JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 1073–1077

Received December 29, 2004
Accepted March 25, 2005
DOI 10.1211/0022357056677
ISSN 0022-3573

Absorption characteristics of model compounds from the small intestinal serosal surface and a comparison with other organ surfaces

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Abstract

We examined the absorption of phenolsulfonphthalein (PSP) and fluorescein isothiocyanate dextrans (FD-4, MW 4400; FD-10, MW 9500; FD-40, MW 40 500) as model compounds through the small intestinal serosal surface. After application to the rat small intestinal serosal surface using a cylindrical diffusion cell, each compound was absorbed at different rates. The absorption ratios in 6 h after PSP, FD-4, FD-10 and FD-40 application were calculated to be 89.2, 34.6, 14.9 and 2.1% of dose, respectively. Elimination profiles of PSP, FD-4 and FD-10 from the small intestinal serosal surface obeyed first-order kinetics. Moreover, we calculated the apparent permeability coefficient P_{app} for comparison to other organ surfaces. The kidney had the highest absorption efficiency, as shown by having more than 1.5 times significantly higher P_{app} values of PSP, FD-4 and FD-10. Similar to the other organ surfaces, a correlation was observed between the P_{app} of the small intestine and the molecular weight of these hydrophilic compounds. In addition, the small intestine is likely to contribute largely to hydrophilic compound absorption from the peritoneal cavity, judging from absorption clearance, CL_a, calculated using the peritoneal organ surface area.

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Acknowledgments and funding:
We wish to thank Yukari Ide and Kayoko Tsuda for their skilled technical assistance. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, by a Grant-in-Aid from the Uehara Memorial Foundation, by a Grant-in-Aid from the Nakatomi Foundation and by a Grant-in-Aid for Scientific Research from the President of Nagasaki University.

Introduction

The peritoneal cavity is a useful space for intraperitoneal (i.p.) chemotherapy of cancers restricted to the peritoneal cavity, such as peritoneal carcinomatosis and ovarian cancer. The clarification of drug absorption characteristics from the peritoneal cavity would improve peritoneal chemotherapy. Intraperitoneally administered drugs are possibly absorbed from the peritoneum surrounding these peritoneal organs and the abdominal wall. Previously, we reported on drug absorption from the liver surface (Nishida et al 1994), kidney surface (Nishida et al 2004), serosal stomach surface (Mukai et al 1999; Nakamura et al 1999) and serosal caecal surface (Nishida et al 2002) in rats utilizing the diffusion cell, and demonstrated the possibility that the peritoneal organ surface was largely responsible for drug absorption from the peritoneal cavity. Because the small intestine has the largest peritoneal area (Flessner 1996), its contribution to drug absorption from the peritoneal cavity might be considerable.

In this study, we examined the absorption of model compounds with different molecular weights after their application to the rat small intestinal serosal surface by utilizing a diffusion cell. Phenolsulfonphthalein (PSP) and fluorescein isothiocyanate dextrans (FDs) were selected as model compounds because their absorption characteristics from other organ surfaces have been studied (Nishida et al 1995, 1996, 2002, 2004; Mukai et al 1999). Furthermore, we compared the absorption rates among peritoneal organs using quantitative pharmacokinetic parameters.

Materials and Methods

Chemicals

PSP (MW 354) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). FDs (FD-4, MW 4400; FD-10, MW 9500; FD-40, MW 40 500) were obtained from the Sigma Chemical Co. (St Louis, MO). All other chemicals were of reagent grade.

Animal experiments

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

Male Wistar rats (260–290 g) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.) and their body temperatures were kept at 37 °C by a heat lamp during the experiments. The left femoral artery was cannulated with a polyethylene tube (i.d. 0.5 mm, o.d. 0.8 mm; Dural Plastics, Dural, Australia). An incision was made in the middle of the abdomen and the common bile duct was cannulated with a polyethylene tube (i.d. 0.28 mm, o.d. 0.61 mm; Becton Dickinson & Co., Parsippany, NJ).

The drug solutions were prepared in isotonic phosphate buffer (pH 7.4) to yield a concentration of 30 mg mL^{-1} and were administered as follows. A cylindrical diffusion cell (i.d. 4 mm, area 0.13 cm^2) was attached to the rat small intestinal serosal surface with the biocompatible glue Aron Alpha (Sankyo Co. Ltd, Tokyo, Japan). The drug solution (1 mg in 0.0334 mL) was added directly to the diffusion cell. The top of the diffusion cell was sealed with aluminium foil to prevent evaporation.

Blood samples (200 μL) were collected at selected times after dosing from the heparinized cannula inserted into the femoral artery over a 6-h period, and were centrifuged at 15 000 rpm for 5 min. Bile samples were collected at appropriate time intervals for 6 h. At 6 h after the application, urine was collected directly from the bladder with a syringe, and the drug solution remaining in the diffusion cell was withdrawn. Certain experiments were carried out up to 0.5, 1, 2, 4 and 6 h after drug application.

Analysis

The concentrations of free PSP in the plasma, bile, urine and the solution remaining in the diffusion cell were determined spectrophotometrically at 560 nm after dilution with 1 M NaOH solution by modifying the method previously described (Hart & Schanker 1966). The total concentrations of free PSP and its metabolite were similarly measured after acid hydrolysis (1 M HCl at 100 °C for 30 min). The concentration of the PSP metabolite was estimated from the difference between these values.

The concentration of FDs as fluorescence was measured using a spectrophotofluorometer at excitation and emission wavelengths of 489 and 515 nm, respectively, by modifying the method previously described (Kurtzhals et al 1989).

Analytical validation information is as follows. The limits of quantification of PSP, FD-4, FD-10 and FD-40 were 0.010, 0.0027, 0.0010 and 0.0038 μg mL^{-1}, respectively. The reproducibility of quantification was guaranteed by the inter-day coefficient of variation (PSP, 1.1%; FD-4, 0.9%; FD-10, 1.4%; FD-40, 3.8%) and the intra-day coefficient of variation (PSP, 0.9%; FD-4, 0.4%; FD-10, 1.2%; FD-40, 1.2%). The linearity of the calibration curve was guaranteed by the correlation coefficient ($R^2 > 0.997$). In addition, the validation data were not influenced by the presence of endogenous compounds.

Statistical analysis

In all cases, post-hoc comparisons of the means of individual groups were performed using Tukey's test, following a one-way ANOVA. A significance level of $P < 0.05$ denoted significance in all cases. All values were expressed as the mean value ± standard error (s.e.) of at least four different independent experiments.

Results

Possibility of drug absorption from the rat small intestinal serosal surface

Figure 1 shows the plasma concentration profiles of PSP, FD-4 and FD-10 after application to the rat small intestinal serosal surface at a dose of 1 mg using the diffusion cell. After absorption from the small intestinal serosal surface each model compound appeared in the plasma. FD-4 and FD-10 appeared in the plasma at significantly lower concentrations than PSP at each time point (Figure 1).

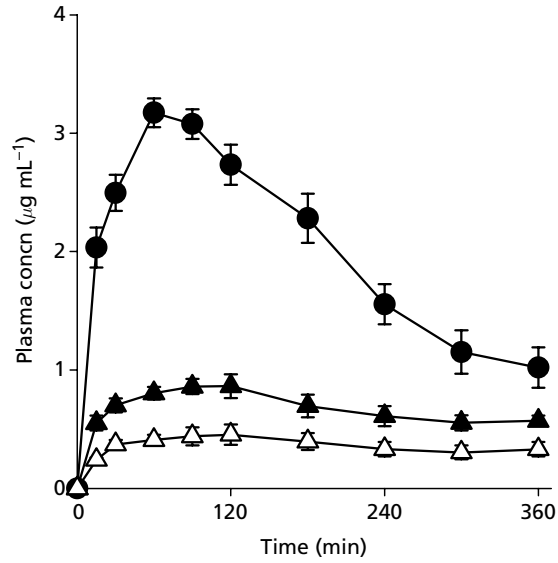

Figure 1 Plasma concentration profiles of PSP (●), FD-4 (▲) and FD-10 (△) after application to the rat small intestinal serosal surface at a dose of 1 mg. Each point represents the mean ± s.e. of six experiments.

Recovery of model compounds in the diffusion cell, bile and urine

Table 1 lists the recovery of model compounds in the diffusion cell, bile and urine 6 h after application to the small intestinal serosal surface at a dose of 1 mg. The absorption ratio (percentage of dose) in 6 h of model compounds from the small intestinal serosal surface was calculated from the amount recovered from the diffusion cell at 6 h after application. The absorption ratio of PSP (89.2%) was significantly larger than FD-4 (34.6%), FD-10 (14.9%) and FD-40 (2.1%).

After application to the small intestinal serosal surface, model compounds were excreted into the bile and urine as listed in Table 1. On the other hand, FD-4 and FD-10 absorbed from the small intestinal serosal surface were mainly excreted into the urine, and their biliary excretion was significantly low compared to PSP.

Elimination profiles of PSP, FD-4 and FD-10 from the small intestinal serosal surface

We studied the time courses of PSP, FD-4 and FD-10 remaining in the diffusion cell. The remaining amount (percentage of dose) of PSP was significantly smaller than for FD-4 and FD-10 at each time point. As illustrated in Figure 2, semi-log plots of the time courses gave straight lines (correlation coefficient $R^2 = 0.973$ for PSP, 0.988 for FD-4 and 0.988 for FD-10). This suggests that drug absorption from the rat small intestinal serosal surface proceeds via a first-order process. The absorption rate constants (k_a) of PSP, FD-4 and FD-10 were calculated to be 6.10×10^{-3}, 1.13×10^{-3} and $0.45 \times 10^{-3}\,min^{-1}$, respectively.

Discussion

Because the small intestine occupies about 40% of the total peritoneal area in rats (Flessner 1996), we considered that its contribution to drug absorption from the peritoneal cavity was remarkable. Flessner (1996) reported that absorption from the peritoneal cavity was dependent on the surface area exposed to the solution. To obtain information about drug absorption characteristics after i.p. administration, as a first step we compared the drug absorption rates from several organ surfaces, such as the liver (Nishida et al 1996), kidney (Nishida et al 2004), stomach (Mukai et al 1999) and caecum (Nishida et al 2002), by employing the apparent permeability coefficient P_{app}. P_{app} was calculated as the absorption clearance per application area, according to the equation:

$$P_{app} = \frac{k_a \cdot V_a}{A_{cell}} \quad (1)$$

where V_a is the application volume of the drug solution and A_{cell} is the application area of the diffusion cell.

Table 2 summarizes the P_{app} of PSP, FD-4 and FD-10 after application to several organ surfaces. The P_{app} values of PSP, FD-4 and FD-10 were not significantly changed for the small intestine, liver, stomach and caecum while the kidney had the largest P_{app} values of PSP, FD-4 and FD-10, as shown by a more than 1.5 times significantly higher P_{app} value compared to other organ surfaces. These results suggest that the kidney has the highest absorption efficiency for various molecular weight compounds among these organ surfaces.

Flessner measured the mass transfer rates of mannitol to the plasma from fluid in diffusion chambers affixed to the peritoneal surfaces of the rat caecum, liver, stomach and abdominal wall, and determined that the rates of mannitol transport were similar for all four organs (Flessner 1996). The present result is in agreement with this previous study by Flessner (1996) on the similar P_{app} of the rat caecum, liver and stomach. However, we additionally indicated higher P_{app} for the kidney surface of several model compounds with different molecular weights.

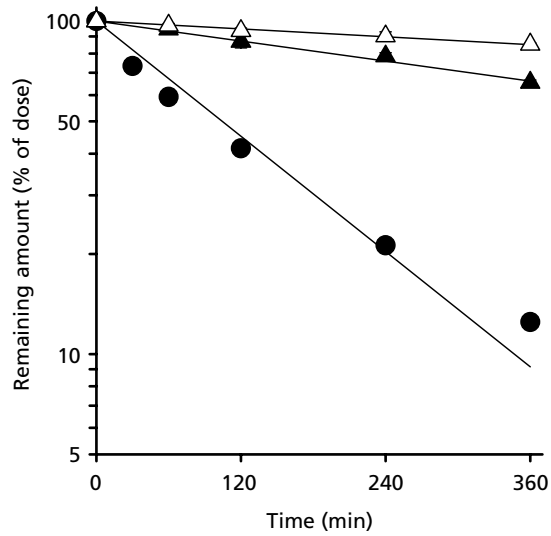

Figure 2 Semi-log plots of the PSP (●), FD-4 (▲) and FD-10 (△) amount remaining in the diffusion cell after application to the rat small intestinal serosal surface at a dose of 1 mg. Each point represents the mean ± s.e. of four experiments. When no error bar is given, the s.e. is smaller than the symbol used.

Table 1 Recovery (percentage of dose) of model compounds 6 h after application to the small intestinal serosal surface at a dose of 1 mg in rats

Compound	n	Diffusion cell (%)	Bile (%)	Urine (%)
PSP	6	10.8 ± 1.3	61.9 ± 4.0[a]	9.1 ± 3.5[a]
FD-4	6	65.4 ± 1.1	0.9 ± 0.2	21.4 ± 5.1
FD-10	6	85.1 ± 0.8	0.4 ± 0.1	12.2 ± 0.8
FD-40	4	97.9 ± 0.4	N.D.[b]	N.D.[b]

Each value is the mean ± s.e. of at least four experiments. [a]The biliary and urinary recoveries of PSP represent the total amount of free PSP and its metabolite. [b]FD-40 could not be detected in the bile and urine.

Table 2 Permeability coefficient (P_{app}) and absorption clearance (CL_a) of model compounds after application to several organ surfaces at a dose of 1 mg in rats

Organ	P_{app} (μm min^{-1})[a]			MW limit[b]	A_{organ}[c] (cm^2)	CL_a (μL min^{-1})[d]		
	PSP	FD-4	FD-10			PSP	FD-4	FD-10
Small intestine	15.9 ± 0.8 (6)	2.62 ± 0.10 (6)	1.18 ± 0.07 (6)	44 800	186.3 ± 7.5 (6)	296.2 ± 15.4 (6)	48.8 ± 1.9 (6)	22.0 ± 1.3 (6)
Liver	10.9 ± 1.4 (4)	2.56 ± 0.17 (5)	1.51 ± 0.18 (5)	71 200	71.7 ± 1.9 (6)	77.9 ± 9.8 (4)	18.4 ± 1.2 (5)	10.8 ± 1.3 (5)
Kidney	24.7 ± 0.8 (4)	7.11 ± 0.25 (4)	3.90 ± 0.18 (4)	130 900	13.0 ± 1.0 (6)	32.1 ± 1.1 (4)	9.2 ± 0.3 (4)	5.1 ± 0.2 (4)
Stomach	12.3 ± 0.9 (7)	2.47 ± 0.21 (5)	0.76 ± 0.21 (6)	23 700	16.3 ± 0.7 (6)	20.1 ± 1.5 (7)	4.0 ± 0.3 (5)	1.2 ± 0.3 (6)
Caecum	11.2 ± 0.9 (8)	2.11 ± 0.30 (5)	0.83 ± 0.13 (5)	35 500	25.2 ± 2.0 (6)	28.2 ± 2.4 (8)	5.3 ± 0.8 (5)	2.1 ± 0.3 (5)

Each value is the mean ± s.e. of at least four experiments. The number of experiments is indicated in parentheses. [a]P_{app} was calculated using k_a according to equation 1. k_a values were obtained previously: liver (Nishida et al 1995, 1996), kidney (Nishida et al 2004), stomach (Mukai et al 1999) and caecum (Nishida et al 2002)). [b]MW limit was calculated by the intercept of the x-axis on the relationship between P_{app} and $1/\sqrt{MW}$. [c]A_{organ} (peritoneal absorption area) of rats was reported in the literature (Flessner 1991). [d]CL_a was calculated according to equation 3.

We have clarified that the absorption rates from several organ surfaces were dependent on the molecular weights of the model compounds (Nishida et al 1996, 2002, 2004; Mukai et al 1999). We then compared the absorption rates of model compounds with different molecular weights from the small intestinal serosal surfaces. The following equation has been proposed with respect to drug absorption from the gastrointestinal mucosa via passive diffusion (Koizumi et al 1964a, b):

$$\frac{1}{\sqrt{MW} \cdot P_{app}} = A + \frac{B}{P_a} \quad (2)$$

where P_a represents the partition coefficient, and constants A and B are the correction factors to P_a and constants for diffusion, respectively.

Because each model compound has a high hydrophilicity (partition coefficient between n-octanol and water < 0.08), P_a as a lipophilic index of each model compound is very small. This might enable us to assume that each P_a value is approximately identical. Then, the right-hand side of equation 2 can be transformed as a fixed number. A linear relationship (correlation coefficient $R^2 = 0.997$) was observed between the P_{app} values and the $1/\sqrt{MW}$ values of PSP, FD-4, FD-10 and FD-40 for the small intestinal serosal surface, similar to liver (Nishida et al 1996), kidney (Nishida et al 2004), stomach (Mukai et al 1999) and caecum (Nishida et al 2002). This suggests that these hydrophilic model compounds were absorbed from the rat small intestinal serosal surfaces membranes via simple passive diffusion by the paracellular pathway, similar to other organ surfaces.

Table 2 lists the molecular weights when the P_{app} values are 0 (MW limit), calculated using the intercept of the x-axis ($P_{app} = 0$) on the relationship between the P_{app} and $1/\sqrt{MW}$. We considered that these values are equivalent to the limits of the molecular weights of the drugs that can be absorbed from each organ surface in rats. The limits of the molecular weights were different among the organ surfaces.

Furthermore, we calculated absorption clearance (CL_a), according to equation 3, to estimate the contribution ratio for each organ to absorption from the peritoneal cavity.

$$CL_a = P_{app} \cdot A_{organ} \quad (3)$$

CL_a is the absorption clearance on the peritoneal organ itself from the peritoneal cavity. A_{organ} is the peritoneal surface area of an organ, which was previously measured (Flessner 1991). In this case, we assumed that each organ would contribute to drug absorption from the peritoneal cavity according to its proportion of peritoneal surface area in contact with the peritoneal fluid. As listed in Table 2, CL_a values for the small intestine with every model compound were the significantly highest among these organ surfaces, probably by virtue of having the largest peritoneal area in the peritoneal cavity. These results suggest that absorption from the small intestinal serosal surface contributes the most to hydrophilic drug absorption from the peritoneal cavity.

In conclusion, we clarified the absorption characteristics of model compounds with different molecular weights from the rat small intestinal serosal surface by the paracellular pathway. These results should be useful to estimate overall hydrophilic drug absorption rates after i.p. administrations.

References

Flessner, M. F. (1991) Peritoneal transport physiology: insights from basic research. *J. Am. Soc. Nephrol.* **2**: 122–135

Flessner, M. F. (1996) Small-solute transport across specific peritoneal tissue surfaces in the rat. *J. Am. Soc. Nephrol.* **7**: 225–233

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

Koizumi, T., Arita, T., Kakemi, K. (1964a) Absorption and excretion of drugs. XIX. Some pharmacokinetic aspects of absorption and excretion of sulfonamides. (1). Absorption from rat stomach. *Chem. Pharm. Bull.* **12**: 413–420

Koizumi, T., Arita, T., Kakemi, K. (1964b) Absorption and excretion of drugs. XX. Some pharmacokinetic aspects of absorption and excretion of sulfonamide. (2). Absorption from rat small intestine. *Chem. Pharm. Bull.* **12**: 421–427

Kurtzhals, P., Larsen, C., Johansen, M. (1989) High-performance size-exclusion chromatographic procedure for the determination of fluoresceinyl isothiocyanate dextrans of various molecular masses in biological media. *J. Chromatogr.* **491**: 117–127

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., 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. (1995) 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. (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., Nose, S., Kuma, A., Mukai, T., Kawakami, S., Nakashima, M., Sasaki, H., Sakaeda, T., Nakamura, J. (2002) Absorption characteristics of model compounds with different molecular weights from the serosal caecal surface in rats. *J. Pharm. Pharmacol.* **54**: 1005–1009

Nishida, K., Tomiyama, N., Mukai, T., Nakashima, M., Sasaki, H., Nakamura, J. (2004) Absorption characteristics of compounds with different molecular weights after application to the unilateral kidney surface in rats. *Eur. J. Pharm. Biopharm.* **58**: 705–711