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Absorption characteristics of model compounds with different molecular weights from the serosal caecal surface in rats

Koyo Nishida, Seiichi Nose, Akiko Kuma, Takahiro Mukai, Shigeru Kawakami, Mikiro Nakashima, Hitoshi Sasaki, Toshiyuki Sakaeda and Junzo Nakamura

Abstract

The purpose of this study was to clarify the absorption characteristics of drugs across the serosal caecal surface membrane, occupying a large absorption area in the peritoneal cavity in rats. Absorption of phenolsulfonphthalein (PSP) and fluorescein isothiocyanate dextrans (FDs) as model drugs after application to the rat serosal caecal surface was investigated using a cylindrical diffusion cell. PSP was absorbed from the rat serosal caecal surface, followed by appearance in the plasma and bile. The time course of the remaining PSP amount in the diffusion cell obeyed first-order kinetics, and the rate constant, K_a, was calculated to be 8.01×10^{-3} min^{-1}. No significant difference was seen in the absorption ratio of PSP, which was approximately 90% in 6 h for three doses (0.3, 0.5 and 1 mg), suggesting linear absorption. Moreover, the absorption ratios of FDs from the rat serosal caecal surface at 3 h decreased with an increase in the molecular weight (24.7% for FD-4, 12.8% for FD-10 and 3.4% for FD-40).

School of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

K. Nishida, S. Nose, A. Kuma, T. Mukai, S. Kawakami, J. Nakamura

Department of Hospital Pharmacy, School of Medicine, Kobe University, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

T. Sakaeda

Department of Hospital Pharmacy, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

M. Nakashima, H. Sasaki

Correspondence: K. Nishida, School of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.
E-mail: koyo-n@net.nagasaki-u.ac.jp

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Introduction

The peritoneal cavity is a potential space for peritoneal dialysis as a long-term renal replacement therapy and intraperitoneal chemotherapy for cancer restricted to the peritoneal cavity (e.g. ovarian carcinoma). Although the intraperitoneal route of drugs has attracted attention, it has not been clarified whether drug absorption from the peritoneal cavity occurs through specific organs. We previously examined the absorption characteristics of several compounds from the liver surface (Nishida et al 1994, 1995a, b, 1996, 1997, 2000) and serosal stomach surface (Mukai et al 1999; Nakamura et al 1999) in rats. Among the peritoneal organs in rats, the caecum is well developed and should play an important role in peritoneal drug transport because of its large surface area. Although the surface area of the serosal caecum in the human peritoneal cavity is proportionally much smaller, the absorption mechanism from the serosal caecal surface needs to be examined to estimate the overall in-vivo absorption rate of a drug after intraperitoneal administration in humans by the extrapolation of animal data.

In the present study, we investigated the absorption of an organic anion, phenolsulfonphthalein (PSP), as a model after application to the rat serosal caecal surface. Furthermore, we selected three fluorescein isothiocyanate dextrans (FDs) with different molecular weights as model macromolecules to examine molecular weight dependency.

Materials and Methods

Chemicals

PSP was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). FDs with average molecular weights of 4400 (FD-4), 11 000 (FD-10), and 40 500 (FD-40) were obtained

from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of reagent grade.

In-vivo experiment

All animal experiments conformed to the Guidelines for Animal Experimentation in Nagasaki University.

Male Wistar rats (250–316 g) were housed in cages in an air-conditioned room and maintained on standard rat foods, with water freely available. The left femoral artery and common bile duct of rats, previously anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.), were cannulated with polyethylene tubes. A cylindrical diffusion cell (6 mm i.d., area 0.28 cm^2) was designed to fit over the rat serosal caecal membrane. The diffusion cell was attached to the rat serosal caecal surface with the biocompatible adhesive Aron Alpha (Sankyo, Tokyo, Japan). The in-vivo experiments were performed under closed cavity conditions, except for the region where the diffusion cell was attached.

Each compound was dissolved in 0.05 mL isotonic phosphate buffer (pH 7.4) and added to the diffusion cell directly. After application to the rat serosal caecal surface, plasma, bile, urine and the remaining solution in the diffusion cell were sampled at predetermined times.

Analytical methods

The concentrations of model compounds in the plasma, bile, urine and remaining solution in the diffusion cell were determined as follows. The concentration of free PSP was determined spectrophotometrically at 560 nm after dilution with 1 M NaOH. The total concentration of free PSP and its metabolite was measured in the same manner after acid hydrolysis (2 M HCl at 100°C for 30 min) (Hart & Schanker 1966). The concentration of PSP metabolite (glucuronic acid conjugate) was estimated from the difference between these values. The PSP metabolite could not be detected in plasma.

The concentrations of FDs as fluorescence in the solution remaining in the diffusion cell were measured by a spectrophotofluorometer at excitation and emission wavelengths of 489 and 515 nm, respectively.

Calculation of moment parameters

The plasma concentration–time profiles and biliary excretion rate–time curves of free PSP and its metabolite were analysed based on statistical moment theory. Moment parameters for the plasma concentration profile of free PSP (AUC_p, MRT_p) and those for biliary excretion rate–time curves of free PSP ($AUC_{b,f}$, $MRT_{b,f}$) and its metabolite ($AUC_{b,m}$, $MRT_{b,m}$) were calculated using a linear trapezoidal formula and extrapolation to infinity based on a monoexponential equation (Yamaoka et al 1978).

The overall absorption and excretion process can be evaluated with moment parameters. In particular, AUC_p and MRT_p are useful parameters for roughly evaluating the drug absorbability from the peritoneal cavity with regard to extent and rate, respectively. Also, AUC_b and MRT_b are useful parameters for roughly evaluating the biliary excretion of drug with regard to extent and rate, respectively.

Statistical analysis

Statistical analysis was performed by applying the unpaired Student's t-test and $P < 0.05$ was considered to be statistically significant. All values were expressed as the mean value ± s.e. of at least four experiments.

Results and Discussion

The established experimental system, utilizing a cylindrical diffusion cell attached to the rat serosal caecal surface, enabled us to examine drug absorption from the serosal caecal surface without interference by absorption from other sites. We selected PSP as a model low molecular drug because the disposition characteristics have been investigated previously (Enna & Schanker 1973; Nishida et al 1989; Kakutani et al 1992).

Plasma concentration and biliary excretion of PSP after application to the serosal caecal surface

Figure 1A shows the plasma concentration profile of PSP after application to the rat serosal caecal surface at doses of 0.3, 0.5 and 1 mg. PSP appeared in the plasma, suggesting drug absorption from the rat serosal caecal surface. The plasma concentration of PSP after application to the rat serosal caecal surface reached a maximum approximately 1 h after dosing (Figure 1A). After absorption from the rat serosal caecal surface, PSP was excreted into the bile, as shown in Figure 1B. The metabolite of PSP (glucuronic acid conjugate) was also excreted into the bile (Figure 1C). Therefore, PSP demonstrated fast absorption from the serosal caecal surface, although it is poorly absorbed from the gastrointestinal mucosa because it is highly ionized and has a very small partition coefficient at physiological pH (Shanker et al 1958).

PSP absorbed from the serosal caecal surface tended to be excreted into the urine as compared with intravenous administration (Table 1). The difference in the MRT_p values between the serosal caecal surface application and intravenous administration corresponded to the mean absorption time from the serosal caecal surface. The mean absorption time of PSP after application to the rat serosal caecal surface was calculated to be 75.8 min from the MRT_p values given in Table 2.

Dose dependency of PSP absorption from the serosal caecal surface

As shown in Figures 1A–C, the plasma concentration profiles of free PSP and the biliary excretion rate–time

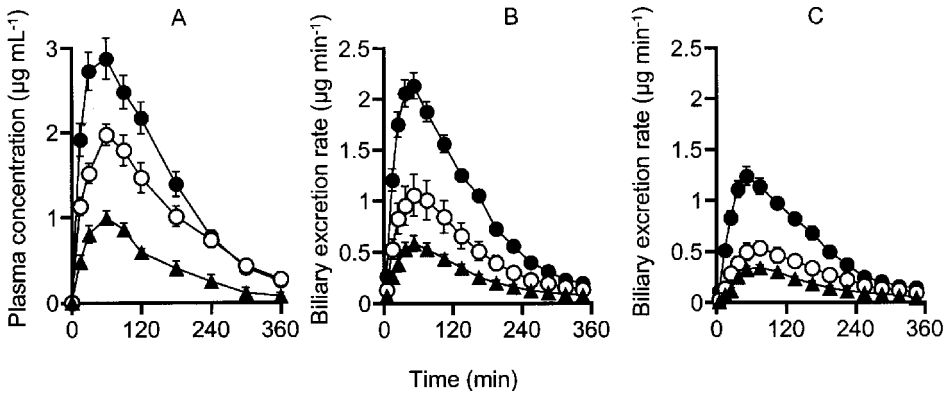

Figure 1 Plasma concentrations of free phenolsulfonphthalein (PSP; A) and biliary excretion rates of free PSP (B) and its metabolite (C) after application to the rat serosal caecal surface at doses of 0.3 (▲), 0.5 (○) and 1 mg (●). Each point represents the mean±s.e. of at least five experiments.

Table 1 Recovery (% of dose) of free phenolsulfonphthalein and its metabolite at 6 h after application to the rat serosal caecal surface or after intravenous administration.

	Dose (mg)	Diffusion cell	Bile			Urine		
		Free	Total	Free	Metabolite	Total	Free	Metabolite
Application to serosal caecal surface	0.3	14.3±3.0	49.6±2.4	30.6±2.4	19.0±1.5	35.3±3.4	14.8±2.8	20.5±2.4
	0.5	9.9±1.9	54.9±6.2	35.2±6.4	19.7±2.4	30.1±4.7	12.3±3.7	17.7±3.0
	1	10.4±1.7	54.1±2.1	33.6±1.5	20.6±1.6	29.4±2.5	20.7±2.5	8.7±1.2***,†
Intravenous administration	1	–	69.2±4.0	42.1±2.2	27.1±3.3	23.1±5.7	16.2±4.9	6.9±1.0

Each value is the mean±s.e. of at least five experiments. Significantly different compared with 0.3 mg (***$P < 0.001$) and 0.5 mg (†$P < 0.05$).

Table 2 Moment parameters of phenolsulfonphthalein after application to the rat serosal caecal surface or after intravenous administration.

	Dose (mg)	AUC_p ($\mu g\ mL^{-1}\ min$)	AUC_p/dose ($min\ mL^{-1}$)	MRT_p (min)	$AUC_{b,f}$ (μg)	$MRT_{b,f}$ (min)	$AUC_{b,m}$ (μg)	$MRT_{b,m}$ (min)
Application to serosal caecal surface	0.3	178.1±18.2	0.59±0.06	160.5±30.8	102.5±8.6	163.7±28.9	67.7±7.5	168.5±23.7
	0.5	339.3±47.9	0.68±0.10	180.9±21.0	188.9±34.5	152.2±7.6	111.6±10.2	194.5±21.6
	1	545.0±30.5	0.55±0.03	151.7±15.9	354.7±16.7	139.9±4.5	220.7±17.1	149.2±9.1
Intravenous administration	1	1026.3±132.1	1.03±0.13	75.9±9.0	457.3±32.6	58.4±4.6	301.2±38.5	80.1±6.4

AUC_p, MRT_p, moment parameters for the plasma concentration profile of free PSP; $AUC_{b,f}$, $MRT_{b,f}$, moment parameters for biliary excretion rate–time curves of free PSP; $AUC_{b,m}$, $MRT_{b,m}$, moment parameters for bililary excretion rate-time curves of the PSP metabolite. Each value is the mean±s.e. of at least five experiments.

curves of free PSP and its metabolite were almost identical at doses of 0.3, 0.5 and 1 mg.

The recoveries (% of dose) of free PSP and its metabolite in bile, urine and the diffusion cell at doses of 0.3, 0.5 and 1 mg are given in Table 1. Absorption ratios of PSP in 6 h, calculated from the remaining amount of PSP in the diffusion cell, were 85.7, 90.1 and 89.6% at doses of 0.3, 0.5 and 1 mg, respectively, indicating that PSP absorption from the rat serosal caecal surface shows no saturation over the dose range tested. The decrease in the urinary recovery ratio of the PSP metabolite could be responsible for the saturation of the metabolic process of PSP at a high dose. Moreover, the PSP renal clearance after application to the rat serosal caecal surface at a dose of 1 mg

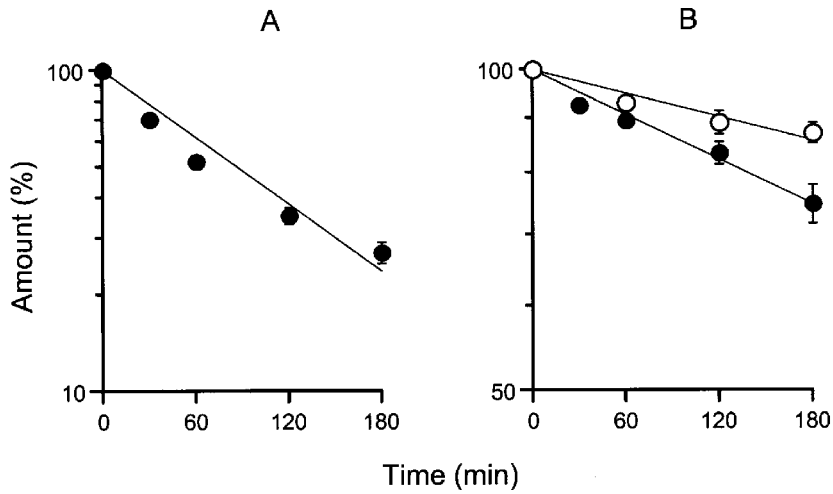

Figure 2 Semi-log plots of the remaining amount of free phenolsulfonphthalein (●; A), fluorescein isothiocyanate dextran with an average molecular weight of 4400 (●) and fluorescein isothiocyanate dextran with an average molecular weight of 11000 (○) (B) in the diffusion cell after application to the rat serosal caecal surface at a dose of 1 mg. Each point represents the mean ± s.e. of at least four experiments.

(0.384 mL min^{-1}) was significantly declined ($P < 0.05$) compared with at a dose of 0.3 mg (0.808 mL min^{-1}). This suggests that the urinary secretion of PSP is also saturable.

Moment parameters for the plasma concentration profiles of free PSP and biliary excretion rate–time curves of free PSP and its metabolite are given in Table 2. No significant difference was seen in the AUC_p/dose value among the three doses, suggesting linear absorption of PSP from the rat serosal caecal surface. Accordingly, a specific transport mechanism such as active transport might not be involved in PSP absorption from the rat serosal caecal surface, similar to other organ surfaces (Nishida et al 1995a; Mukai et al 1999).

Time course of PSP amount in the diffusion cell after application to the serosal caecal surface

To assess the absorption characteristics from the rat serosal caecal surface, we studied the time course of the PSP amount in the diffusion cell (Figure 2A). A semi-log plot of the remaining PSP amount in the diffusion cell until 180 min gave a straight line (correlation coefficient 0.94) as shown in Figure 2A, indicating that PSP absorption from the rat serosal caecal surface proceeds via first-order kinetics. Its first-order absorption rate constant, K_a, was calculated to be 8.01×10^{-3} min^{-1}.

Absorption of FDs with different molecular weights from the serosal caecal surface

Since there was a significant relationship between the molecular weight and absorption rate constant after application of model compounds to the liver and serosal stomach surface (Nishida et al 1996; Mukai et al 1999), the molecular weight would appear to play an important role in drug absorption from the serosal caecal surface. We selected FDs as model macromolecules, and examined the molecular weight dependence of drug absorption from the serosal caecal surface in rats, because dextrans are reasonably resistant to metabolic degradation and their in-vivo fate has been characterized fully in rats (Nishida et al 1991; Mehvar & Shepard 1992; Mehvar et al 1994).

Figure 1B shows the time courses of FD-4 and FD-10 recovery in the diffusion cell until 180 min after application to the rat serosal caecal surface. This suggests that their absorption from the rat serosal caecal surface proceeds via first-order kinetics, similar to the small molecule, PSP (Figure 2A). The K_a values for FD-4 and FD-10 were calculated to be 1.62×10^{-3} and 0.85×10^{-3} min^{-1}, respectively. The absorption ratios from the rat serosal caecal surface in 3 h were calculated from the amount recovered from the diffusion cell as 73.0% for PSP, 24.7% for FD-4, 12.8% for FD-10 and 3.4% for FD-40. Accordingly, comparison of the absorption of several model compounds with different molecular weights demonstrated that the absorption ratio decreased with an increase in the molecular weight.

Comparison of the absorption rates of PSP among the liver, stomach and caecal surfaces

We compared the absorption rate of PSP from the other previously investigated organ surfaces, namely, liver (Nishida et al 1995a) and stomach (Nakamura et al 1999), based on the clearance concept. The K_a values of PSP from the surface of the liver (Nishida et al 1995a) and stomach (Nakamura et al 1999) were obtained by the time courses of PSP recovery in the diffusion cell. We derived the apparent permeability coefficients (P_{app}) representing drug absorbability from the organ surface. P_{app} was calculated by:

$$P_{app} = (K_a \times V_a)/\text{area}$$

where V_a and area are the application volume and effective application area of the diffusion cell, respectively.

The P_{app} values of PSP from the liver, stomach and caecal surface were calculated to be 10.9, 12.3 and 11.2 l m min^{-1}, respectively. No significant difference was seen in P_{app} among the three organ surfaces, implying that these surface membranes are broadly uniform with respect to absorption characteristics.

In conclusion, the absorption characteristics of model compounds from the serosal caecal surface in rats are useful to estimate the overall drug absorption rate after intraperitoneal administration.

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