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Inhibition of the Hepatic Uptake of Paracetamol Sulphate by Anionic Compounds

NORIKO SAKUMA-SAWADA, SATOFUMI IIDA, TAKASHI MIZUMA, MASAHIRO HAYASHI* AND SHOJI AWAZU

Department of Biopharmaceutics, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432–1 Horinouchi, Hachioji, Tokyo 192–03, Japan

Abstract

The effect of anionic compounds on the hepatic uptake of paracetamol sulphate, a conjugative metabolite of paracetamol, has been studied.

Hepatic uptake of paracetamol sulphate by isolated hepatocytes was inhibited by bromosulphophthalein, dibromosulphophthalein and *p*-nitrophenyl sulphate, but not by probenecid or cholic acid. Bromosulphophthalein and dibromosulphophthalein also inhibited the uptake of paracetamol sulphate in the rat isolated perfused liver. Saturable uptake of paracetamol sulphate was also observed in the absence of inorganic sulphate. The uptake of paracetamol sulphate was reduced by 1 and 10 mM inorganic sulphate. Bromosulphophthalein and *p*-nitrophenyl sulphate inhibited the uptake of paracetamol sulphate in the absence of inorganic sulphate.

These results indicate that paracetamol sulphate shares a transporter with bromosulphophthalein, dibromosulphophthalein and *p*-nitrophenyl sulphate, all of which contain the sulphate or sulphonate group. Therefore, the sulphate or sulphonate moiety might be crucial for interaction with the transporter, and mutual inhibition of hepatic uptake among these compounds is likely.

Paracetamol, an analgesic-antipyretic agent, is metabolized primarily to sulphate and glucuronide conjugates in the liver in man (Caldwell et al 1982), rat and mouse (Moldeus 1978). The hepatic uptake of paracetamol sulphate and paracetamol glucuronide (Iida et al 1989) and their deconjugation to paracetamol have been observed in isolated hepatocytes. An experiment with isolated perfused rat liver showed the retarded influx (Miyauchi et al 1988) or carrier-mediated entry (Ratna et al 1993) of 4-methylumbelliferone sulphate to liver. The deconjugation of 4-methylumbelliferone conjugates and the conjugation of 4-methylumbelliferone has also been reported (Ratna et al 1993). Since the report of the carrier-mediated uptake of sulphate conjugates of harmol in isolated hepatocytes (Sundheimer & Brendel 1983), there have been few reports of other studies on the interaction of hepatic uptake between sulphate conjugates and other compounds. The process of uptake of drug sulphate metabolite is a prerequisite for deconjugation to the parent drug and its subsequent metabolism, and thereby affects the pharmacokinetics of the drug and its metabolite in the body. Therefore, the inhibitory effects of hepatic uptake of paracetamol sulphate by anionic compounds were studied.

Materials and Methods

Materials

Paracetamol was purchased from Wako Pure Chemicals (Osaka, Japan). Paracetamol sulphate was synthesized by the method of Burkhardt & Wood (1929) as reported previously (Mizuma et al 1985; Iida et al 1989). HPLC of purified paracetamol (colourless needles, mp 138–140°C) showed a single peak. Treatment of paracetamol sulphate with sulphatase resulted in the formation of paracetamol. ^{1}H NMR spectroscopy of paracetamol sulphate in D_2O showed 3 × H of CH_3CO at 2·13 ppm and 4 × H of benzene ring at 7·33 ppm. Bromosulphophthalein, dibromosulphophthalein, *p*-nitrophenyl sulphate, bovine serum albumin (fraction V) and collagenase (type I) were obtained from Sigma (St Louis, MO). Silicone oil (KF961, 100) was purchased from Shin-Etsu (Tokyo, Japan). Other chemicals were of analytical grade.

Uptake by isolated hepatocytes

The hepatic uptake of paracetamol sulphate was studied by the method described previously (Iida et al 1989). Briefly, rat hepatocytes were isolated from Wistar male rats, 250–300 g (Japan SLC, Shizuoka, Japan) by the method of Baur et al (1975) with slight modification as reported previously (Mizuma et al 1982). The isolated hepatocytes were suspended in reaction medium (pH 7·4) containing 4% bovine serum albumin at 2×10^6 cells mL^{-1}. The reaction medium was identical with that reported previously, except for the medium used for study of the effect of inorganic sulphate, for which sodium sulphate was added to Ringer's buffer (pH 7·4). After pre-incubating hepatocyte suspension (2×10^6 cells mL^{-1}; 2 mL) at 37°C for 5 min, the uptake experiment was started by adding paracetamol sulphate solution (final concentration, 1–10 mM; 2 mL) or a mixed solution of paracetamol sulphate and inhibitors at appropriate concentration (indicated in figure and table legends) to the cell suspension (final cell concentration, 1×10^6 cells mL^{-1}). The reaction mixture (1 mL) was periodically sampled up to 2 min after the start, and was centrifuged by the centrifugal silicone filtration method (Schwarz et al 1975) for separation of hepatocytes from reaction medium. The separated hepatocytes were resuspended in purified water (0·5 mL) and mixed with perchloric acid (25%; 0·1 mL)

*Present address: M. Hayashi, Department of Pharmaceutics, Science University of Tokyo, 12 Ichigaya Funagawara-Machi, Shinjuku-ku, Tokyo 162, Japan.

Correspondence: S. Awazu, Department of Biopharmaceutics, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432–1 Horinouchi, Hachioji, Tokyo 192–03, Japan.

containing 4-fluorophenol (internal standard) and centrifuged at 3000 rev min^{-1} for 5 min to precipitate protein. The resulting supernatant was further filtered through a membrane filter (0·45 μM) immediately before HPLC analysis. Paracetamol sulphate was determined by reversed-phase HPLC (Moldeus 1978). The detection limit of the HPLC assay was 0·8 μM.

Transport by isolated perfused liver

Perfusion experiments for hepatic uptake were performed by the method of Meijer et al (1981). Briefly, livers from male Wistar rats, 250–300 g, were perfused using a recirculating system. Paracetamol sulphate dissolved in Krebs–Henseleit bicarbonate buffer (pH 7·4) was perfused at 20 mL min^{-1} at 37°C. Perfusate was periodically sampled. Paracetamol sulphate in the perfusate was assayed by the HPLC method described above. Bile flow was used as a measure of liver viability.

Data analysis

Statistical treatment of data was performed by use of Student's *t*-test.

Results

Effects of anionic compounds on uptake of paracetamol sulphate by isolated hepatocytes

Table 1 shows the effect of anionic compounds on the initial uptake (amount) of paracetamol sulphate by isolated hepatocytes. The hepatic uptake of paracetamol sulphate was significantly reduced in the presence of bromosulphophthalein, dibromosulphophthalein and *p*-nitrophenyl sulphate, whereas it was not significantly reduced by cholic acid or probenecid.

Effects of anionic compounds on uptake of paracetamol sulphate by isolated perfused liver

Fig. 1 shows the time-course of paracetamol sulphate concentration in the recirculated perfusate in the isolated perfused liver experiment. The concentrations of paracetamol sulphate in the perfusate in the presence of bromosulphophthalein and dibromosulphophthalein were significantly higher than in the control, indicating inhibition of paracetamol sulphate uptake.

Table 1. Inhibition by anionic compounds of the uptake of paracetamol sulphate by isolated hepatocytes.

Conditions	Rate of uptake (nmol $(10^6$ cells$)^{-1}$ min^{-1})
Control	5·97 ± 0·74
+ bromosulphophthalein (1 mM)	0·88 ± 0·35*
+ dibromosulphophthalein (1 mM)	0·20 ± 0·13*
+ cholic acid (1 mM)	5·32 ± 1·32
+ probenecid (1 mM)	4·91 ± 0·14
+ *p*-nitrophenyl sulphate (10 mM)	1·02 ± 0·55*

The paracetamol sulphate concentration was 10 mM. Data are means ± s.e. (n = 3 or 4). *$P < 0·01$, significantly different compared with control.

Effect of inorganic sulphate (ion) on uptake of paracetamol sulphate

The initial rate of uptake of paracetamol sulphate by isolated hepatocytes in the absence of inorganic sulphate is shown in Table 2. The relationship between the rate of uptake and the concentration of paracetamol sulphate was non-linear, although the rate of uptake increased with increasing of paracetamol sulphate concentration. The uptake of paracetamol sulphate in the presence of 1 and 10 mM inorganic sulphate was significantly lower than that in the absence of inorganic sulphate (Table 3).

Table 2. Concentration-dependence of the rate of uptake of paracetamol sulphate by isolated hepatocytes in the absence of inorganic sulphate.

Paracetamol sulphate (mM)	Rate of uptake (nmol $(10^6$ cells$)^{-1}$ min^{-1})
1·0	0·518 ± 0·079
2·5	1·36 ± 0·34
5·0	2·05 ± 0·49
10·0	3·58 ± 0·36

Data are means ± s.e. (n = 3 or 4).

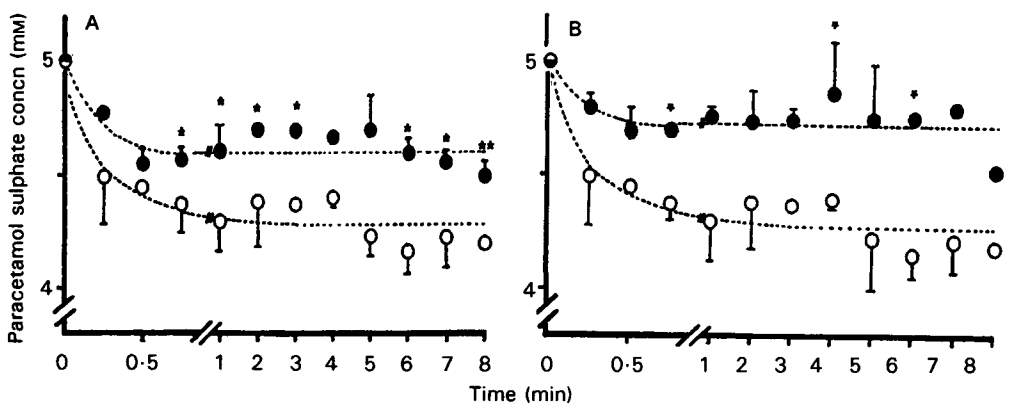

FIG. 1. Effect of A. bromosulphophthalein (1 mM) and B. dibromosulphophthalein (1 mM) on the uptake of paracetamol sulphate by isolated perfused liver. Data represent means ± s.e. (n = 3 or 4). *$P < 0·05$, **$P < 0·01$, significantly different compared with control (○).

Table 3. Effect of inorganic sulphate on the uptake of paracetamol sulphate (5 mM) by isolated hepatocytes.

Conditions	Uptake
Control	100 ± 9·0
+ inorganic sulphate (1 mM)	77·1 ± 10·0*
+ inorganic sulphate (10 mM)	78·4 ± 16·1*

Data (means ± s.e., n = 3 or 4) are percentages compared with the mean value of the control. *$P < 0.05$, significantly different compared with control.

Table 4. Effect of anionic compounds of the uptake of paracetamol sulphate (5 mM) by isolated hepatocytes in the absence of inorganic sulphate.

Conditions	Uptake
Control	100 ± 9·7
+ bromosulphophthalein (5 μM)	45·0 ± 6·5**
+ bromosulphophthalein (50 μM)	55·6 ± 8·2*
+ taurocholic acid (5 μM)	91·3 ± 11·5
+ taurocholic acid (25 μM)	71·4 ± 7·8
+ taurocholic acid (50 μM)	105·8 ± 15·1
+ *p*-nitrophenyl sulphate (5 μM)	89·5 ± 6·5
+ *p*-nitrophenyl sulphate (25 μM)	59·1 ± 4·6**
+ *p*-nitrophenyl sulphate (50 μM)	21·4 ± 6·6**

Data (means ± s.e., n = 3 or 4) are percentages compared with the mean value of the control. *$P < 0.05$, **$P < 0.01$, significantly different compared with control.

Effects of anionic compounds on uptake of paracetamol sulphate by isolated hepatocytes in the absence of inorganic sulphate

The uptake of paracetamol sulphate by isolated hepatocytes was significantly reduced by bromosulphophthalein or *p*-nitrophenyl sulphate in the absence of inorganic sulphate. However, the uptake of paracetamol sulphate by isolated hepatocytes was not significantly reduced by taurocholic acid (Table 4).

Discussion

The initial uptake of paracetamol sulphate by isolated hepatocytes was significantly reduced in the presence of bromosulphophthalein, dibromosulphophthalein, and *p*-nitrophenyl sulphate (Table 1). The inhibition by bromosulphophthalein and dibromosulphophthalein of the uptake of paracetamol sulphate was also observed in experiments on the isolated perfused liver (Fig. 1). It is reported that bromosulphophthalein (Schwenk et al 1976) and dibromosulphophthalein (Blom et al 1981) are transported by a carrier in isolated rat hepatocytes. Therefore, it was hypothesized that paracetamol sulphate was transported by the same carrier as bromosulphophthalein and dibromosulphophthalein. Paracetamol sulphate or bromosulphophthalein inhibited the hepatic uptake of *p*-nitrophenyl sulphate in isolated rat hepatocytes (unpublished data), supporting this hypothesis. In contrast, the uptake of paracetamol sulphate was not inhibited by cholic acid or probenecid, which have been reported to be transported by a carrier (Anwer & Hegner 1978; Gigon & Guarino 1970) for hepatic uptake.

Because paracetamol sulphate interacted with *p*-nitrophenyl sulphate during uptake, the effect of inorganic sulphate on the hepatic uptake of paracetamol sulphate was studied. The relationship between the concentration of paracetamol sulphate and the initial rate of uptake by isolated hepatocytes in the absence of inorganic sulphate (Table 2) indicated that hepatic uptake of paracetamol sulphate was by saturable transport and passive transport. Furthermore, the uptake of paracetamol sulphate was significantly reduced in the presence of 1 and 10 mM inorganic sulphate (Table 3). Cheng & Levy (1980) reported the hepatic transport of inorganic sulphate and Hungentobler & Meier (1986) showed that the hepatic uptake of inorganic sulphate was inhibited by bromosulphophthalein. Therefore, it was considered that paracetamol sulphate and inorganic sulphate shared a common transporter for uptake. Inhibition of the uptake of paracetamol sulphate by bromosulphophthalein and dibromosulphophthalein was shown in isolated hepatocytes (Tables 1 and 4) and isolated perfused liver (Fig. 1). The uptake of paracetamol sulphate was inhibited by *p*-nitrophenyl sulphate (Tables 1 and 4), of which hepatic uptake was inhibited by paracetamol sulphate (unpublished data). These results let us propose that the sulphate or sulphonate moiety might be a primary factor in the binding to the transporter. Hassen et al (1996) suggested the existence of multiple pathways for uptake of oestrone sulphate, harmol sulphate and 4-methylumbelliferyl sulphate, which share a common transporter. On the other hand, the uptake of paracetamol sulphate was not significantly reduced by cholic acid (Table 1) or taurocholic acid (Table 4), which are transported by carrier in the sinusoidal rat liver plasma membrane (Meier et al 1984). Therefore, it was concluded that paracetamol sulphate was transported by a transporter but not by the bile-acid transporter, and that paracetamol sulphate and *p*-nitrophenyl sulphate shared the same transporter for hepatic uptake. It is suggested that the sulphate conjugative metabolites of drugs interact with each other on hepatic uptake, affecting their sequential metabolism.

In conclusion, this study has shown that hepatic uptake of paracetamol sulphate is performed by a transporter, but not by the bile-acid transporter. Paracetamol sulphate, bromosulphophthalein, dibromosulphophthalein and *p*-nitrophenyl sulphate share the same transporter for hepatic uptake.

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