline (50 mg kg^{-1}) was administered intravenously via the caudal vein. Activated charcoal suspended in water (150 mg mL^{-1}) was administered orally through a catheter at an initial dose of 300 mg at time zero and additional doses of 150 mg at 1, 2, 3, and 4 h after the dose of aminophylline. In control experiments, only 2 mL water was given immediately after i.v. administration of aminophylline. Blood samples (200 μL) were collected periodically from the tail vein.

Protein binding study

The serum protein binding of theophylline was determined by an ultrafiltration technique using Centrifree MPS-3 (Amicon Co., Lexington). Aminophylline (50 mg kg^{-1}) was administered intravenously to rats via the caudal vein. Blood samples were collected by cardiocentesis, centrifuged and serum (1 mL) further ultrafiltrated at 1000 *g* for 15 min. The filtrate was stored at −20°C until assay.

Analytical method

Theophylline in the perfusate was determined by high pressure liquid chromatography (HPLC) with 8-chlorotheophylline as an internal standard. A 2 mL portion of the sample was extracted with 5 mL chloroform containing the internal standard after addition of 1 mL 1 M HCl. Separation was performed with a LiChrosorb RP-18 column (5 μm, 4·6 i.d. × 250 mm). The mobile phase consisted of acetate buffer, pH 4·0 and methanol (3:2), and was pumped at a flow rate of 1·0 mL min^{-1}. Theophylline was detected by measured absorbance at 272 nm. Theophylline in serum and bile were measured by a homogeneous immunoassay technique (Ames TDA, Miles Sankyo Co., Tokyo) using drug-free human serum. This assay showed a coefficient of variation less than 3% within the range 5–40 μg mL^{-1}. Concentrations above 40 μg mL^{-1} were also assayed after dilution with buffer solution.

Pharmacokinetic analysis

The serum half-life ($t_{\frac{1}{2}}$) was determined by least-squares regression analysis of the elimination curve of serum levels. The elimination rate constant (k_{el}) was calculated from the relation $k_{el} = 0{\cdot}693/t_{\frac{1}{2}}$. An intercept of ordinate at time zero was used to estimate the initial serum concentration (C_o) of theophylline. The apparent volume of distribution ($Vd = dose/C_o$), the total body clearance ($Cl_{tot} = k_{el}V_d$), and the area under the serum concentration-time curve extrapolated to time infinity ($AUC = C_o/k_{el}$) were also calculated. The paired *t*-test was used to assess the effect of charcoal treatment on the pharmacokinetic parameters. The intestinal and biliary clearance values of theophylline were calculated by dividing the amount of theophylline excreted into the perfusate or the bile in 2 h by AUC from 0 to 2 h, respectively. Although aminophylline was injected, dose is shown in terms of theophylline.

Results

Dose-dependent elimination of theophylline from serum

The time courses of serum theophylline levels after i.v. administration of aminophylline at the dose of 10, 25, and 50 mg kg^{-1}, are shown in Fig. 1. Serum theophylline levels declined apparently obeying first-order kinetics until 6 h after i.v. administration, and showed dose-dependent pharmacokinetics, as the elimination half-life of theophylline in serum increased with increase in dose. This finding suggests dose-dependent changes in metabolism and excretion which may include exsorption into the intestinal lumen.

Transport of theophylline into intestinal lumen

The exsorption rate of theophylline into the perfusate across the small intestinal membrane and the concentrations of theophylline in the serum and bile were examined using the doses from 10 to 50 mg kg^{-1} of aminophylline by the in-situ single pass perfusion technique. As shown in Fig. 2, serum concentrations of the drug increased with increasing dose of aminophylline, although the serum elimination of theophylline did not clearly show dose-dependency, possibly because of a short sampling period under the in-situ experimental condition compared with that in-vivo.

The exsorption rate of theophylline into the perfusate increased with increase in serum level which itself increased with increase in dose of aminophylline. It was also observed that after i.v. administration of aminophylline, theophylline was rapidly excreted in the bile. Theophylline levels in the bile at each dose were higher than those in the serum. Thus, it is suggested that theophylline is transported into the intestinal lumen via the mucosal membrane of the bile duct to an appreciable extent.

The relationship between the amount exsorbed in 120 min and the dose of aminophylline administered is shown in Fig. 3. When the dose of aminophylline was increased five-fold from 10 to 50 mg kg^{-1}, the amount of theophylline exsorbed in 120 min was proportionally increased from 450 to 2300 μg. The percentages of dose transported into the perfusate and the bile in 120 min are shown in Fig. 4. The percentage of the dose exsorbed appeared to show a tendency to increase with the increase in the dose of the drug, but there was no clear-cut difference. The average amounts of theophylline exsorbed into the perfusate were 12·1, 13·9, 15·0, and 14·6% at 10, 20 30, and 50 mg kg^{-1} doses, respectively. The amount of the

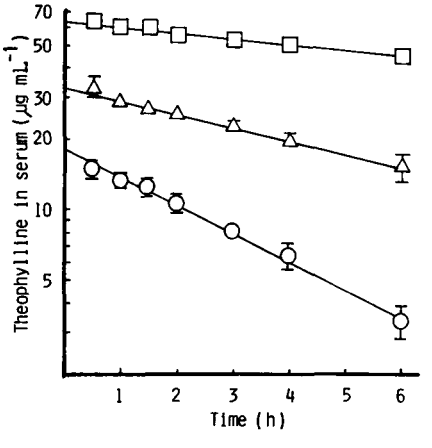

FIG. 1. The serum concentration-time curves of theophylline following i.v.administration of aminophylline to rats. Each point represents the mean ± s.e.m. of 5 rats. ○ 10 mg kg^{-1}; △ 25 mg kg^{-1}; □ 50 mg kg^{-1}.

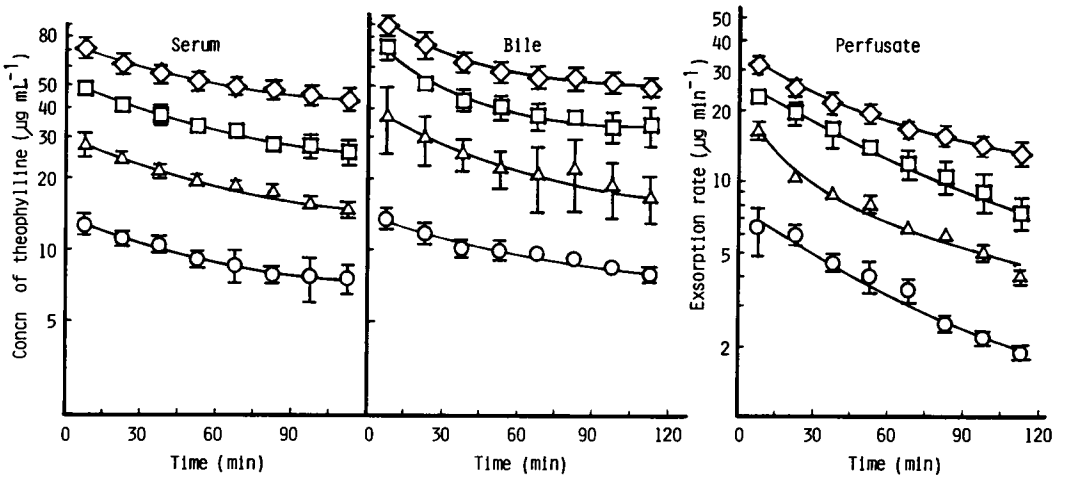

FIG. 2. The concentrations of theophylline in the serum and bile and the exsorption rate of theophylline into the perfusate after i.v. administration of aminophylline (10–50 mg kg^{-1}) to rats studied by the in-situ single pass perfusion method. The perfusate was composed of isotonic phosphate buffer, pH 6·0. Each point represents the mean ± s.e.m. of 3–5 rats. ○ 10 mg kg^{-1} (reported earlier by Arimori & Nakano 1985); △ 20 mg kg^{-1}; □ 30 mg kg^{-1}; ◇ 50 mg kg^{-1}.

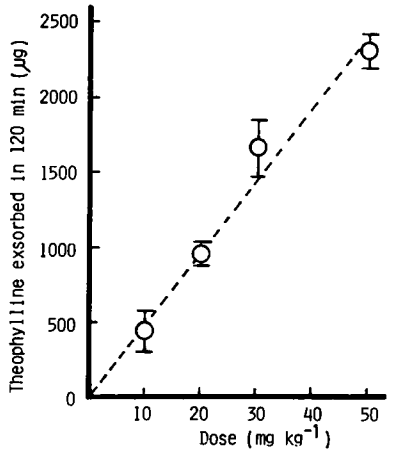

FIG. 3. Effect of dose on the amount of theophylline exsorbed into the perfusate after i.v. administration of aminophylline (10–50 mg kg^{-1}) to rats. Each point represents the mean ± s.e.m. of 3–5 rats. The data at 10 mg kg^{-1} dose are from Arimori & Nakano (1985). The equation of the line is $y = 47{\cdot}5x + 26{\cdot}0$, $r^2 = 0{\cdot}984$.

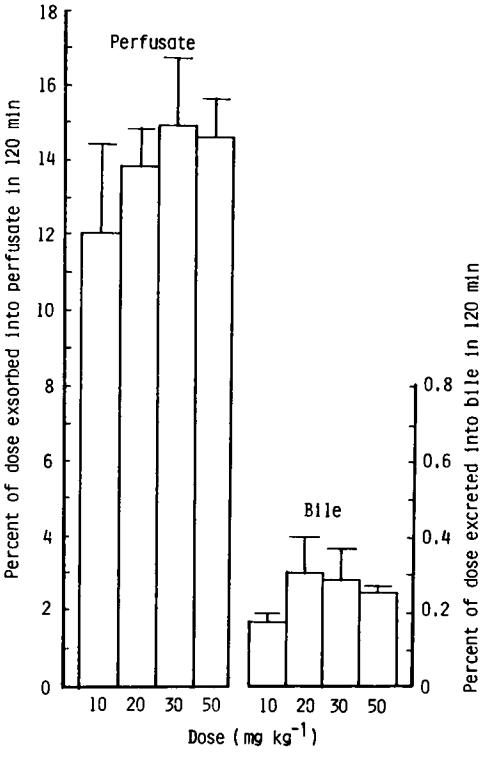

FIG. 4. Percentage of theophylline exsorbed into the perfusate and excreted into the bile in 120 min after i.v. administration of aminophylline (10–50 mg kg^{-1}) to rats. Each bar represents the mean ± s.e.m. of 3–5 rats. The data at 10 mg kg^{-1} are from Arimori & Nakano (1985).

drug excreted into the bile was much smaller than that exsorbed into the perfusate. The amounts of theophylline excreted into the bile were 0·17, 0·30, 0·29, and 0·25% after 10, 20, 30, and 50 mg kg^{-1} doses, respectively. Thus, the intestinal lumen exhibits an exsorption/excretion function for theophylline.

To express the ability of a system to excrete the drug, the concept of intestinal and biliary clearance was used. Fig. 5 shows the relationship between intestinal and biliary clearance values of theophylline and the dose of aminophylline administered to rats. Both clearance values are defined as volumes of serum from which the drug is removed by exsorption or excretion into the intestine or bile, respectively, in 1 h. Both clearance values were little different over the dose range studied (Fig. 5). Moreover, intestinal clearance values were about 55-fold higher than biliary clearance values. These results suggest that the intestinal lumen may be considered to be one of the routes of drug distribution, in other words, a route of drug removal unless it is reabsorbed.

Effect of activated charcoal on theophylline clearance

Fig. 6. shows the time course of serum theophylline levels after i.v. administration of aminophylline (50 mg kg^{-1}) to

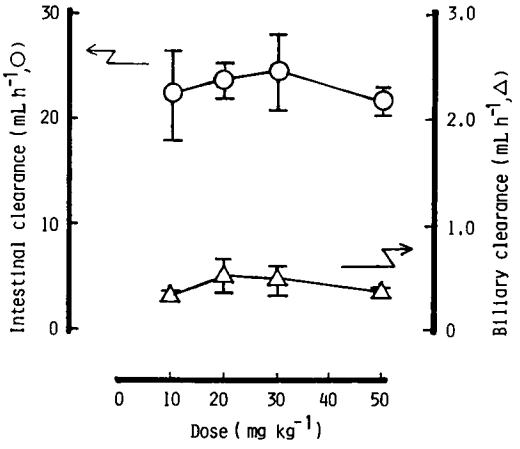

FIG. 5. Dose-dependency in intestinal and biliary clearance of theophylline after i.v. administration of aminophylline (10–50 mg kg^{-1}) to rats. Each point represents the mean ± s.e.m. of 3–5 rats.

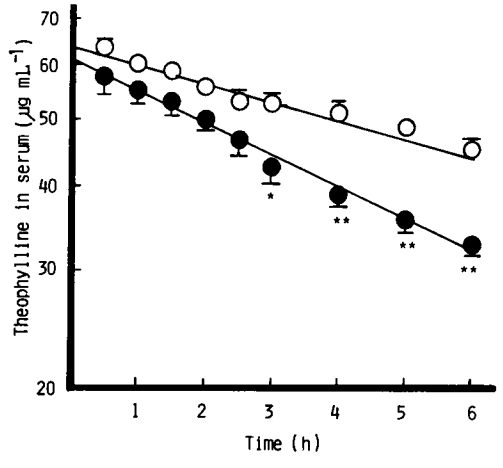

FIG. 6. The serum concentrations of theophylline after i.v. administration of aminophylline (50 mg kg^{-1}) to rats with (●) or without (○) treatment with activated charcoal. Each point represents the mean ± s.e.m. of 4 rats. (*) $P < 0.05$; (**) $P < 0.01$.

rats with or without oral activated charcoal treatment. Oral administration of multiple doses of activated charcoal reduced serum theophylline levels compared with the corresponding control experiments. Its mean serum level at 6 h was significantly decreased from 45·0 to 32·8 μg mL^{-1} by multiple doses of activated charcoal. Pharmacokinetic parameters after 50 mg kg^{-1} aminophylline are shown in Table 1. With oral administration of activated charcoal $t_{\frac{1}{2}}$ and AUC were decreased to 52·3 and 50·0%, of their original control values, respectively, while Cl_{tot} was increased to 188% compared with the corresponding controls. V_d was not significantly different between the treated and control rats.

Discussion

Our previous report (Arimori & Nakano 1986) has indicated that multiple oral doses of activated charcoal enhance the clearance of i.v. administered drugs. The mechanism has been confirmed by in-situ exsorption studies to be adsorption of the drug secreted into the g.i. tract.

Table 1. Pharmacokinetic parameters of theophylline after i.v. administration of aminophylline (50 mg kg^{-1}) to rats with or without treatment with activated charcoal.

Pharmacokinetic parameters	Control	Treatment with activated charcoal
$t_{\frac{1}{2}}$ (h)	12·7 ± 2·34	6·63 ± 1·07*
V_d (L kg^{-1})	0·675 ± 0·013	0·708 ± 0·036
Cl (mL kg^{-1} h^{-1})	39·9 ± 5·39	74·9 ± 4·58**
AUC (μg h mL^{-1})	1159 ± 204	579 ± 36·3*

Each value represents the mean ± s.e.m. of 4 rats.
* $P < 0.05$; ** $P < 0.01$.

Since the elimination rate constant of theophylline was dose-dependent between 10 and 50 mg kg^{-1} (Fig. 1), it was expected that the clearance of the drug at a high dose, because of its decreased endogenous clearance, would be increased to a greater extent by oral activated charcoal than that at a low dose.

Radomski et al (1984) have proposed that the total clearance of a drug during treatment with activated charcoal is the sum of the normal endogenous clearance and the clearance through the g.i. tract by adsorption on to charcoal. They reported that repeated oral doses of activated charcoal had been more effective in decreasing the serum half-life of theophylline in persons with a long endogenous serum half-life of the drug (e.g. patients with hepatic cirrhosis). Park et al (1985) have also reported that activated charcoal has a relatively small effect in increasing digoxin elimination in normal subjects who have short digoxin half-lives, but a significant effect in increasing digoxin elimination in the subject with renal failure.

Our results showed that the amount of theophylline exsorbed into the intestinal lumen was increased in proportion to dose, although intestinal and biliary clearance were approximately constant. If activated charcoal produces a constant intestinal drug clearance, the endogenous drug clearance may be more enhanced after a high dose of drug than a low dose. We previously demonstrated that the exsorption rate of phenytoin from blood into the intestinal lumen, which shows the dose-dependent kinetics, was increased with increase in dose of drug (Arimori & Nakano 1987). When the dose of phenytoin was increased five-fold, the amount exsorbed in 120 min was increased eight-fold. Moreover, intestinal and biliary clearance values were increased with increase in dose. However, the fraction of the drug exsorbed per dose was much smaller than that of theophylline.

We have suggested that the dose-dependent increase in the exsorption of phenytoin is due to the saturation of serum protein binding of the drug which is highly bound. Percentage binding of theophylline was lower (62% in the present study) than that of phenytoin (about 90%, Lund et al 1971). Since saturation of serum protein binding does not take place in theophylline, and unbound fraction of theophylline does not increase as much as that of phenytoin. We showed that $t_{\frac{1}{2}}$, AUC and Cl_{tot} after i.v. aminophylline at the dose of 10 mg kg^{-1} reported by us earlier (Arimori & Nakano 1986) were 4·6 and 2·8 h, 138 and 88 μg h mL^{-1}, and 67 and 101 mL $kg^{-1}h^{-1}$ without and with the charcoal treatment, respect-

ively. Comparison of the present study with the earlier one also revealed that the effect of oral administration of multiple doses of activated charcoal was much greater with 50 mg kg^{-1} doses of aminophylline than that with 10 mg kg^{-1} doses (48% decrease in $t_{\frac{1}{2}}$ at 50 mg kg^{-1} vs 39% decrease at 10 mg kg^{-1}, 50% decrease in AUC at 50 mg kg^{-1} vs 37% decrease at 10 mg kg^{-1} and 188% increase in Cl_{tot} at 50 mg kg^{-1} vs 152% increase at 10 mg kg^{-1}).

In conclusion, as exsorption of theophylline from blood into the g.i. tract is increased proportionally with an increase in the dose and the endogenous clearance of the drug is decreased at higher doses as seen in a theophylline overdose, intestinal dialysis by oral administration of activated charcoal may be a reasonable method to shorten the period of intoxication in a patient with theophylline poisoning.

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