JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 736–740

Received November 8, 2010
Accepted February 22, 2011
DOI
10.1111/j.2042-7158.2011.01276.x
ISSN 0022-3573

Short Communication

Relationship between lipophilicity and absorption from the liver surface of paraben derivatives and antipyrine in rats

Koyo Nishida[a], Masanori Kobayashi[a], Hirotaka Miyamoto[a], Naoki Yoshikawa[a], Shintaro Fumoto[a], Hitoshi Sasaki[b] and Junzo Nakamura[a]

[a]Department of Clinical Pharmacy, Graduate School of Biomedical Sciences, Nagasaki University and [b]Department of Hospital Pharmacy, Nagasaki University Hospital, Nagasaki, Japan

Abstract

Objectives The importance of drug lipophilicity on absorption from the liver surface was examined in rats using paraben derivatives, antipyrine, Sudan III, and Sudan blue.
Methods The log partition coefficient (PC) of *n*-octanol/water ranged from −1.39 to 4.62. The compounds were applied to the rat liver surface using a cylindrical diffusion cell (i.d. 9 mm).
Key findings The rate of absorption at 15 min was calculated to be 13.9% for paraben, much lower than that for its derivatives methylparaben, propylparaben and butylparaben (~80%). The obtained first-order absorption rate constant (k_a) of paraben, methylparaben, propylparaben and antipyrine increased according to lipophilicity. Further lipophilicity resulted in a fall in k_a, implying the importance of affinity for lipids and water in absorption from the liver surface. Thus, a compound with a log PC of around 2.5 is considered to have maximum absorbability from the rat liver surface. A good relationship ($r^2 = 0.97$) was recognized between the log k_a and log reciprocal value with the square root of molecular weight of the compounds with a log PC below 2.5.
Conclusions The rate of absorption of a drug from the liver surface could be estimated from physicochemical properties such as lipophilicity and molecular weight.
Keywords absorption; antipyrine; lipophilicity; liver surface; paraben

Introduction

We have developed a new method of direct application to the liver that may be useful for drug delivery, and reported that application to the liver surface could achieve a site-selective delivery of drugs to the liver, including the anticancer drug 5-fluorouracil.[1,2] Moreover, we have examined the factors determining the absorption of hydrophilic compounds from the rat liver surface such as passive transport,[3] protein binding[4] and molecular weight.[5] However, we had not yet studied the absorption and distribution characteristics of lipophilic compounds after application to the liver surface. The importance of lipophilicity as the prime determinant of the diffusion process in biological membranes has been widely accepted. Although a relationship between lipophilicity and the rate of absorption from other membranes has been reported,[6–8] few papers have discussed the effect of lipophilicity on drug absorption across membranes in organs in the peritoneal cavity, such as the liver. As the peritoneum is used clinically for patients requiring treatment for bacterial peritonitis and those requiring peritoneal chemotherapy against peritoneal metastasis from gastrointestinal or ovarian cancer, it is important to examine the effect of lipophilicity. The relationship between lipophilicity and the overall rate of absorption from the peritoneal membrane has been examined for barbiturate derivatives[9] and xanthines.[10]

In this study, we chose paraben derivatives as models with different lipophilicity, and examined the effect of lipophilicity on drug absorption from the rat liver surface. Moreover, we studied the intrahepatic distribution of antipyrine as a fluid marker in the liver to ensure that the well systemic absorption with high hepatic concentration.

Correspondence: Koyo Nishida, Department of Clinical Pharmacy, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.
E-mail: koyo-n@nagasaki-u.ac.jp

Materials and Methods

Materials

p-Hydroxybenzoic acid (paraben), *p*-hydroxybenzoic acid butyl ester (butylparaben), Sudan III, *n*-octanol, phenacetin and oleic acid were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). *p*-Hydroxybenzoic acid methyl ester (methylparaben), *p*-hydroxybenzoic acid propyl ester (propylparaben), and antipyrine were obtained from Sigma Chemical Co. (St Louis, MO, USA). Sudan blue was purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). All other chemicals were reagent grade products.

Measurement of drug lipophilicity in buffer solution

Lipophilicity in a buffer solution was measured as reported previously.[6] The paraben derivative or antipyrine was dissolved in phosphate buffer saturated with *n*-octanol. The solution (4 ml) and a blank buffer solution were mixed for 20 min in an incubator. The mixture was separated into water and lipid layers, and incubated for 1 h at 37°C. The concentration in each layer was determined, and a partition coefficient (PC) was calculated using the following equation:

$$PC = (C_{init} - C_w)/C_w \quad (1)$$

where C_{init} and C_w represent the initial and final concentration in the water phase, respectively.

In the case of Sudan blue and Sudan III, the PC was calculated by the fragment method,[11] considering substitution constants in the chemical structure, because they did not dissolve in water.

Animal experiments

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

Male Wistar rats (230–280 g) were anaesthetized with sodium pentobarbitone (50 mg/kg, i.p.) and the left femoral artery was cannulated with a polyethylene tube (i.d. 0.5 mm, o.d. 0.8 mm; Dural Plastics, Dural, Australia). The body temperature of the rats was kept at 37°C with a heat lamp during the experiment. The solution to be administered was prepared in an isotonic phosphate buffer (pH 7.4). Sudan III and Sudan blue were dissolved in oleic acid, as they are insoluble in water.

A cylindrical diffusion cell (i.d. 9 mm, area 0.64 cm^2) was attached to the rat liver surface with an adhesive chemical, Aron Alpha (Daiichi Sankyo Co., Ltd, Tokyo, Japan). The drug solution (0.1 ml) was added to the diffusion cell directly.

The solution remaining in the diffusion cell was withdrawn at predetermined times. Experiments were carried out at 2, 5, 15, 30, 60, 120 and 180 min after the application. In certain experiments with antipyrine, 200 µl of blood was collected at selected times from the heparinized cannula inserted into the femoral artery over 180 min and centrifuged at 16 000*g* for 5 min. Then, the liver was perfused with saline and subjected to extraction, followed by homogenization.

Analytical methods

Paraben derivatives

The concentration of paraben and its derivatives was determined by high-performance liquid chromatography (HPLC). The sample (0.2 ml) was added to diethyl ether (7 ml). The extracted residue was dissolved in 300 µl of methanol. Samples were injected onto the HPLC column (Cosmosil packed column 5C18-MS-II, 4.6 mm i.d. × 150 mm; Nacalai Tesque, Inc.). A HPLC system (LC-10AS; Shimadzu Co., Ltd, Kyoto, Japan) with a variable wavelength UV detector (SPD-10A; Shimadzu) was used in reverse-phase mode. The detector wavelength, flow rate and column temperature were set at 254 nm, 0.5 ml/min and 25°C, respectively. The mobile phase consisted of 10 mM sodium acetate buffer (pH 4.0). Propylparaben or methylparaben was used as an internal standard.

Sudan blue and Sudan III

After appropriate distillation with acetone, the absorbance of Sudan blue and Sudan III was determined at 640 and 500 nm, respectively, by spectrophotometry.

Antipyrine

Antipyrine was analysed as described previously,[12] using HPLC spectrophotometry under the same conditions as for paraben. The solution remaining in the diffusion cell was dissolved in phosphate buffer. Then, 0.15 ml was added to 0.15 ml of methanol containing phenacetin as an internal standard. For the plasma samples, 0.2 ml of 0.05 M NaOH and 1 ml of dichloromethane were added to 0.1 ml of plasma for extraction, and incubation was carried out for 10 min with 1 ml of dichloromethane. The dichloromethane phase (0.5 ml) was extracted, 0.15 ml of methanol containing phenacetin and 0.15 ml of phosphate buffer were added, and the sample was assayed.

For the liver homogenate, the excised liver was homogenized in two-times its weight of cold distilled water. Then, 1 ml of 0.05 M NaOH and 5 ml of dichloromethane were added to 1 ml of the homogenate for extraction. An assay was then carried out as for the plasma.

Statistical analysis

The statistical analysis of antipyrine concentrations in the liver (applied lobe *versus* non-applied lobes) was performed with the Wilcoxon signed-rank test. $P < 0.05$ was considered to be statistically significant. All results for animal experiments were expressed as the mean ± SE of at least three determinations.

Results and Discussion

Lipophilicity of the model compounds

The log PC (*n*-octanol/water) values of the compounds are given in Table 1. Those of the paraben derivatives increased according to the length of the carbon chain. As shown in Table 1, there were no significant differences in log PC between experimental and calculated values by the fragment method.[11] The log PC values of Sudan blue and Sudan III were extremely high (>4).

Rate of absorption from the rat liver surface

The rate of absorption (% of dose) over 15 min was calculated from the amount remaining in the diffusion cell, and was 13.9% for paraben, 75.2% for methylparaben, 82.4% for propylparaben and 78.2% for butylparaben.

Figure 1a shows the amount of paraben and its esters remaining in the diffusion cell after application to the rat liver surface. The decrease in each compound was linear on a semi-log scale, indicating first-order absorption from the liver surface. A similar trend was observed for antipyrine (Figure 1b).

The first-order absorption rate constants (k_a) of paraben and its ester compounds, and antipyrine were calculated from the linear regression curves (Figure 1a and 1b), and are given in Table 1. With respect to Sudan III and Sudan blue, the k_a values were calculated from the amount remaining in the diffusion cell at 360 min (Table 1). The k_a values of compounds with a log PC of <2.5 increased according to the log PC value (Table 1), indicating that lipophilicity is an important determining factor of absorption from the liver surface. However, the k_a of butylparaben (0.101 min^{-1}) was not as great as that of propylparaben (0.108 min^{-1}) despite its estimation from the log PC value as illustrated in Figure 2. Furthermore, k_a values of highly lipophilic compounds (Sudan III and Sudan blue) were considerably decreased, probably because they were dissolved in oleic acid. This implies a reduction in absorption from the liver surface in the case of highly lipophilic compounds. Accordingly, compounds with a log PC of around 2.5 are considered to show maximum absorbability from the rat liver surface. Further lipophilicity resulted in a fall in k_a, indicating the importance of affinity for lipids and solubility in water to absorption from the liver surface. The decrease in absorption of highly lipophilic compounds is considered to be due to high affinity with cell contents and low affinity with blood circulation after absorption from the liver surface, similar to the case with other membranes.[13]

Table 1 Physicochemical properties and first-order absorption rate constant of paraben derivatives and other model compounds

Compound	MW	log PC	k_a ($min^{-1} \times 10^{-3}$)
Paraben	139.1	−1.39 (–0.70)	9.10
Methylparaben	152.1	1.66 (1.69)	99.08
Propylparaben	180.2	2.47 (2.85)	107.89
Butylparaben	194.2	3.41 (3.31)	100.93
Antipyrine	188.2	0.19 (0.17)	31.12
Sudan blue	342.4	ND (4.19)	0.43
Sudan III	352.4	ND (4.62)	0.97

ND, not detected; PC, partition coefficient. The first-order absorption rate constant (k_a) was calculated from a linear regression curve or the amount remaining in the diffusion cell at 360 min. The log PC values in parentheses were calculated by the fragment method.[11]

Relationship between lipophilicity and absorption rate from the rat liver

In our previous report,[5] we derived a relationship between the k_a and the reciprocal of the square root of molecular weight ($1/\sqrt{Mw}$) of hydrophilic compounds such as FITC dextrans with different molecular weights. Since the absorption rate is likely to be proportional to lipophilicity (PC), we examined the correlation between the k_a and $PC/\sqrt{Mw}$ of compounds with a log PC of <2.5 (except for highly lipophilic compounds, butylparaben, Sudan III and Sudan blue).

Figure 2 illustrates the correlation between the k_a and $PC/\sqrt{Mw}$ on both log scales. There was a slight gap in linearity with respect to butylparaben. A good linear relationship was observed (correlation coefficient: $r^2 = 0.97$), with a log PC of ≤2.5, for the three types of parabens and antipyrine. In addition, in the case of phenolsulfonphthalein examined previously,[3] the MW was 354.4 and the log PC value

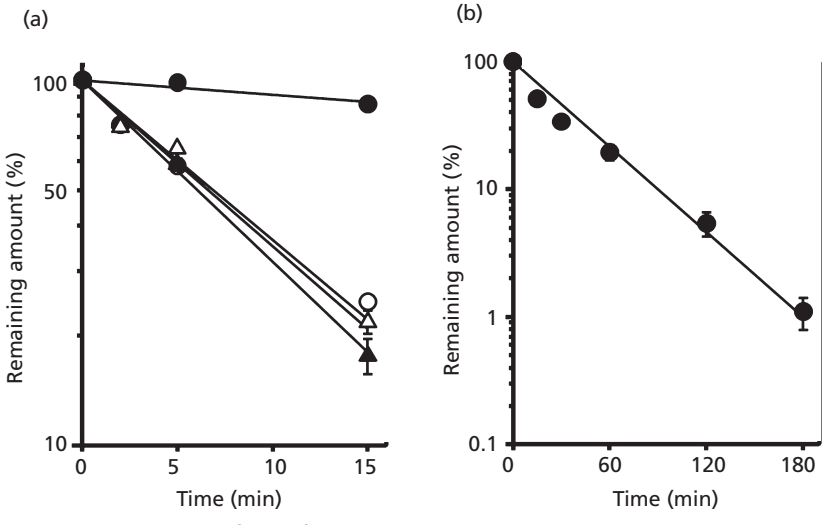

Figure 1 Amount of paraben and its derivatives, and antipyrine, remaining in the diffusion cell after application to the rat liver surface. Semi-log plots of the amount of paraben (●), methylparaben (○), propylparaben (▲), butylparaben (△) (a) and antipyrine (●) (b) remaining in the diffusion cell after application to the rat liver surface at doses of 20 μg (a) and 1 mg (b). Each point represents the mean ± SE of at least three experiments.

was −1.09. The experimentally obtained k_a value of 6.9×10^{-3} min^{-1}[3] was in good agreement with the value extrapolated from the regression curve in Figure 2 (7.9×10^{-3} min^{-1}). This suggests that the drug absorption rate from the rat liver surface could be estimated according to physicochemical properties such as lipophilicity and molecular weight.

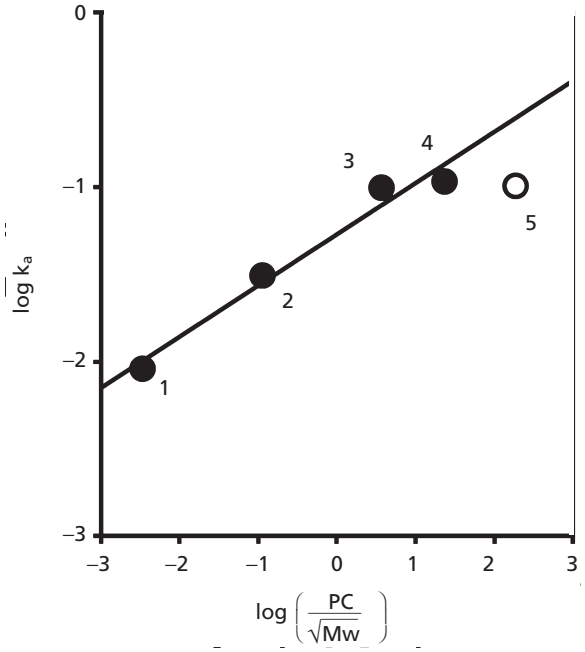

Figure 2 Relationship between first-order absorption rate constants k_a and $PC/\sqrt{Mw}$ of the model compounds. 1, paraben; 2, antipyrine; 3, methylparaben; 4, propylparaben; 5, butylparaben. The correlation coefficient (r) was calculated by closed symbols (1–4).

Fate of antipyrine after its application to the rat liver surface

We studied the fate of antipyrine as a marker of body fluid after its application to the rat liver surface, to examine the intrahepatic distribution of lipophilic compounds *in vivo*. Figure 3a shows the plasma–concentration profile of antipyrine. Antipyrine appeared in the plasma immediately after its application to the liver surface, with a maximum concentration at 60 min.

Figure 3b illustrates the distribution of antipyrine in the applied (diffusion cell attachment lobe) and non-applied liver lobes at 60, 120 and 180 min after application of antipyrine to the rat liver surface. The concentrations of antipyrine in the non-applied lobes were similar among the time points (60, 120 and 180 min). On the other hand, the concentration in the applied lobe was maximal at 120 min, and was higher than that of the non-applied lobe at 60 and 120 min by 1.8-times, although this was not significant ($P = 0.10$), as shown in Figure 3b. This could imply the preferential distribution of lipophilic compounds such as antipyrine into the applied region after application to the rat liver surface.

Conclusions

The relationship between lipophilicity and absorption from the liver surface of paraben derivatives and antipyrine was clarified, leading to estimations of drug absorption rates by utilizing physicochemical factors such as lipophilicity and molecular weight.

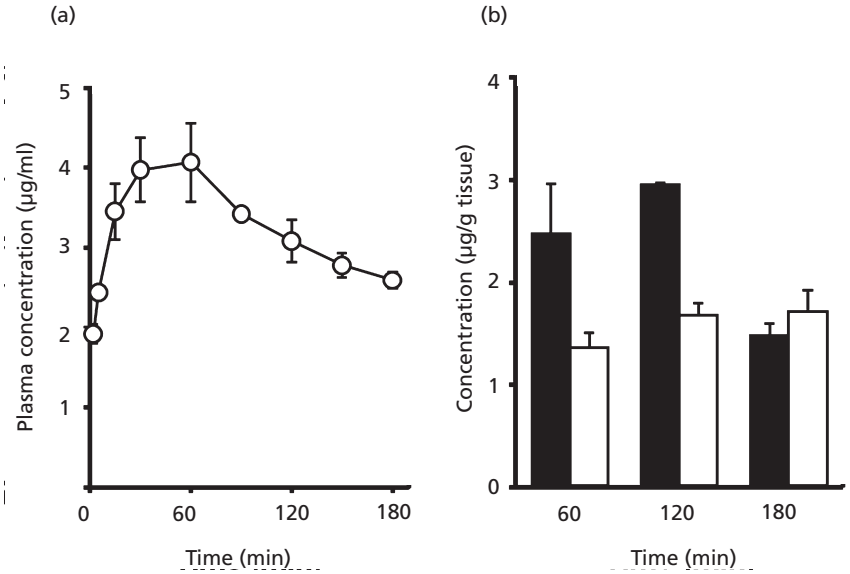

Figure 3 Fate of antipyrine after its application to the rat liver surface. Plasma–concentration profile of antipyrine (a) and concentration of antipyrine in the applied liver lobe (■) and non-applied liver lobes (□) (b) after application to the rat liver surface at a dose of 1 mg. Each point or bar represents the mean ± SE of three experiments.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

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.

Acknowledgements

We thank Momoko Matsui for skilled technical assistance.

References

1. Kodama Y *et al*. Absorption and distribution characteristics of 5-fluorouracil (5-FU) after an application to the liver surface in rats in order to reduce systemic side effects. *Biol Pharm Bull* 2008; 31: 1049–1052.
2. Nishida K *et al*. Regional delivery of model compounds and 5-fluorouracil to the liver by their application to the liver surface in rats: its implication for clinical use. *Pharm Res* 2005; 22: 1331–1337.
3. Nishida K *et al*. Mechanism for drug absorption from rat-liver surface membrane: effect of dose and transport inhibitors on the pharmacokinetics of phenol red. *J Pharm Pharmacol* 1995; 47: 227–231.
4. Nishida K *et al*. 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* 1995; 18: 1548–1550.
5. Nishida K *et al*. Absorption characteristics of dextrans with different molecular weights from the liver surface membrane in rats: implications for targeting to the liver. *J Drug Target* 1996; 4: 141–150.
6. Koizumi T *et al*. Absorption and excretion of drugs. XIX. Some pharmacokinetic aspects of absorption and excretion of sulfonamides. (1). Absorption from rat stomach. *Chem Pharm Bull* 1964; 12: 413–420.
7. Koizumi T *et al*. Absorption and excretion of drugs. XX. Some pharmacokinetic aspects of absorption and excretion of sulfonamide. (2). Absorption from rat small intestine. *Chem Pharm Bull* 1964; 12: 421–427.
8. Schanker LS *et al*. Absorption of drugs from the rat small intestine. *J Pharmacol Exp Ther* 1958; 123: 81–88.
9. Nakashima E *et al*. Quantitative relationship between structure and peritoneal membrane transport based on physiological pharmacokinetic concepts for acidic drugs. *Drug Metab Dispos* 1995; 23: 1220–1224.
10. Kuzuya T *et al*. Structure-related pharmacokinetics of xanthines after direct administration into the peritoneal cavity of rats. *Biol Pharm Bull* 1997; 20: 1051–1055.
11. Leo A *et al*. Calculation of hydrophobic constant (log P) from pi and f constants. *J Med Chem* 1975; 18: 865–868.
12. De Beer JO *et al*. Simple reversed-phase high-performance liquid chromatographic determination of antipyrine in rabbit plasma for pharmacokinetic studies. *J Chromatogr* 1984; 307: 475–480.
13. Houston JB *et al*. A re-evaluation of the importance of partition coefficients in the gastrointestinal absorption of nutrients. *J Pharmacol Exp Ther* 1974; 189: 244–254.