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

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Shortened title: Drug absorption from serosal **cecal** surface

Abstract

The purpose of this study is to clarify the absorption characteristics of drugs across the serosal **cecal** surface membrane occupying a large absorption area in the peritoneal cavity **in rats**. Absorptions of phenolsulphonphthalein (PSP) and fluorescein isothiocyanate-dextrans (FDs) as model drugs after application to the rat serosal **cecal** surface were investigated in rats, employing a cylindrical diffusion cell. PSP was absorbed from the rat serosal **cecal** 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 its rate constant K_a was calculated to be $8.01 \times 10^{-3} \text{ min}^{-1}$. No significant difference was seen in the absorption ratio of PSP which was approximately 90 % in 6 h among three doses (0.3, 0.5 and 1 mg), suggesting a linearity of absorption. Moreover, the absorption ratios of FDs from the rat serosal **cecal** 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).

Introduction

The peritoneal cavity is a potential space for peritoneal dialysis as a long-term renal replacement therapy and intraperitoneal (i.p.) chemotherapy of cancer restricted to the peritoneal cavity e.g. ovarian carcinoma, and so on. Although the i.p. 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 (Nakamura et al 1999; Mukai et al 1999) in rats as a series of investigations. Among the peritoneal organs in rats, the cecum 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 cecum in the human peritoneal cavity is proportionally much smaller, the absorption mechanism from the serosal cecal surface needs to be examined to estimate the overall in-vivo absorption rate of a drug after i.p. administration in human beings from the extrapolation of animal data such as rats.

In the present study, we investigated the absorption of an organic anion phenolsulphonphthalein as a model after application to the rat serosal cecal surface. Furthermore, we selected three fluorescein isothiocyanate-dextran with different molecular weights as model macromolecules to examine the molecular weight dependency.

Materials and Methods

Chemicals

Phenolsulphonphthalein (PSP) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Fluorescein isothiocyanate-dextran (FDs) with average molecular weights of 4,400 (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 in the present study conformed to the Guideline 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 and water freely available. The left femoral artery and common bile duct of the rats previously anaesthetized with sodium pentobarbitone (50 mg kg⁻¹, i.p.) were cannulated with polyethylene tubes. A cylindrical diffusion cell (i.d. 6 mm, area 0.28 cm²) was designed to fit over the rat serosal cecal membrane. The diffusion cell was attached to the rat serosal cecal surface with adhesive chemical Aron Alpha (Sankyo, Tokyo, Japan). The in vivo experiments was performed under closed cavity conditions except for the region where the diffusion cell was attached. A schematic drawing outlining the set up of the in vivo experiment was shown in Fig. 1.

Each compound was dissolved in 0.05 mL of isotonic phosphate buffer (pH 7.4) and added to the diffusion cell directly. After application to the rat serosal cecal surface, plasma, bile, urine and the remaining solution in the diffusion cell were sampled at the specified 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 they were subjected to 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 the 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 profiles and biliary excretion rate-time curves of free PSP and its metabolite were analyzed based on statistical moment theory. Moment parameters for 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 infinite time 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 unpaired Student's *t*-test. $P < 0.05$ was

considered to be statistically significant. All values were expressed as the mean value \pm

standard error of at least four experiments.

Results and Discussion

We established the experimental system utilizing a cylindrical diffusion cell attached to the rat serosal **cecal** surface. This experimental system enables us to examine drug absorption from the serosal **cecal** surface without interference by absorption from other sites. We selected PSP as a low molecular weight model because their 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 **cecal** surface

Figure 2A shows the plasma concentration profile of PSP after application to the rat serosal **cecal** surface at doses of 0.3, 0.5 and 1 mg. PSP appeared in the plasma, suggesting the occurrence of drug absorption from the rat serosal **cecal** surface. The plasma concentration of PSP after application to the rat serosal **cecal** surface reached a maximum about 1 h after dosing (Fig. 2A). After absorption from the rat serosal **cecal** surface, PSP was excreted into the bile as shown in Fig. 2B. The metabolite of PSP (glucuronic acid conjugate) was also excreted into the bile (Fig. 2C). Therefore, PSP demonstrated fast absorption from the serosal **cecal** 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 **cecal** surface tended to be excreted into the urine, compared with i.v. administration (Table 1). The difference in the MRT_p values between the serosal **cecal** surface application and i.v. administration corresponded to the mean time value for the absorption from the serosal **cecal** surface (MAT). MAT of PSP after application to the rat serosal **cecal** surface was calculated to be 75.8 min from the MRT_p values listed in Table 2.

Dose-dependency on PSP absorption from serosal cecal surface

As shown in Figs 2A, 2B and 2C, the shapes of plasma concentration profiles of free PSP and the biliary excretion rate-time curves of free PSP and its metabolite were almost identical among 0.3, 0.5 and 1 mg.

Recoveries (% of dose) of free PSP and its metabolite in the bile, urine and 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 the PSP absorption from the rat serosal cecal surface shows no saturation within the dose range used. The decrease in the urinary recovery ratio of 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 cecal surface at a dose of 1 mg ($0.384 \text{ mL min}^{-1}$) was significantly declined ($P < 0.05$), compared to 0.3 mg ($0.808 \text{ mL min}^{-1}$). 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 listed in Table 2. No significant difference was seen in the AUC_p/dose value among the three doses, suggesting the linearity of PSP absorption from the rat serosal cecal surface. Accordingly, a specific transport mechanism such as active transport might not be involved in PSP absorption from the rat serosal cecal 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 cecal surface

To assess the absorption characteristics from the rat serosal cecal surface, we studied the time course of the PSP amount in the diffusion cell (Fig. 3A). 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 Fig. 3A, indicating that the PSP absorption from the rat serosal **cecal** surface proceeds via first-order kinetics. Its first-order absorption rate constant K_a was calculated to be $8.01 \times 10^{-3} \text{ min}^{-1}$.

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

Since there was a significant relation 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 appeared to play an important role in drug absorption from the serosal **cecal** surface. We selected FDs as model macromolecules, and examined the molecular weight dependence of drug absorption from the serosal **cecal** surface in rats because dextrans are fairly 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 2B shows the time courses of FD-4 and FD-10 recovery in the diffusion cell until 180 min after application to the rat serosal **cecal** surface. This suggests that their absorption from the rat serosal **cecal** surface proceeds via first-order kinetics, similar to a small molecule PSP (Fig. 3A). The K_a values for FD-4 and FD-10 were calculated to be 1.62×10^{-3} and $0.85 \times 10^{-3} \text{ min}^{-1}$, respectively. The absorption ratios from the rat serosal **cecal** 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 the increase in the molecular weight.

Comparison of the absorption rates of PSP among the liver, stomach and cecal surface

We compared the absorption rate of PSP from the other previously investigated organ surfaces

such as liver (Nishida et al 1995a) and stomach (Nakamura et al 1999) based on the clearance concept. The K_a values of PSP from the other organ surfaces of liver (Nishida et al 1995a) and stomach (Nakamura et al 1999) have been 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

$$P_{app} = \frac{K_a \cdot V_a}{Area}$$

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

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

In conclusion, the absorption characteristics of the model compounds from the serosal cecal surface in rats would be useful to estimate the overall drug absorption rate after i.p. administration.

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References

- Enna, S. J., Schanker, L. S. (1973) Phenol red absorption from the rat lung: evidence of carrier transport. *Life Sci.* **12**: 231-239
- Hart, L. G., Schanker, L. S. (1966) The chemical forms in which phenol red is secreted into the bile of rats. *Proc. Soc. Exp. Biol. Med.* **123**: 433-435
- Kakutani, T., Endo, K., Nara, E., Nakazora, S., Hashida, M. (1992) Effect of chronic administration of phenobarbital on the hepatobiliary transport of phenol red: assessment by statistical moment analysis. *Pharm. Res.* **9**: 908-914
- Mehvar, R., Shepard, T. L. (1992) Molecular-weight-dependent pharmacokinetics of fluorescein-labeled dextrans in rats. *J. Pharm. Sci.* **81**: 908-912
- Mehvar, R., Robinson, M. A., Reynolds, J. M. (1994) Molecular weight dependent tissue accumulation of dextrans: in vivo studies in rats. *J. Pharm. Sci.* **83**: 1495-1499
- Mukai, T., Tsurumaru, A., Mera, K., Nishida, K., Nakamura, J., Sasaki, H., Sakaeda, T. (1999) Absorption characteristics of model drugs from the gastric serosal surface in rats. *Pharm. Pharmacol. Commun.* **5**: 609-614
- Nakamura, J., Tsurumaru, A., Mera, K., Mukai, T., Nishida, K., Sasaki, H. (1999) Absorption of drugs applied to the gastric serosal surface in rats. *Pharm. Pharmacol. Commun.* **5**: 519-522
- Nishida, K., Tonegawa, C., Kakutani, T., Hashida, M., Sezaki, H. (1989) Statistical moment analysis of hepatobiliary transport of phenol red in the perfused rat liver. *Pharm. Res.* **6**: 140-146
- Nishida, K., Mihara, K., Takino, T., Nakane, S., Takakura, Y., Hashida, M., Sezaki, H. (1991) Hepatic disposition characteristics of electrically charged macromolecules in rat *in vivo* and in the perfused liver. *Pharm. Res.* **8**: 437-444
- Nishida, K., Sato, N., Sasaki, H., Nakamura, J. (1994) Absorption of organic anions as model

- drugs following application to rat liver surface in-vivo. *J. Pharm. Pharmacol.* **46**: 867-870
- Nishida, K., Sato, N., Sasaki, H., Nakamura, J. (1995a) Mechanism for drug absorption from rat-liver surface membrane: effect of dose and transport inhibitors on the pharmacokinetics of phenol red. *J. Pharm. Pharmacol.* **47**: 227-231
- Nishida, K., Sato, N., Sasaki, H., Nakamura, J. (1995b) Effect of albumin on the absorption of phenol red, bromphenol blue and bromosulphonphthalein as model drugs from the liver surface membrane in rats. *Biol. Pharm. Bull.* **18**: 1548-1550
- Nishida, K., Sato, N., Sasaki, H., Nakamura, J. (1996) Absorption characteristics of dextrans with different molecular weights from the liver surface membrane in rats: implications for targeting to the liver. *J. Drug Target.* **4**: 141-150
- Nishida, K., Sato, N., Nakakoga, Y., Mukai, T., Sasaki, H., Nakamura, J. (1997) Effect of application volume and area on the absorption of phenol red, as a model drug, from the liver surface in rats. *J. Pharm. Pharmacol.* **49**: 976-980
- Nishida, K., Nakakoga, Y., Sato, N., Kawakami, S., Mukai, T., Sasaki, H., Sakaeda, T., Nakamura, J. (2000) Effect of viscous additives on drug absorption from the liver surface in rats using phenol red as a model. *Eur. J. Pharm. Biopharm.* **50**: 397-402
- Shanker, L. S., Tocco, D. J., Brodie, B. B., Hogben, C. A. M. (1958) Absorption of drugs from the rat small intestine. *J. Pharmacol. Exp. Ther.* **123**: 81-88
- Yamaoka, K., Nakagawa, T., Uno, T. (1978) Statistical moments in pharmacokinetics. *J. Pharmacokinet. Biopharm.* **6**: 547-558

Table 1 Recovery (% of dose) of free PSP and its metabolite at 6 h after application to the rat serosal **cecal** surface or i.v. administration

| Dose (mg) | Diffusion cell | Bile | | | Urine | | |
|---|----------------|--------------|--------------|--------------|--------------|--------------|------------------------------|
| | Free | Total | Free | Metabolite | Total | Free | Metabolite |
| Application to serosal cecal 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 |
| I.v. 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 from 0.3 mg (^{***} $P < 0.001$) and 0.5 mg ([§] $P < 0.05$).

Table 2 Moment parameters of PSP after application to the rat serosal **cecal** surface or i.v. administration

| Dose (mg) | AUC _p (µg 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 cecal 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 |
| I.v. 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 |

Each value is the mean ± s.e. of at least five experiments.

Figure caption

Figure 1 A schematic drawing of in vivo experiment. A cylindrical diffusion cell was attached to the rat serosal cecal surface using Aron Alpha biocompatible glue. All dimensions are approximate.

Figure 2 Plasma concentrations of free PSP (A) and biliary excretion rates of free PSP (B) and its metabolite (C) after application to the rat serosal cecal surface at doses of 0.3 (▲), 0.5 (○) and 1 mg (●). Each point represents the mean \pm s.e. of at least five experiments.

Figure 3 Semi-log plots of the remaining amount of free PSP (●) (A), FD-4 (●) and FD-10 (○) (B) in the diffusion cell after application to the rat serosal cecal surface at a dose of 1 mg. Each point represents the mean \pm s.e. of at least four experiments.

Fig. 1

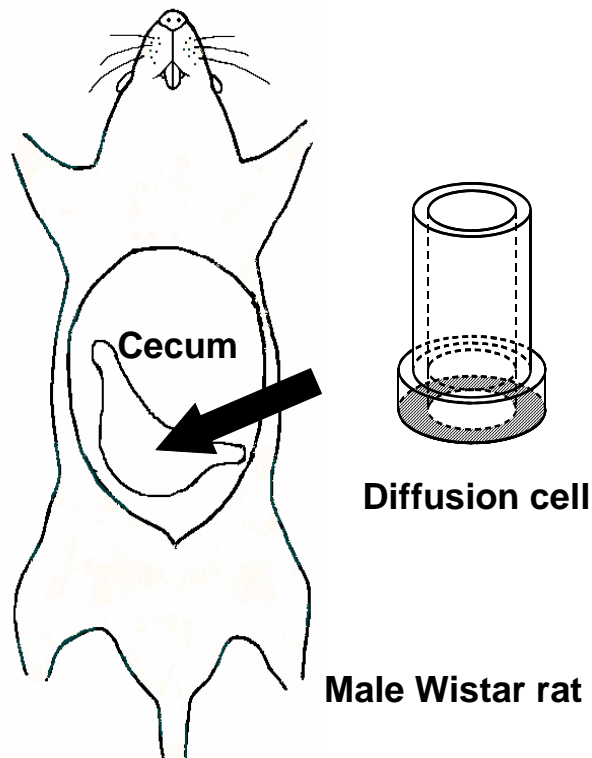


Fig. 2

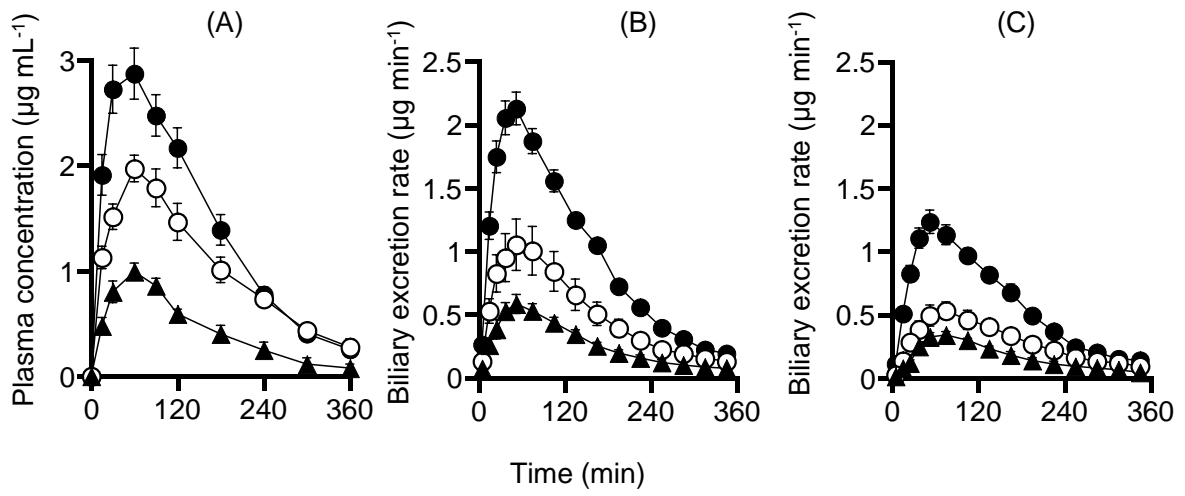


Fig. 3

