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Kinetic Evidence for Facilitated Peritoneal Transport of Benzoic acid in Rats

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Abstract

To evaluate the dose dependency in apparent peritoneal permeability (P_d) of benzoic acid as a model compound for a monocarboxylic acid transport system, a kinetic model, which involves changes in the volume and osmolality of the dialysate as well as the diffusion and convection of drugs across the peritoneum, was applied.

We compared the P_d value of benzoic acid to that of phenobarbital which is a more lipophilic drug than benzoic acid. The concentration-time courses of phenobarbital in both peritoneal cavity and serum after the intraperitoneal administration with various doses were parallel according to dose, whereas those of benzoic acid varied in a dose-dependent manner. Using the values of unbound fraction (f_u), the value of P_d for unbound drugs was estimated. The P_d values of benzoic acid at 20 $\mu g\,mL^{-1}$ was three times the value determined at 1000 $\mu g\,mL^{-1}$.

We suggest that certain facilitated transport systems constitute the mechanism of enhanced peritoneal membrane permeability of benzoic acid.

Continuous ambulatory peritoneal dialysis (CAPD) is an accepted alternative to haemodialysis in the treatment of end-stage renal failure. Numerous peritoneal transport studies indicate that a rapid disappearance of drugs from the peritoneal cavity following intraperitoneal application combined with an insufficient drug elimination by CAPD may be explained by basic pharmacokinetic considerations (Keller et al 1990). Unidirectional peritoneal transport of a particular drug has so far not been demonstrated (Deguchi et al 1988; Nakashima et al 1988; Sato et al 1988; Nolph et al 1989).

The peritoneal membrane consists of the visceral peritoneum, which covers the visceral organs such as the intestine, kidneys, and liver, and the parietal peritoneum, which lines the inner surface of the abdominal wall. Accumulating evidence indicates that there are many carrier-mediated transport systems for drugs in the liver as well as intestine. Recently, a carrier mediated transport system specific for benzoic acid, as representative of monocarboxylic acids, in intestinal transport has been demonstrated (Tsuji et al 1994). If drugs can penetrate the surface membranes of these organs, it is reasonable to consider that the rate-limiting steps of drug disappearance from the peritoneal cavity may be influenced by the absorption routes in each organ. Recent studies indicate that the liver plays an important role in drug disposition from the peritoneal cavity through the liver surface (Flessner & Dedrick 1994; Nishida et al 1994). These findings raise the possibility that facilitated transport systems may contribute to the peritoneal absorption of drugs. In the present study, we examined the transport of benzoic acid as a model drug, after intraperitoneal administration to rats.

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Materials and Methods

Materials

Sodium phenobarbital and benzoic acid were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All other chemicals were of reagent grade and were used without further purification.

Analytical methods

The determination of the dialysate volume and osmolality in the serum and dialysate samples were described in a previous report (Nakashima et al 1988). The HPLC conditions for phenobarbital were: mobile phase, 35% methanol-0·01 M sodium biphosphate; flow rate 1·0 $mL\,min^{-1}$; sample volume, 10–30 μL. For benzoic acid and paracetamol the mobile phase, 25% acetonitrile-0·01 M sodium phosphate buffer (pH 3·0) and 20% acetonitrile-0·01 M sodium biphosphate were used, respectively. Shim-pack CLC-ODS (150 mm × 6 mm, Shimadzu) was used as a stationary phase. The regression lines were linear for drug concentrations more than 0·78 $\mu g\,mL^{-1}$ ($r > 0·999$).

Animal experiments

Adult male Wistar rats, 230–270 g (Japan SLC Inc., Shizuoka, Japan) were used in all experiments. All animal experiments were approved by the Institutional Animal Care and Use committee and complied with the standards set out in the Guideline for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University. The rats were anaesthetized with diethyl ether during surgery. The operating procedure employed was essentially the same method as previously described (Nakashima et al 1988). Briefly, polyethylene catheters were inserted into the peritoneal cavity (multiholed tubing, 3 mm i.d.) for instillation and sampling of the peritoneal fluid and the bilateral

jugular veins were exposed for blood sampling. The whole operation was finished within 20 min. Body temperature was maintained at 37 ± 0·4°C with a heating chamber.

Transport experiments were performed by using isotonic Krebs-Ringer solution (pH 7·4), containing 0·2% blue dextran (Sigma Chemical Co., St Louis, MO) as a volume marker, and 20–1000 $\mu g\,mL^{-1}$ of drug was prepared by the same method as described previously (Nakashima et al 1988). At designated times after initiation of dialysis, peritoneal samples (0·6 mL) were collected in a 1·0 mL-syringe by aspiration through the catheter. Blood samples (0·2 mL) were withdrawn from the jugular vein at designated time intervals, and collected in the tubes with a serum separator (Microtainer No. 5960, Becton Dickinson Co., Rutherford, NJ). Serum was separated by centrifugation and stored at −70°C until assayed. For phenobarbital and paracetamol, serum or dialysate was mixed with 1 mL 1 M phosphate buffer (pH 5·0). An internal standard, primidon or barbital, respectively was added and the samples were extracted with ethyl acetate. The samples were centrifuged for 5 min at 3500 rev min^{-1}, and the organic phase was transferred into another series of test tubes for the evaporation. The dried residue was dissolved in 200 μL mobile phase for HPLC. For the benzoic acid, serum was mixed with an equal volume of methanol with *o*-anisic acid as an internal standard. The sample was centrifuged for 10 min at 12 000 rev min^{-1} and supernatant was injected onto the HPLC. The volume, osmolality and drug concentration in the dialysate were simultaneously determined.

Data analysis

The hydrodynamic parameters for the drugs were obtained by simultaneously fitting the profiles of the volume, osmolality and drug concentration in the dialysate to equations A2–4 on the fixed values of hydrodynamic parameters (L_pS, P_s, J_0, σ_d, and σ_s) (Nakashima et al 1988). The data were analysed using a digital computer, FACOM-M760/20, at the Information Processing Center, Kanazawa University. All means are presented with their standard error (mean ± s.e.). Statistical analysis was performed using Student's *t*-test.

Results and Discussion

To evaluate the dose dependency in apparent peritoneal permeability (P_d) of benzoic acid as a model compound for the monocarboxylic acid-transport system, a kinetic model, which involves changes in the volume and osmolality of the dialysate as well as the diffusion of drugs across the peritoneum, was applied (Deguchi et al 1988; Nakashima et al 1988; Sato et al 1988). This model serves to improve our understanding of the relationships between the physiological determinants and peritoneal clearance, and predict changes in the disposition of substrates when homeostasis of the body is perturbed (see Appendix). We compared the P_d value of benzoic acid with that of phenobarbital which is a more lipophilic drug than benzoic acid as listed in Table 1. The concentration–time profiles for the transfer of both phenobarbitone and benzoic acid between the peritoneal cavity and serum after intraperitoneal administration are illustrated in Fig. 1. The drugs disappeared monoexponentially or biexponentially from the peritoneal cavity. The time course of phenobarbitone was not dose-dependent and steady-state serum concentration appeared directly proportional to dose, suggesting linear pharmacokinetics in this dose range. On the other hand, the time courses of benzoic acid showed nonlinear pharmacokinetics. We observed that the binding of benzoic acid to the serum protein was dependent on the total concentration added in serum. As shown in Fig. 2, the unbound fraction of benzoic acid changed from 0·4 to 0·8. The kinetic parameters of the binding to rat-serum protein were estimated by a nonlinear least-squares regression analysis (Metzler et al 1974). An albumin concentration of 470 μM was used for calculation. The values of K_a, n, and α were 3139 ± 247 M^{-1}, 0·94 ± 0·06, and 0·103 ± 0·010 (mean ± s.d.), respectively. No appreciable dependency on drug concentration in the binding of phenobarbital and paracetamol to rat-serum proteins was observed over the concentrations examined in the range of 1–100 $\mu g\,mL^{-1}$, which correspond to the serum concentrations observed in-vivo during the experiments. The values of unbound fraction (f_u) with rat serum protein (0·687 ± 0·021

Table 1. Physicochemical and physiological parameters of acidic drugs.

Drugs	Molecular weight	Apparent partition coefficient in octanol-water at pH 7·4, 37°C (P_{app})	Initial concentration in dialysate ($\mu g\,mL^{-1}$)	Apparent capillary membrane permeability (P_d[a], mL min^{-1})
Benzoic acid	122·12	0·047 ± 0·016	20	3·68 ± 0·21
			50	2·74 ± 0·11
			100	1·71 ± 0·02[b]
			200	2·11 ± 0·05
			1000	1·29 ± 0·02
Phenobarbitone	226·26	12·6 ± 0·6	20	1·74 ± 0·15
			50	1·61 ± 0·07
			200	1·55 ± 0·04
			1000	1·43 ± 0·03
Paracetamol	151·16	2·02 ± 0·02	100	0·726 ± 0·017
Lomefloxacin	351·35	0·045 ± 0·005[c]	100	0·294 ± 0·003[c]

Values are means ± s.d. [a]Values were determined in 250 g rats. [b]Values reported previously (Nakashima et al 1988). [c]Values reported previously (Sato et al 1988).

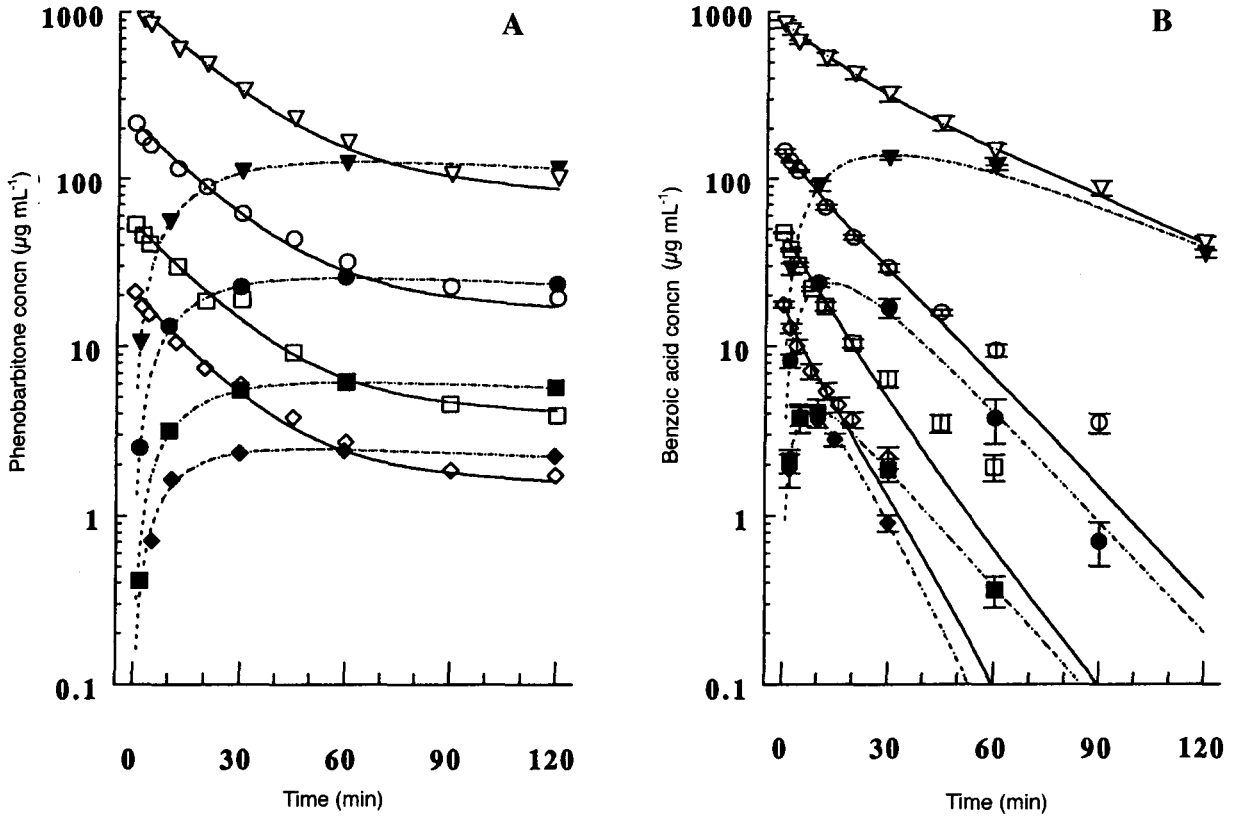

FIG. 1. Model fitted (lines) vs observed serum (closed symbols) and dialysate (open symbols) concentrations after A. intraperitoneal administration of phenobarbital and B. benzoic acid to rats. Initial concentrations of drugs in dialysate were 20 (◇), 50 (□), 200 (○), and 1000 (▽) $\mu g\,mL^{-1}$. Each line was obtained by a nonlinear least squares regression analysis (Metzler et al 1974) fitting all observed data to equations A2, A3, and A4 incorporating the parameters estimated for equation A1. Each value of benzoic acid represents the mean ± s.e. (n = 3–4).

and 0·674 ± 0·051 for phenobarbital and paracetamol, respectively) were used in this study.

Each serum concentration datum in Fig. 1 was treated by nonlinear least-squares regression analysis (Metzler et al 1974) fitting the observed data to equation A1. Estimated parameters were incorporated to estimate the hydrodynamic parameters of the drugs. The solid lines in Fig. 1 represent the fitting curves generated by simultaneously solving the three differential equations (eqns A2–A4) by incorporating equation A1 for the drug concentration in serum. There were no significant differences in the time courses of the osmolality in serum (C^S_{osm}) or dialysate volume between phenobarbitone and benzoic acid. Since C^S_{osm} was determined to be almost 0·3 osm kg^{-1} and was unchanged throughout the transport experiments, C^S_{osm} was regarded as a constant parameter. Using the values of f_u, the serum unbound concentrations ($f_u \cdot C^S_d$) of phenobarbital were determined to be comparable with the dialysate concentrations during the dialysis, so that the bidirectional transfer of drugs through the peritoneal membrane should be taken into consideration. The results indicate that equation A4, in which only the unbound drug is available for the peritoneal membrane transport, fully accounts for the peritoneal transport behaviour of phenobarbital. Since the unbound concentrations ($f_u \cdot C^S_d$) of benzoic acid in serum were considerably lower than the dialysate concentrations in the early phase of dialysis, the transfer of benzoic acid from serum to peritoneal cavity may be negligible for the estimation of P_d.

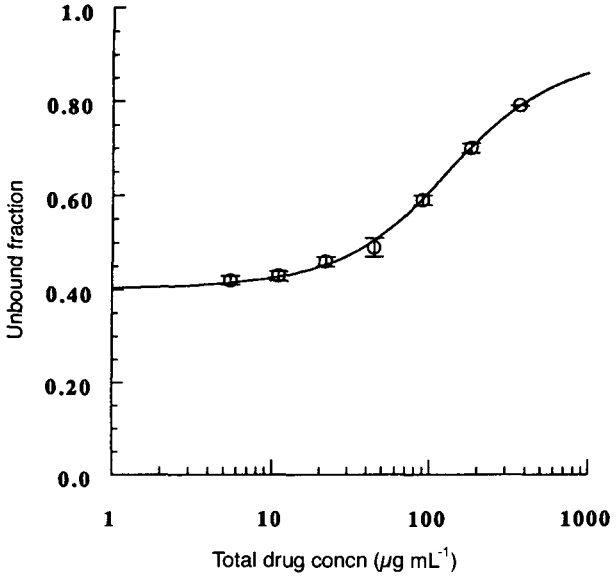

FIG. 2. The relationship between unbound fraction and total concentration of benzoic acid for rat serum in-vitro. Each value represents the mean ± s.e. (n = 3). Solid line shows the curve fitting line.

The estimated hydrodynamic parameters of both phenobarbitone and benzoic acid at various initial dialysate concentrations are listed in Table 1. No significant difference

between doses was noted for P_d values of phenobarbital. However, there were significant differences in P_d values of benzoic acid among doses. The P_d value of benzoic acid at 20 μg mL^{-1} was 3-fold the value determined at 1000 μg mL^{-1}. The P_d values of benzoic acid were more than 4-fold the P_d value of lomefloxacin, the apparent octanol-water partition coefficient of which is similar to that of benzoic acid at pH 7·4 (Table 1) (Sato et al 1988). It is reported that drugs with molecular weights of less than 5200 are able to cross the peritoneal membrane, and drugs with low molecular weights equilibrate rapidly (O'Brien & Mason 1992). As listed in Table 1, the P_d values of benzoic acid were higher than that of paracetamol, which is a more lipophilic drug with a similar low molecular weight.

The above results indicate that benzoic acid was transported through the peritoneal membrane of rats faster than the rates expected from the lipophilicity or molecular weight. It is suggested that certain facilitated transport systems constitute the mechanism of enhanced peritoneal membrane permeability of benzoic acid.

Appendix

Blood concentrations of the drugs (C_d^S) after administration in the peritoneal cavity were explained pharmacokinetically with the following equation:

$$C_d^S = A(e^{-\alpha t} - e^{-\beta t}) \quad \text{(A1)}$$

Concerning the changes in the volume (V), osmolality (C_{osm}), and drug concentration (C_d) in the dialysate after intraperitoneal administration, the differential equations in the final forms can be written as follows:

$$\frac{dV^D}{dt} = -J_V = -J_0 + L_pS \cdot \sigma_s(C_{osm}^D - C_{osm}^S) \quad \text{(A2)}$$

$$\frac{dC_{osm}^D}{dt} = \{J_V(1 - \sigma_s)C_{osm}^D - P_s(C_{osm}^D - C_{osm}^S)\}/V^D \quad \text{(A3)}$$

$$\frac{dC_d^D}{dt} = \{J_V(1 - \sigma_d)C_d - P_d(C_d^D - f_u \cdot C_d^S)\}/V^D$$

$$\cong \{-P_d(C_d^D - f_u \cdot C_d^S)\}/V^D \quad \text{(A4)}$$

where J_V and L_pS represent the net volume flux (mL min^{-1}) and hydraulic conductivity (mL min^{-1} atm^{-1}) across the peritoneum, respectively; C_{osm} and C_d represent the osmolality (osm kg^{-1}) and the concentration of drug, respectively. P_s and σ_s are the apparent capillary membrane permeability and the reflection coefficient of solute, respectively; P_d and σ_d are the apparent capillary membrane permeability and the reflection coefficient of drug, respectively. The superscripts of these terms are: D = dialysate, and S = serum.

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