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Effect of acute renal failure on the clearance and biliary excretion of indocyanine green in perfused rat liver

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Evidence has accumulated that the hepatic uptake of dyes such as indocyanine green (ICG), bromosulphophthalein (BSP) and rose bengal is decreased in rats with renal failure [1–4]. The reason for the diminished uptake of these dyes is unknown; but one possible explanation is that the influx of these dyes into the hepatocyte is competitively inhibited by endogenous metabolites which are retained in uraemic plasma.

Substances present in uraemic plasma inhibit renal tubule transport of organic anions such as *p*-aminobenzoic acid (PABA) [5, 6] and hippuric acid [7]. Many organic anions are known to accumulate in uraemic plasma [8] and Preuss *et al.* [5] have suggested that these may compete for tubular transport. Furthermore, the hepatic uptake of hippuric acid and PABA is also decreased in rats with renal failure [7, 9]. ICG, BSP and rose bengal are anions and may enter the hepatocyte by carrier-mediated transport [10], so it is possible that uraemic metabolites could compete with these dyes for hepatic uptake.

The purpose of this study was to clarify the role of uraemic metabolites as putative competitive inhibitors of the hepatic uptake of ICG. The plasma clearance and biliary excretion of ICG have been determined in livers of control rats and rats with glycerol-induced acute renal failure (ARF) that have been perfused with a medium free of uraemic metabolites.

Materials and methods

Chemicals. ICG U.S.P. was purchased from Hynson, Wescott & Dunning Ltd. (Baltimore, MD). Bovine serum albumin (BSA) and reagents for the assay of urea were obtained from Sigma Chemical Co. All other reagents were available commercially and of analytical grade.

Glycerol-induced ARF. Male Wistar albino rats (300–400 g) were used and ARF was produced by an i.m. injection of 50% (v/v) glycerol in sterile saline (10 ml/kg body wt) [1]. Control rats were injected with saline (10 ml/kg body wt) and both groups of rats were studied 48 hr after i.m. injection.

Liver perfusion. Rats were anaesthetised with pentobarbitone (60 mg/kg body wt. i.p.) and the subsequent operative technique and liver perfusion were essentially as described by Hems *et al.* [11]. The abdomen was opened by a midline incision and cannulae placed in the bile duct and portal vein. The thorax was opened and a blood sample, for plasma urea determination, was taken by left ventricular puncture before a cannula was placed in the inferior vena cava.

The perfusion medium (150 ml) was Krebs–bicarbonate solution containing washed human erythrocytes (packed cell volume, 15–16% v/v) and BSA (2.6% w/v) [11]. The final pH of the medium was adjusted to 7.4 by addition of 1 M $NaHCO_3$. The composition (mM) of the Krebs–bicarbonate solution was: NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 25.0 and glucose 10.0. The perfusion medium was oxygenated with a mixture of 95% O_2 and 5% CO_2.

After completion of surgery the rat was transferred to a thermostated cabinet at 37° and the liver perfused via the portal vein. The height of the perfusate reservoir was adjusted to give sufficient hydrostatic pressure to allow a flow of 12.5 ml/min. This pressure varied between 25–30 cm and did not cause swelling of the liver. The liver was allowed to recover for 30 min and then ICG was added to the collecting vessel as an aqueous solution (7.5 mg/kg body wt; 10 mg/ml). Samples of perfusion medium (0.5 ml) were removed from the collecting vessel at 5, 7.5, 10, 15, 20, 30 and 40 min after the administration of ICG. In another series of experiments bile samples were collected at 10 min intervals for up to 40 min.

Analytical methods. The concentration of ICG in the plasma of the perfusion medium and bile was determined spectrophotometrically at 800 nm as described previously [1, 12]. Plasma urea concentrations were measured using a commercial kit (Sigma No 535).

Data analysis. Plasma concentration–time data were fitted to a monoexponential equation by linear regression and the elimination rate constant (k_{el}), elimination half-life ($t_{0.5}$), volume of distribution (Vd) and plasma clearance (Clp) were calculated as described by Gibaldi and Perrier [13]. Results are given as mean ± S.E.M. and statistical comparisons were made by the non-paired Student's *t*-test.

Results

Rats injected with glycerol had significantly greater ($P < 0.001$) mean plasma urea concentrations (309 ± 48 mg/100 ml; $N = 10$) than rats given i.m. saline (43 ± 3 mg/100 ml; $N = 10$). However, there was no significant difference in either body weight or liver weight between control rats (346 ± 15 g and 11.7 ± 0.4 g respectively; $N = 10$) and rats with ARF (353 ± 12 g and 11.3 ± 0.3 g respectively; $N = 10$).

Figure 1 shows the mean concentration of ICG in plasma perfusing livers from control and uraemic rats. It is clear that in both groups of livers ICG is eliminated in a monoexponential manner. Only at 40 min was the mean concentration of ICG significantly greater ($P < 0.05$) in plasma perfusing uraemic livers, but the fractional rate of dye removal, k_{el}, was significantly smaller in the uraemic group (Table 1). In addition, $t_{0.5}$ and Clp were also decreased while there was no significant difference in the Vd of ICG between control and uraemic groups (Table 1).

ICG could be detected in all the bile samples collected from control livers over the first 10 min. By contrast in three out of four experiments with uraemic livers, no ICG could be detected in bile collected over the same time interval. The cumulative amount of dye excreted was significantly lower in uraemic livers from 20 to 40 min (Fig. 2). There was no significant difference in bile flow rate between control and uraemic livers during any collection interval and the mean bile flow rate over the 40 min collection period was 0.37 and 0.36 ml/hr in control and uraemic livers respectively. These results suggest that the biliary excretion of ICG by uraemic livers is less efficient than in control livers.

Discussion

The concentration of ICG in the plasma of the perfusion medium decreased in a monoexponential manner in both control and uraemic groups. By contrast, ICG shows biexponential kinetics *in vivo* [1, 2]. In perfusion experiments the rate of delivery of ICG to the liver is considerably less than *in vivo* because of a larger initial Vd (135 ml

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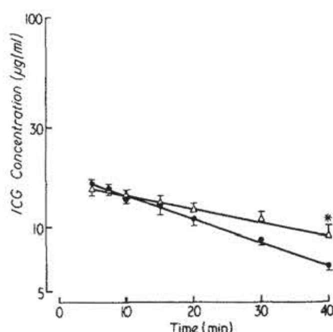

Fig. 1. Concentrations of ICG (7.5 mg/kg) in plasma perfusing livers of control (●) and uraemic (△) rats. Each point is the mean ± S.E.M. obtained in six rats. Key: $P < 0.05$ relative to respective control value.

compared to about 8 ml *in vivo* [1, 2]) which cannot be compensated for by a proportional increase in flow rate. Meijer *et al.* [14] have pointed out that uptake may be further retarded by the relatively large amount of albumin in the perfusion medium. The binding capacity of the medium is likely to be much greater than that of hepatic binding proteins, so that a larger fraction of the dose will remain within the perfusion medium. The combination of decreased delivery of dye and higher binding capacity means that the initial disposition phase will be much less pronounced in perfusion studies than *in vivo* [14].

Livers from rats with ARF have been perfused with a medium free of uraemic metabolites. The results show that these livers were less efficient than control livers at removing ICG from plasma and excreting it into bile.

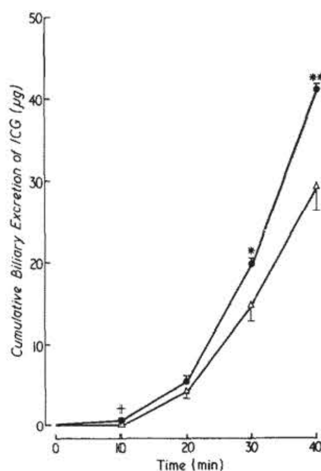

Fig. 2. Cumulative biliary excretion of ICG (7.5 mg/kg) in perfused livers of control (●) and uraemic (△) rats. Each point is the mean ± S.E.M. obtained in four rats. Key † in three out of four experiments with livers of uraemic animals no ICG could be detected in bile collected over the first 10 min; * $P < 0.05$, ** $P < 0.01$ relative to respective control value.

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Table 1. Effect of glycerol-induced acute renal failure on the pharmacokinetics of ICG (7.5 mg/kg) in perfused rat livers*

Pharmacokinetic parameters	Control rats (N = 6)	Uraemic rats (N = 6)
k_{el}/(min)	0.027 ± 0.001	0.015 ± 0.002‡
$t_{0.5}$ (min)	26 ± 1	51 ± 8†
Clp (ml/min/100 g body wt)	1.1 ± 0.1	0.69 ± 0.10‡
Vd (ml)	142 ± 4	155 ± 12

* Results are given as mean ± S.E.M.
† $P < 0.05$.
‡ $P < 0.01$ relative to respective control group.

Moreover, Howie and Burke [9] found decreased uptake of PABA into isolated perfused livers from rats with chronic renal failure, and another study [15] reported that a small number of uraemic metabolites failed to inhibit the uptake of BSP into isolated perfused rat livers. In view of these reports and the evidence presented here, it would seem unlikely that competition by uraemic metabolites could explain the impaired hepatic uptake of PABA or dyes such as ICG and BSP. However, it is possible that uraemic metabolites have a direct effect upon the liver and thereby induce changes in liver function which lead to impaired uptake of dyes.

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