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Communication

Absorption of organic anions as model drugs following application to rat liver surface in-vivo

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Abstract

Absorption of organic anions (phenol red, bromphenol blue and bromsulphonphthalein) has been studied after their application to rat liver surface in-vivo, employing a cylindrical glass cell (i.d. 9 mm, area 0.64 cm²). Every drug appeared gradually in the blood with the peak level at about 1 h, after which its concentration declined slowly. Absorbed model drug was efficiently excreted into the bile. These observations appear to indicate the possibility of drug absorption from liver surface membrane. Absorption ratios of model drugs were estimated to be more than 59 % in 6 h. As to phenol red, its biliary recovery and metabolism ratio did not change as compared with that of i.v. administration.

Introduction

Liver plays an important role in drug disposition in the body, so that there is an increasing interest in improving treatment of liver diseases. It is desired that the administered drug distributes largely into the target site in the liver, as proposed to treat liver diseases e.g. localized tumor. Normal routes of drug administration have difficulty in achieving a local site of action in the liver.

Although direct drug application to the liver surface should yield local drug distribution, drug absorption from liver surface has not been reported in literature.

In the present study, we have examined the absorption of organic anions (phenol red (PR), bromphenol blue (BPB), bromosulphonphthalein (BSP)) as model drugs, after application to rat liver surface.

Materials and methods

Chemicals.

PR and BPB were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). BSP was 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 (230-250 g) were anesthetized with sodium pentobarbital (50 mg kg⁻¹, 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). After the middle abdomen was cut open for about 3 cm, 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, USA). The body temperature of rats was kept at 37°C by a heat lamp during the experiment. The test solution was prepared in an isotonic phosphate buffer (pH 7.4) to yield a concentration of 1 mg/0.1 mL, and administered as follows.

Application to rat liver surface: A cylindrical glass cell (i.d. 9 mm, area 0.64 cm²) was attached to rat liver surface at the area of left lobe with Aron Alpha (Sankyo Co. Ltd, Tokyo, Japan). The drug solution (0.1 mL) was added to the glass cell directly. The top of the glass cell was sealed by a piece of aluminum foil to prevent evaporation of the applied solution.

i.v. administration: The drug solution (0.1 mL) was injected into the jugular vein.

Direct injection into rat liver: The test solution (0.1 mL) was directly injected over 20 sec into the center of liver left lobe by using a syringe at a depth of 3 mm. The midpoint of the injection time was defined to be time 0.

After application of the drug solution, 200 mL of blood sample was collected at selected times from the heparinized cannula inserted into the femoral artery over 4 or 6 h and centrifuged at 15000 rpm for 5 min. Bile samples were collected at appropriate time intervals for 4 or 6 h. At 4 or 6 h after the application, urine was collected from the bladder directly by syringe. Following application to rat liver surface, the remaining dose solution in the glass cell was withdrawn at 6 h after dosing.

Analytical Method.

The concentrations of model drugs in plasma, bile, urine and remaining solution in glass cell were determined as follows.

PR: The concentration of free PR was determined spectrophotometrically at 560 nm after dilution with a 1 M NaOH solution. The total concentration of free PR and its metabolite was measured in the same manner after they were subjected to acid hydrolysis (1 M HCl at 100. C for 30 min) (Hart & Schanker 1966). The concentration of PR metabolite was estimated from the difference between these values.

BPB: The concentration of BPB was determined spectrophotometrically at 600 nm after dilution with an isotonic phosphate buffer (pH 7.4) (Takada et al 1974).

BSP: The concentration of BSP was determined spectrophotometrically at 580 nm after dilution with a 0.1 M NaOH solution (Frezza et al 1974).

Calculation of Moment Parameters.

The plasma concentration profiles and biliary excretion rate-time curves of free PR and its metabolite, BPB and BSP were analyzed based on statistical moment theory. Moment parameters for plasma concentration profile (AUC_p , MRT_p) and those for biliary excretion rate-time curve (AUC_b , MRT_b) are calculated by numeral integration using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation (Yamaoka et al 1978). As to PR, moment parameters for the biliary excretion rate-time curves of free PR ($AUC_{b,f}$, $MRT_{b,f}$) and its metabolite ($AUC_{b,m}$, $MRT_{b,m}$) are calculated independently.

Results and discussion

The main purpose of this study is to examine the possibility of drug absorption from the liver surface. Kinetic analysis of drug absorption from liver surface is of particular interest physiologically.

We selected three organic anions (PR, BPB and BSP) as a model drug because their disposition characteristics in liver had been investigated (Klaassen & Watkins III 1984; Tiribelli et al 1986, 1990; Hart & Schanker 1966; Moller & Sheikh 1983; Kakutani et al 1992).

Fig. 1a shows the plasma concentration profiles of model drugs after application to rat liver surface at a dose of 1 mg. Every model drug appeared in the blood at a low concentration ($< 6.5 \text{ mg mL}^{-1}$). This observation suggests the occurrence of drug absorption from rat liver surface membrane which seems to act as an effective barrier for several molecules to some extent. The plasma concentration of model drugs after application to rat liver surface reached a maximum at about 1 h after dosing and decreased gradually (Fig. 1a), representing the increased residence time in plasma as compared with i.v. administration (Fig. 1b). The prolongation of plasma concentration as compared with i.v. administration was marked in BPB and BSP (Figs. 1a and 1b).

< Fig. 1 >

After absorption from rat liver surface, every model drug was excreted into the bile, as shown in Fig. 2a. Similarly to the plasma concentration profile, the biliary excretion rate after application to rat liver surface showed a delayed pattern as compared with that of i.v. administration (Fig. 2b). The biliary excretion rate patterns differed among the model drugs. As to PR, its metabolite (PR-glucuronic acid conjugate) (Hart & Schanker 1966) was also excreted into the bile.

< Fig. 2 >

Tables 1 and 2 list the recovery (% dose) of model drugs in the bile, urine and glass cell on various administration routes. The extents of absorption were calculated, from the dose and the amount recovered in the glass cell after 6 h, as 91.8 % (PR), 71.6 % (BPB) and 58.6 % (BSP).

PR and its metabolite were excreted into both bile and urine on every administration route (Table 1), whereas BPB and BSP were predominantly excreted into the bile (Table 2). The biliary recovery and metabolism ratios of PR after application to rat liver surface were broadly similar to those of i.v. administration (Table 1). It is supposed that the drug elimination and metabolism processes are not affected by application to liver surface.

Moment parameters are free from the complexities of a pharmacokinetic model, and thus AUC_p and MRT_p can be appropriate parameters for evaluating roughly the drug absorbability from liver surface. Tables 2 and 3 summarize the moment parameters for model drugs after application to rat liver surface. The AUC_p and MRT_p for BSP after application to rat liver surface could not be calculated exactly owing to its low plasma concentration.

The AUC_p values for PR and BPB after application to rat liver surface, which contain large errors, were roughly equal to those of i.v. administration (Tables 2 and 3), supporting the good absorbability from rat liver surface. As expected, the MRT_p values for PR and BPB after application to rat liver surface were fairly larger than those of i.v. administration (Tables 2 and 3).

The ratio of AUC_{b,f} for PR and BPB values following application to rat liver surface to those of i.v. administration were 89.9 and 70.5 % respectively, which were in good agreement with recovery data estimates of absorption (Tables 2 and 3). The MRT_{b,f} and MRT_{b,m} for PR after application to rat liver surface were by respectively, 2.7 and 2.4 times larger than those of i.v. administration (Table 3). Similarly, a marked increase in MRT_b values was

seen with the application of BPB and BSP to rat liver surface (Table 2). From these results, the drug concentration in liver after application to rat liver surface seems to be sustained as compared with that of i.v. administration, although its concentration was not determined directly.

We also examined the disposition characteristics of model drugs after direct injection into rat liver by use of a syringe, for comparison. In this case, the plasma concentration profiles and biliary excretion rate patterns of every model drug (data not shown) were unaltered, as compared with those of i.v. administration (Figs. 1b and 2b). The in-vivo behaviours of model drugs after direct injection into rat liver were considered to be basically similar to those of i.v. administration, judging from the pharmacokinetic parameters (Tables 1-3). It is suggested that the direct injection route is not suitable for target site-specific drug delivery to the liver with a high blood flow, because directly injected drug was rapidly cleared from the injection site, followed by drainage into the systemic circulation.

In conclusion, the present paper provides initial evidence of the drug absorption from rat liver surface. Such information should be useful in the development of new administration route for drug delivery to the target site in liver. However, further work is required to elucidate the mechanism for drug absorption and to examine the drug absorption from the diseased liver surface for clinical application.

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