The potency of both compounds in the inhibition of enzyme in-vitro is of the same magnitude or greater compared with those of the H^+, K^+-ATPase inhibitors omeprazole (Im et al 1985) and SCH 28080 (Scott & Sundell 1985) which exhibited IC50 values of 6·0 and 2·5 μM in our assay system, respectively. The antisecretory and anti-ulcer activity in-vivo, however, was less effective. II, in particular, was inactive in doses up to 200 mg kg^{-1}, p.o. for inhibiting gastric lesions. One possible explanation for the discrepancy observed in-vitro and in-vivo is the poor hydrophobicity of the compounds. Since these compounds act at the cytosolic site after absorption, a poor hydrophobicity may prevent them from reaching the H^+, K^+-ATPase site. Also, the possibility that these compounds may be readily degraded to inactive metabolites cannot be ruled out.

References

Fiske, C. H., Subbarow, Y. (1925) The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375–400

Im, W. B., Sih, J. C., Blakman, D. P., McGrath, J. P. (1985) Omeprazole, a specific inhibitor of gastric (H^+-K^+)-ATPase, is a H^+-activated oxidizing agent of sulfhydryl groups. Ibid. 260: 4591–4597

Kozawa, M., Morita, N., Baba, K., Hata, K. (1978) Chemical components of the roots of Angelica keiskei Koidzumi. II. The structure of the chalcone derivatives. Yakugaku Zasshi 98: 210–214

Kyogoku, K., Hatayama, K., Yokomori, S., Saziki, R., Nakane, S., Sasajima, M., Sawada, J., Ohzeki, M., Tanaka I. (1979) Anti-ulcer effect of isoprenyl flavonoids. II. Synthesis and anti-ulcer activity of new chalcones related to sophoradin. Chem. Pharm. Bull. 27: 2943–2953

Lee, H. C., Forte, J. G. (1978) A study of H^+ transport in gastric microsomal vesicles using fluorescent probes. Biochim. Biophys. Acta 508: 339–356

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265–275

Ray, T. K., Nandi, J. (1986) K^+-Stimulated p-nitrophenyl phosphatase is not a partial reaction of the gastric ($H^+ + K^+$)-transporting ATPase systems. Biochem. J. 233: 231–238

Saccomani, G., Stewart, H. B., Shaw, D., Lewin, M., Sachs, G. (1977) Characterization of gastric mucosal membranes. IX. Fractionation and purification of K^+-ATPase-containing vesicles by zonal centrifugation and free-flow electrophoresis technique. Biochim. Biophys. Acta 465: 311–330

Scott, C. K., Sundell, E. (1985) Inhibition of H^+ K^+ ATPase by SCH 28080 and SCH 32651. Eur. J. Pharmacol. 112: 268–270

Shay, H., Komarov, S. A., Fels, S. S., Meranze, D., Gruenstein, M., Siplet, H. (1945) A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology 5: 43–61

Takagi, K., Okabe, S. (1968) The effects of drugs on the production and recovery processes of the stress ulcer. Japan. J. Pharmacol. 18: 9–18

Tanaka, S., Kuwai, Y., Tabata, M. (1987) Isolation of monoamine oxidase inhibitors from Glycyrrhiza uralensis roots and the structure-activity relationship. Planta Med. 53: 5–8

Wagner, H., Geyer, B., Fiebig, M., Kiso, Y., Hikino, H. (1986) Isobutrin and butrin, the antihepatotoxic principles of Butea monosperma flowers. Ibid. 52: 77–79

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Comparison of intestinal and peritoneal dialysis of theophylline and phenobarbitone in rats

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Abstract—Intestinal dialysis of drugs by oral administration of activated charcoal has been compared with peritoneal dialysis in rats. The average amounts of theophylline transported over 120 min into the intestinal lumen and the peritoneal cavity were 15·7 and 16·5% of the intravenous dose (10 mg kg^{-1}), respectively, showing no significant difference, whereas the amount of the same intravenous dose of phenobarbitone transported from the blood into the intestinal lumen (7·8%) was significantly smaller than that entering the peritoneal cavity (12·5%). The net water flux showed that secretion predominated in the peritoneal transport whilst absorption predominated in the intestinal transport for both drugs. However, the net water flux in the intestinal lumen after intravenous theophylline (as aminophylline) was significantly smaller than that following phenobarbitone. The differences in transport across the two membranes could be due to differences in the intrinsic properties of the membranes, such as the surface area, the thickness of the membrane and the distribution of blood vessels. Differences could also be due to differences in the pharmacological effects of the drugs.

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In acute drug overdoses, haemoperfusion, haemodialysis, peritoneal dialysis, and combined haemodialysis-haemoperfusion have been used as methods of haemopurification (Weinberger & Hendeles 1980; Gibson 1981). Oral administration of activated charcoal has also been employed to inhibit absorption of excess drugs from the gastrointestinal (GI) lumen into the blood. The GI mucous membranes have a large surface area when considered as a whole. In particular, the total absorptive or exsorptive area of the small intestine has been calculated to be about 200 m^2 in an adult human, and is far larger than that of the peritoneal membrane (about 2 m^2, Csaky 1984).

We have previously reported that intravenously administered drugs are generally transported into the small intestinal lumen and the bile in rats, and that drugs can be removed by adsorption onto orally administered activated charcoal (Arimori & Nakano 1986a, 1987, 1988a, b). However, GI dialysis by oral administration of activated charcoal has not been clearly established as an effective means of removing drugs which have been parenterally administered or have already been absorbed into the systemic circulation from the GI tract. The present study compared GI

dialysis with peritoneal dialysis, which has been established as a valuable means of haemopurification in drug overdoses, for theophylline and phenobarbitone.

Materials and methods

Materials. Aminophylline (Neophyllin) and theophylline were purchased from Eisai Co. (Tokyo) and Wako Pure Chemical Industries (Osaka), respectively. Phenobarbitone sodium was obtained from Daiichi Seiyaku Co. (Tokyo). Scintillation fluid (Instagel) was from United Technologies (Packard, Illinois, USA). All other chemicals used were of analytical grade.

Intestinal dialysis. Male Wistar rats, 220–360 g, were fasted overnight with free access to water. Intestinal exsorption experiments were performed using an in-situ single-pass perfusion technique (Arimori & Nakano 1985). Lactated Ringer solution (37°C) was perfused at 1·3 mL min^{-1} from the duodenum through the small intestine to the ileocaecal junction using a perfusion pump. Theophylline (as aminophylline) or phenobarbitone was injected over about 1 min at a dose of 10 mg kg^{-1} into the right femoral vein. Blood samples and dialysate were collected periodically.

Peritoneal dialysis. A small incision was made in the abdomen and 20 mL of lactated Ringer solution (37°C) was injected into the peritoneal cavity. The dialysate was exchanged for the corresponding volume of new dialysate every 15 min. In the control group of rats, the same operation was performed without injection of the dialysate. Administration of drugs and collection of samples were performed as above.

Water flux. The rate of water movement from the blood into the GI lumen or peritoneal cavity and from the lumen or peritoneal cavity back to the blood was calculated from changes of concentrations in ^3H_2O (0·74 MBq) and phenol red (10 μg mL^{-1}), used as a non-absorbable marker.

Analytical methods. Theophylline and phenobarbitone in the serum and dialysate were determined by high-pressure liquid chromatography (Arimori & Nakano 1985, 1986b). Phenol red was determined spectrophotometrically by absorbance at 550 nm after addition of 4 mL 1M NaOH to 0·5 mL of dialysate. For the assessment of ^3H_2O concentrations, 5 mL of Instagel scintillation fluid was added to 200 μL of dialysate and radioactivity determined in a liquid scintillation counter.

Pharmacokinetic analysis. The intestinal and peritoneal clearance values of theophylline and phenobarbitone were calculated by dividing the overall amount of the drugs transferred into both dialysates in 120 min by the appropriate value for an area under the serum concentration-time curve of the drugs, obtained over the same period of time. The unpaired *t*-test was used to evaluate significant differences.

Results and discussion

Fig. 1 shows the transfer rate of theophylline and phenobarbitone from the blood into the GI lumen and the peritoneal cavity following intravenous administration (10 mg kg^{-1}). The transfer rate of theophylline into the intestinal lumen was similar to that into the peritoneal cavity, with the average amounts of the drug transported into the intestinal and peritoneal dialysates in 120 min 15·7 and 16·5%, respectively. The transfer rate of phenobarbitone into the lumen was significantly lower than that into the peritoneal cavity ($P<0·01$); the average amounts of the drug transported into the intestinal and peritoneal dialysates in 120 min were 7·8 and 12·5%, respectively.

The intestinal and peritoneal clearances of theophylline were 33·8 and 28·0 mL h^{-1}, respectively, and were not significantly different while the intestinal and peritoneal clearances of phenobarbitone were 12·7 and 22·6 mL h^{-1}, respectively.

Table 1 shows water movement into the GI lumen and peritoneal cavity after intravenous administration of aminophylline or phenobarbitone at a dose of 10 mg kg^{-1}. Water movement from the blood into the lumen was less than that from the blood into the peritoneal cavity in both cases. The net water flux showed that secretion predominated in the peritoneal cavity, while absorption predominated in the lumen for both drugs. However, the net water flux into the lumen after intravenous administration of theophylline was significantly smaller than that following phenobarbitone.

Differences in transport across each of the membranes could be due to differences in their intrinsic properties, such as the surface area, the thickness of the membrane, and distribution of blood vessels. They could also be due to the different pharmacological effects of the drugs. The small intestine has a much larger surface area than the peritoneal membrane and therefore the intestine would be expected to be a more permeable or exsorbable organ compared with the peritoneum. Published studies have demonstrated that the net water flux causes a drag effect on permeability of some drugs (Ochsenfahrt & Winne 1974a, b; Kitazawa et al 1975, 1977; Karino et al 1982a, b).

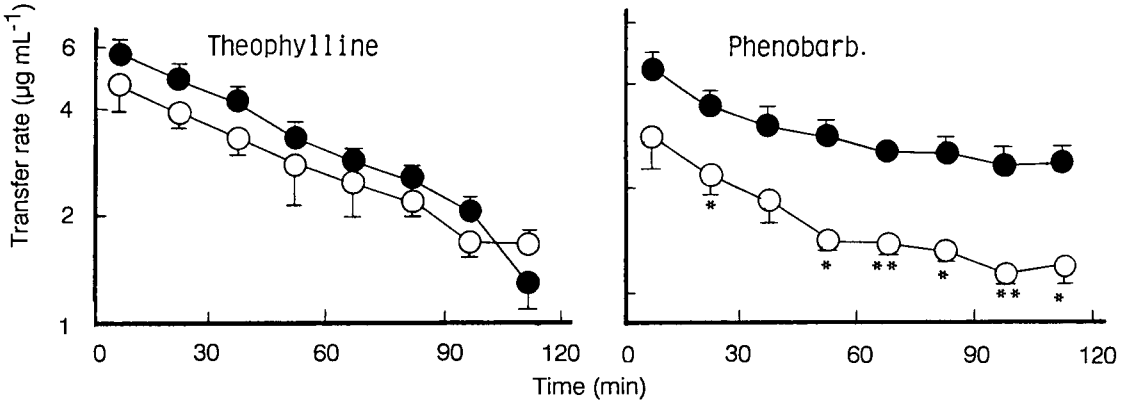

FIG. 1. The transfer rate of theophylline and phenobarbitone from the blood into the intestinal lumen (○) and the peritoneal cavity (●) after intravenous administration of theophylline (as aminophylline) and phenobarbitone (10 mg kg^{-1}) to rats. Each point represents the mean ± s.e.m. of 4 rats. * $P<0·05$, ** $P<0·01$.

Table 1. Water flux into the intestinal lumen or the peritoneal cavity after intravenous administration of theophylline (as aminophylline) and phenobarbitone (10 mg kg^{-1}).

	Water flux from blood to intestinal lumen or peritoneal cavity (mL h^{-1})	Water flux from intestinal lumen or peritoneal cavity to blood (mL h^{-1})	Net water flux[a] (mL h^{-1})
Intestinal lumen			
Theophylline	34·4 ± 0·32	34·5 ± 1·00	−0·10 ± 1·29
Phenobarbitone	35·9 ± 3·80	39·6 ± 4·64	−3·67 ± 0·90[d]
Peritoneal cavity			
Theophylline	49·6 ± 0·32[b]	44·1 ± 1·27[b]	+5·47 ± 1·55[c]
Phenobarbitone	53·8 ± 1·31[b]	48·0 ± 1·65	+5·81 ± 0·83[b]

Each value represents the mean ± s.e.m. of 5 rats. [a]Positive (+) signs indicate secretion and negative (−) signs indicate absorption. [b]$P < 0.01$, [c]$P < 0.05$ vs respective drug in intestinal lumen. [d]$P < 0.05$ vs theophylline in intestinal lumen.

For example, Kitazawa et al (1975) reported that in rats absorption of sulphanilamide, sulphisoxazole, and metoclopramide increased with increasing transmucosal fluid movement from the lumen to the blood and decreased when the movement of water was directed from the blood to the lumen.

During perfusion with lactated Ringer solution in the present study, the net water fluxes were from the GI lumen to the blood and from the blood to the peritoneal cavity (Table 1). This indicates that the effect of solvent drag upon permeability of the intestinal membrane is less than that on permeability of the peritoneal membrane and may be one of the reasons why the amount of phenobarbitone transported from the blood into the GI lumen was less than that entering the peritoneal cavity.

The greater exsorption of theophylline into the GI lumen may be due to the pharmacological effects of this drug. It has been shown that theophylline causes net water secretion by increasing mucosal cyclic AMP levels via phosphodiesterase inhibition (Waldman et al 1977; Beubler 1980). As shown in Table 1, with theophylline, the net intestinal water flux (or the net water absorption from the lumen to the blood) was smaller than with phenobarbitone, although intravenous administration of aminophylline (10 mg kg^{-1}) did not cause net water absorption to convert into net secretion. Therefore, theophylline produces an environment in which it is easier for the drug to enter the GI lumen. Accordingly, the GI lumen may have a more noticeable exsorptive function in a theophylline overdose compared with other drug overdoses.

More data on the transport of other drugs are needed to better compare the efficacy of removal by intestinal and peritoneal dialysis.

References

Arimori, K., Nakano, M. (1985) Transport of theophylline from blood to the intestinal lumen following i.v. administration to rats. J. Pharmacobio-Dyn. 8: 324–327

Arimori, K., Nakano, M. (1986a) Accelerated clearance of intravenously administered theophylline and phenobarbital by oral doses of activated charcoal in rats. A possibility of the intestinal dialysis. Ibid. 9: 437–441

Arimori, K., Nakano, M. (1986b) Transport of phenobarbitone into the intestinal lumen and the biliary tract following i.v. administration to rats. J. Pharm. Pharmacol. 38: 391–393

Arimori, K., Nakano, M. (1987) The intestinal dialysis of intravenously administered phenytoin by oral activated charcoal in rats. J. Pharmacobio-Dyn. 10: 157–165

Arimori, K., Nakano, M. (1988a) Dose-dependency in the exsorption of theophylline and the intestinal dialysis of theophylline by oral activated charcoal in rats. J. Pharm. Pharmacol. 40: 101–105

Arimori, K., Nakano, M. (1988b) Transport of furosemide into the intestinal lumen and the lack of effect of gastrointestinal dialysis by charcoal in rats with acute renal failure. J. Pharmacobio-Dyn. 11: 1–8

Beubler, E. (1980) Influence of vasoactive intestinal polypeptide on net water flux and cyclic adenosine 3′, 5′-monophosphate formation in the rat jejunum. Naunyn-Schmiedeberg's Arch. Pharmacol. 313: 243–247

Csaky, T. Z. (1984) Intestinal absorption of xenobiotics. In: Csaky, T. Z. (ed.) Pharmacology of Intestinal Permeation II.: Handbook of Experimental Pharmacology. 70/II, Springer-Verlag, Berlin, pp 1–30

Gibson, T. P. (1981) Hemoperfusion for drug intoxication: what is it, what does it do, and how much do we know about it? Pharm. Int. 2: 14–17

Karino, A., Hayashi, M., Horie, T., Awazu, S., Minami, H., Hanano, M. (1982a) Solvent drag effect in drug intestinal absorption. I. Studies on drug and D_2O absorption clearances. J. Pharmacobio-Dyn. 5: 410–417

Karino, A., Hayashi, M., Awazu, S., Hanano, M. (1982b) Solvent drag effect in drug intestinal absorption. II. Studies on drug absorption clearance and water influx. Ibid. 5: 670–677

Kitazawa, S., Ito, H., Sezaki, H. (1975) Transmucosal fluid movement and its effect on drug absorption. Chem. Pharm. Bull. 23: 1856–1865

Kitazawa, S., Johno, I., Ito, H. (1977) Studies on characteristics of drug exsorption across the membrane of rat small intestine. Ibid. 25: 2812–2820

Ochsenfahrt, H., Winne, D. (1974a) The contribution of solvent drag to the intestinal absorption of the basic drugs amidopyrine and antipyrine from the jejunum of the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 281: 175–196

Ochsenfahrt, H., Winne, D. (1974b) The contribution of solvent drag to the intestinal absorption of the acidic drugs benzoic acid and salicylic acid from the jejunum of the rat. Ibid. 281: 197–217

Waldman, D. B., Gardner, J. D., Zfass, A. M., Makhlouf, G. M. (1977) Effects of vasoactive intestinal peptide, secretin, and related peptides on rat colonic transport and adenylate cyclase activity. Gastroenterology 73: 518–523

Weinberger, M., Hendeles, L. (1980) Role of dialysis in the management and prevention of theophylline toxicity. Dev. Pharmacol. Ther. 1: 26–30