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Pharmacokinetics and biliary excretion of bromosulphophthalein, [^{3}H]-ouabain and [^{3}H]-taurocholic acid in rats with glycerol-induced acute renal failure

C.J. Bowmer & M.S. Yates

Department of Pharmacology, Worsely Medical and Dental Building, The University, Leeds, LS2 9JT

1 The pharmacokinetics and biliary excretion of bromosulphophthalein (BSP), ouabain and taurocholic acid (TChA) have been studied in rats with glycerol-induced acute renal failure (ARF).

2 In rats with ARF, the hepatic uptake and initial biliary excretion of BSP were decreased. In addition, the rate of BSP conjugation with glutathione by rat liver homogenates was also decreased. This latter change may contribute to the initial decrease in the biliary excretion of BSP.

3 No change was found in the hepatic uptake and biliary excretion of ouabain, but the area under the concentration-time curve was increased and the plasma clearance (Clp) decreased in rats with ARF. This decrease in Clp was not due to reduced renal excretion.

4 The decreased Clp of ouabain in rats with ARF may come from reduced tissue binding and a concomitant decrease in its volume of distribution (Vd).

5 The hepatic handling of TChA appeared unaltered in ARF, but the rate constant for the terminal part of the concentration-time curve (β) was decreased. This change probably resulted from a large increase in Vd in rats with ARF.

6 It is concluded that the decreased uptake of BSP was not due to a non-specific disturbance of hepatocyte function in ARF because the hepatic handling of ouabain and TChA were unaltered.

Introduction

The hepatic transport of endogenous and exogenous substances probably involves a multiplicity of routes both at the initial uptake and final biliary excretion steps. Evidence for this comes largely from studies of competition for transport between pairs of substances. These studies have revealed a number of different routes for organic anions (Alpert *et al.*, 1969; Scharschmidt *et al.*, 1975; Schwenk *et al.*, 1976), cations (Solomon & Schanker, 1963) and uncharged molecules (Kupferberg & Schanker, 1968; Klassen, 1978).

Experimental studies have established that the hepatic uptake of indocyanine green (ICG), an organic anion used to study liver function, is decreased in rats with acute and chronic renal failure (Bowmer *et al.*, 1982a; Yates *et al.*, 1983a,b,c). Moreover, the initial biliary excretion of ICG is decreased, resulting in a delay in the excretion of ICG into bile (Bowmer *et al.*, 1983a). However, little is known about whether these changes in hepatic function are restricted to ICG, or if uptake and biliary excretion of other substances are similarly affected in renal failure.

The purpose of this study was to investigate the effect of glycerol-induced acute renal failure (ARF) on the hepatic uptake and biliary excretion of bromosuphophthalein (BSP), ouabain and taurocholic acid (TChA). BSP and ICG appear to have a common transport route (Scharschmidt *et al.*, 1975; Schwenk *et al.*, 1976); but ouabain and TChA have transport routes separate from each other (Meijer *et al.*, 1976; Klassen, 1978) and from BSP (Schwenk *et al.*, 1976). Ouabain and TChA were also chosen because they are not biotransformed in the rat (Cox *et al.*, 1959; Hoffman *et al.*, 1975). A preliminary account of some of this work has been given (Bowmer *et al.*, 1982b; 1983b).

Methods

Induction of acute renal failure

The method for the production of glycerol-induced ARF has been described in detail elsewhere (Bowmer *et al.*, 1982a). Male Wistar albino rats (250–350 g) were denied access to water for 24 h and ARF was produced by intramuscular injection of 50% v/v glycerol in sterile saline (0.9%w/v NaCl solution), 10 ml kg^{-1}. Control rats were injected with saline, 10 ml kg^{-1}. Both groups of rats were studied 48 h after their respective injections.

Experimental protocol

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.): a tracheal cannula was inserted and artificial respiration maintained with a Miniature Ideal Pump (BioScience) (ventilation rate 80 strokes min^{-1}; stroke volume 10 ml kg^{-1}). Cannulae were also inserted into the left jugular vein, right carotid artery and common bile duct. Rectal temperature was maintained at 37°C by means of a heating lamp.

All compounds were dissolved in saline and injected i.v. over 15–20 s. The dose of BSP was 25 mg kg^{-1}. Ouabain was mixed with [^{3}H]-ouabain and administered at a dose of 0.1 mg kg^{-1}; 15 μCi kg^{-1}. Similarly, TChA was mixed with [^{3}H]-TChA and given at 5 mg kg^{-1}; 10 μCi kg^{-1}. Heparinized blood samples (0.1 ml) were removed at suitable times for 70 min with BSP and for 60 min with ouabain and TChA. After each sample was collected, blood was replaced with an equal volume of saline. Bile was collected over 5 or 10 min intervals for 1 h; over 20 min intervals for the second hour and over 30 min intervals for the third hour. Bile volume was measured gravimetrically assuming a density of 1.0 for rat bile.

The urinary excretion of [^{3}H]-ouabain was estimated by collecting urine directly from the bladder of anaesthetized rats as described by Hirom *et al.* (1976). In a separate series of experiments the kinetics of [^{3}H]-ouabain were determined in the absence of any urinary excretion. These experiments were performed in rats whose renal pedicles (renal artery, vein and ureter) were ligated 10 to 15 min before administration of [^{3}H]-ouabain.

Hepatic uptake of [^{3}H]-ouabain in vivo

Slices of liver (30–65 mg) were removed from the left, median and right lobes of anaesthetized rats at 2.5, 5, 7.5, 10 and 20 min after injection of [^{3}H]-ouabain (Meijer *et al.*, 1975). Each slice was blotted, weighed and solubilized in 0.7 ml FisoSolve (Fisons Ltd). The cumulative amount of tissue removed as a percentage of total liver weight was $1.8 \pm 0.2\%$ ($n = 6$) for control rats and $1.8 \pm 0.4\%$ ($n = 6$) for rats with ARF. Meijer *et al.* (1975) found that [^{3}H]-ouabain was taken up uniformly into slices taken at random from different liver lobes. Preliminary experiments confirmed this, so it was assumed that uptake measured in one slice was representative of uptake into the entire liver.

Analysis of bromosulphophthalein in plasma and bile

Samples (50 μl) of plasma and bile were diluted with an appropriate volume of 0.1 M NaOH and the absorbance measured at 575 nm. Plasma samples were also read at 395 nm to correct for haemoglobin contamination ($E_{395} \times 0.093$ = haemoglobin contribution to extinction at 575 nm). The absorption spectrum of the glutathione conjugate of BSP in alkali is almost identical to that of BSP between 500 to 620 nm (Goldstein & Combes, 1966) so the total amount of BSP in bile was measured.

Hepatic glutathione (GSH)

Liver homogenates were prepared by the procedure of Akerboom & Sies (1981) and the fluorometric method of Cohn & Lyle (1966) was used to determine the the GSH content of rat liver.

Hepatic glutathione-S-transferase activity

The spectrophotometric assay of Goldstein & Combes (1966) was used to measure the rate of BSP conjugation with GSH by rat liver homogenates.

Measurement of radioactivity

Radioactivity in aliquots (50 μl) of plasma and bile was measured in a Beckman LS 330 scintillation counter. Samples were counted in plastic insert vials (Sterilin Ltd) using 5 ml of FisoFluor I liquid scintillator (Fisons Ltd). Urine samples (20–200 μl) were counted in plastic scintillation vials (LIP Ltd) with 15 ml of scintillator. Radioactivity in digested pieces of liver was measured after addition of 15 ml of scintillator followed by 0.5 ml 5 M acetic acid. Counting efficiency was assessed by automatic external standard channels ratio and, where appropriate, with internal standards of [^{3}H]-*n*-hexadecane (Amersham International PLC).

Pharmacokinetic calculations

The plasma concentration-time data for BSP were fitted to a biexponential equation by non-linear least squares regression analysis (Snedecor & Cochran, 1967). Data were analysed using a two compartment

model with elimination of BSP from the peripheral compartment (Richards *et al.*, 1959). In this model k_{12} is the apparent first order rate constant for transfer of dye from plasma to liver; k_{21} the rate constant for return of BSP to plasma and k_{23} the rate constant for excretion into bile. These rate constants together with the apparent volume of the central compartment (Vc); the area under the plasma concentration-time curve ($AUC_{0\to\infty}$) and plasma clearance (Clp) were calculated using the equations given by Gibaldi & Perrier (1975). The apparent volume of distribution at steady-state (Vdss) was calculated as:-

$$Vdss = Vc\left(\frac{k_{12}+k_{21}+k_{23}}{k_{21}+k_{23}}\right)$$ (Bowmer *et al.*, 1982a)

The disappearance of [^{3}H]-ouabain and [^{3}H]-TChA from plasma was analysed using the 'CSTRIP' programme (Sedman & Wagner, 1976). This indicated that their decay was at least triexponential and so these data were not subjected to compartmental analysis. Instead the kinetics of [^{3}H]-ouabain and [^{3}H]-TChA were described in terms of (1) the rate constant for the terminal part of the concentration-time curve (β); (2) the apparent volume of distribution (Vd) which was calculated from:-

$$Vd = \frac{Dose}{AUC_{0\to\infty}\beta}$$ (Gibaldi & Perrier, 1975)

and (3) the plasma clearance (Clp). The area under the plasma concentration-time curve from 0 to 60 min was calculated using the trapezoidal rule and the area under the plasma concentration-time profile from the last observation (Cp_{60}) to infinity was estimated by:-

$$AUC_{60\to\infty} = \frac{Cp_{60}}{\beta}:$$ (Benet & Galeazzi, 1979)

The $AUC_{0\to\infty}$ was the sum of the two areas.

Results are expressed as mean ± s.d. and statistical comparison was made by the non-paired Student's *t* test.

Materials

BSP and ouabain were obtained from Sigma Chemical Co. and TChA was bought from CP Laboratories Ltd (Bishop Stortford, U.K.). GSH and *o*-phthalaldehyde were obtained from BDH Ltd. [G-^{3}H]-ouabain (37 Ci/mmol) and [G-^{3}H]-TChA (6.6 Ci/mmol), all of stated radioactive purity >97%, were purchased from Amersham International PLC and New England Nuclear Ltd respectively, and were used without further purification.

Results

Intramuscular injection of glycerol resulted in a uraemic state characterized by at least a four fold increase in plasma urea concentration. Mean body weight, wet liver weight and liver weight to body weight ratio were not significantly different between any group of control or uraemic rats used. These results are similar to those previously obtained in our laboratory (Bowmer *et al.*, 1982a; Yates *et al.*, 1983a,b).

Bromosulphophthalein kinetics

Figure 1 shows the mean plasma concentration-time data obtained after i.v. administration of BSP (25 mg kg^{-1}) to control and uraemic rats. Plasma concentrations between 5 to 15 min were significantly elevated ($P<0.05$) in the rats with ARF which suggests that the initial disappearance of BSP was delayed in the uraemic rats. The half-life of this initial disappearance phase, $T_{0.5}\alpha$, was significantly prolonged ($P<0.01$) and the rate constants k_{12} and k_{21} were decreased ($P<0.05$) in uraemic rats (Table 1). There was no significant change in the half-life of the terminal elimination phase, $T_{0.5}\beta$, k_{23}; Vdss or Clp, but Vc was significantly larger ($P<0.05$) in the uraemic rats.

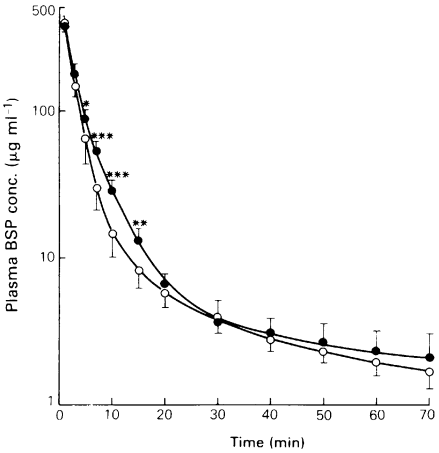

Figure 1 Plasma concentrations of bromosulphophthalein (BSP, 25 mg kg^{-1} i.v.) in control rats (○) and rats with acute renal failure (●). Values are mean ($n=7$); s.d. shown by vertical lines. Significantly different from control vaues: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 1 Effect of glycerol-induced acute renal failure on the pharmacokinetics of bromosulphophthalein (BSP, 25 mg kg^{-1} i.v.)

Pharmacokinetic parameters	*Control rats* ($n=7$)	*Uraemic rats* ($n=7$)
$T_{0.5}\alpha$ (min)	1.4 ± 0.2	2.4 ± 0.7**
$T_{0.5}\beta$ (min)	27 ± 10	53 ± 34
k_{12} (min^{-1})	0.49 ± 0.08	0.30 ± 0.07***
k_{21} (min^{-1})	0.0073 ± 0.0033	0.0043 ± 0.0013*
k_{23} (min^{-1})	0.029 ± 0.009	0.020 ± 0.014
Vc (l kg^{-1})	0.039 ± 0.003	0.066 ± 0.031*
Vp (l kg^{-1})	0.58 ± 0.18	1.0 ± 0.6
Vd_{ss} (l kg^{-1})	0.61 ± 0.18	1.1 ± 0.6
Clp (ml min^{-1} kg^{-1} body wt)	15 ± 2	14 ± 3

Results are given as mean ± s.d.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ relative to respective control group.
k_{12} = rate constant for transfer from plasma to liver; k_{21} = rate constant for return of BSP to plasma; k_{23} = rate constant for excretion into bile; Vc = apparent volume of the central compartment; Vp = apparent volume of the peripheral compartment and Vd_{ss} = apparent volume of distribution at steady-state; Clp = plasma clearance.

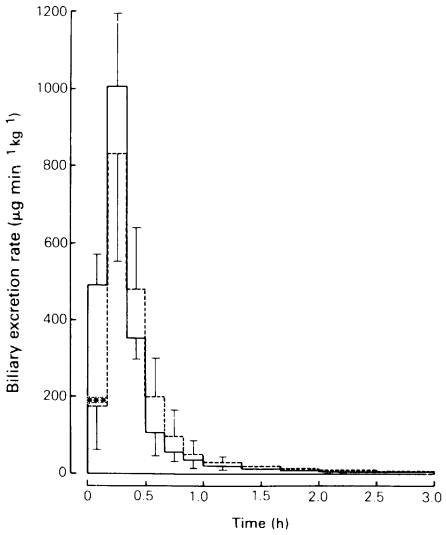

Figure 2 Biliary excretion profile of bromosulphophthalein (BSP, 25 mg kg^{-1} i.v.) in control rats (unbroken line ——) and rats with acute renal failure (broken line - - - -). Values are mean ($n=6$); s.d. shown by vertical lines. Significantly different from control values: ***$P<0.001$.

In uraemic rats the percentage recovery of BSP from bile over 3 h (80 ± 9%; $n=6$) and overall bile flow rate (3.9 ± 0.9 ml h^{-1} kg^{-1}; $n=6$) were not significantly different from control values (87 ± 6% and 4.5 ± 0.9 ml h^{-1} kg^{-1}; $n=6$ respectively). However, the biliary excretion rate during the first 10 min after injection of BSP in uraemic rats (175 ± 115 μg min^{-1} kg^{-1}; $n=6$) was significantly slower ($P<0.001$) than in controls (493 ± 75 μg min^{-1} kg^{-1}; $n=6$) (Figure 2). This initial delay in the biliary excretion of BSP was not caused by decreased bile flow rate at this particular time, because flow rate in rats with ARF (3.3 ± 1.8 ml h^{-1} kg^{-1}; $n=6$) was not significantly different from that in controls (4.2 ± 0.5 ml h^{-1} kg^{-1}; $n=6$). At all other intervals there was no difference in biliary excretion rates between the two groups of rats (Figure 2).

Bromosulphophthalein conjugation

The ability of livers from uraemic rats to conjugate BSP with exogenous GSH was significantly decreased ($P<0.01$). In uraemic rats the *in vitro* glutathione-S-transferase activity was 3.3 ± 0.5 μmol g^{-1} min^{-1} ($n=7$) whereas enzyme activity in controls was 3.9 ± 0.3 μmol g^{-1} min^{-1} ($n=7$). In a separate series of experiments the endogenous GSH content of livers from the uraemic group (3.2 ± 0.5 μmol g^{-1}; $n=8$) tended to be smaller than that in controls (3.7 ± 0.5 μmol g^{-1}; $n=8$). However, this difference was not statistically significant.

Ouabain kinetics

In rats with intact renal pedicles. The disappearance of [^{3}H]-ouabain (0.1 mg kg^{-1} i.v.) from plasma in both control and uraemic rats is shown in Figure 3. At all sample times, mean radioactivity was greater ($P<0.05$) in uraemic than in control plasma. As a result, the $AUC_{0\to\infty}$ was larger ($P<0.02$) in the uraemic group and there was a concomitant decrease ($P<0.02$) in Clp (Table 2). By contrast, no significant change in either β or Vd was observed (Table 2).

Figure 4 shows that between 5 and 20 min following injection of [^{3}H]-ouabain, a significantly greater ($P<0.01$) percentage of the injected dose was found in livers of uraemic rats. However, the percentage of [^{3}H]-ouabain excreted into bile after 1 h was not significantly different between control (43 ± 7%; $n=6$) and uraemic (49 ± 7%; $n=6$) rats. In addition,

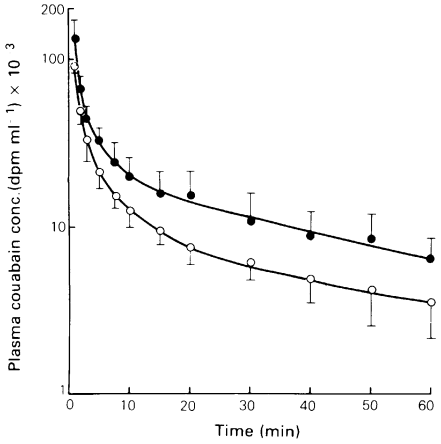

Figure 3 Plasma concentrations of ouabain (0.1 mg kg^{-1}; 15 μCi kg^{-1}) in control rats (○) and rats with acute renal failure (●). Values are mean (n = 6); s.d. shown by vertical lines. All concentrations in plasma from uraemic rats were significantly different from control values: $P < 0.05$.

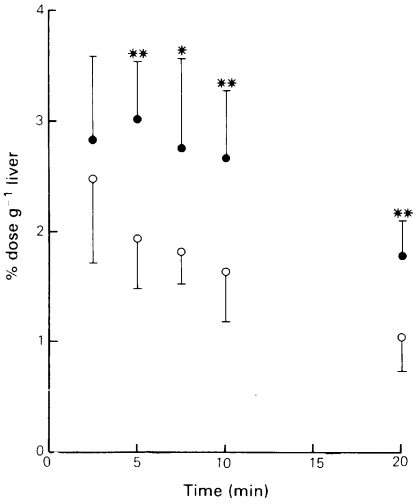

Figure 4 Comparison of the hepatic content of ouabain (0.1 mg kg^{-1}; 15 μCi kg^{-1}; i.v.) in control rats (○) and rats with acute renal failure (●). Values are mean (n = 6); s.d. shown by vertical lines. Significantly different from control values: $^{*}P < 0.05$; $^{**}P < 0.01$.

there was no difference in either biliary excretion rate, over any collection interval, or overall bile flow rate between the two groups of animals, so decreased or delayed biliary excretion cannot account for the greater portion of [^{3}H]-ouabain in uraemic livers. In control rats the mean liver to plasma ratio (dpm g^{-1}/dpm ml^{-1}) of [^{3}H]-ouabain between 5 to 20 min was 12 ± 2 (n = 4) whilst in uraemic rats its value was 11 ± 2 (n = 4). It seems likely, therefore, that the increased levels of ouabain in uraemic livers were a result of correspondingly higher plasma levels.

The percentage of the dose excreted into urine over 1 h was very variable in both groups. In controls the median was 8.0% with a range from 2.4 to 13% and in the uraemic group the median was 1.6% and range from 0.01 to 5.2%. Using the Wilcoxon rank test, the renal excretion of [^{3}H]-ouabain was found to be significantly less ($P < 0.01$) in uraemic rats.

In rats with ligated renal pedicles. These experiments were done to investigate the possibility that decreased renal excretion of ouabain was the cause of

Table 2 Effect of glycerol-induced acute renal failure on the pharmacokinetics of [^{3}H]-ouabain (0.1 mg kg^{-1}; 15 μCi kg^{-1}) in non-ligated and renal pedicle-ligated rats

	$AUC_{0\to\infty}$ (dpm min ml^{-1}) × 10^{-6}	β (min^{-1})	Clp (ml min^{-1} kg^{-1})	Vd (l kg^{-1})
Non-ligated				
Control (n = 6)	0.89 ± 0.23	0.020 ± 0.007	35 ± 10	1.8 ± 0.6
Uraemic (n = 6)	1.6 ± 0.5**	0.017 ± 0.006	21 ± 6**	1.4 ± 0.4
Ligated				
Control (n = 4)	1.5 ± 0.2‡	0.0071 ± 0.0017‡	21 ± 3†	3.0 ± 0.3‡
Uraemic (n = 4)	5.2 ± 2.1**‡	0.0035 ± 0.0023*‡	7.6 ± 4.0***‡	2.4 ± 0.3*‡

Results are given as mean ± s.d.
$^{*}P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$ relative to respective control group.
$^{\dagger}P < 0.05$; $^{\ddagger}P < 0.01$ relative to control and uraemic non-ligated rats.

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