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SHORT COMMUNICATIONS

Delayed biliary excretion of indocyanine green in rats with glycerol-induced acute renal failure

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A previous report from our laboratory showed that the plasma clearance of indocyanine green (ICG) was significantly decreased in rats with glycerol-induced acute renal failure (ARF) [1]. Furthermore, the apparent first-order rate constants for the uptake of ICG from plasma into the liver and removal of ICG from liver into bile were also decreased. These observations suggest that the biliary excretion of ICG may be decreased in rats with ARF. Consequently, we have investigated the effect of glycerol-induced ARF upon the biliary excretion of ICG.

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 were used and ARF was induced by an i.m. injection of glycerol [50% (v/v) glycerol in sterile saline, 10 ml/kg body wt] [2]. Control rats (sham injected) were injected with saline (10 ml/kg body wt) and both groups of rats were studied 48 hr after the i.m. injection of either glycerol or saline.

Collection of bile. Rats were anaesthetised with sodium pentobarbital (60 mg/kg body wt i.p.) and cannulae were inserted into the left jugular vein and common bile duct. The rectal temp was maintained at 37° by means of a heating lamp.

The control bile flow was determined in both groups of rats for 20 min; ICG (7.5 mg/kg) was then administered via the jugular cannula as an aqueous solution. Bile was collected for 10-min intervals for the first hour; for 20-min intervals for the second hour and thereafter for 30-min intervals. After each sample of bile had been collected, an equal volume of saline was infused through the jugular cannula.

Analytical methods. Dye standards were prepared by dissolving ICG in distilled water containing 4% (w/v) BSA. The dye–BSA solutions (50 μl) were diluted with human plasma (100 μl) and rat bile (50 μl). A 50-μl aliquot of this solution was added to 3 ml of distilled water and the absorbance measured at 800 nm. The final concentration range of dye standards was 0.15–2.1 μg/ml. The coefficient of variation of seven samples with a mean concentration of 1.98 μg/ml was 2.5%, and of seven samples with a mean concentration of 0.30 μg/ml 3.3%. The recovery of ICG added to rat bile was 100.3 ± 3.9% (N = 6).

For the assay of ICG in bile, 50 μl of bile were diluted with an appropriate volume of a human plasma–BSA mixture (2:1 by volume); 50 μl of the resultant solution were then added to 3 ml of distilled water and the absorbance measured at 800 nm. Blanks were prepared by diluting 50 μl of bile, collected prior to the injection of ICG, in an identical way to that for bile containing ICG. It was necessary to use a human plasma–BSA mixture to stabilize the dye during dilution of the bile samples.

Statistical analysis. Results are expressed as means ± S.D. and statistical comparison was made by the non-paired Student's *t*-test.

Results and discussion

Table 1 shows that 48 hr after the injection of glycerol, the rats were severely uraemic. However, there was no significant difference in either the wet liver wt or the mean bile flow, over the 4-hr collection, between control and uraemic rats.

The total percentage recovery of ICG from bile, after 4 hr, was not significantly different between the two groups of rats (Table 1, Fig. 1 upper), but the rate of biliary excretion in the uraemic rats [13.1 ± 11.4 μg/min/kg (N = 7)] was significantly lower ($P < 0.01$) than that in control

Table 1. Body weight, liver weight, plasma urea concentration, bile flow rate and percentage biliary recovery of ICG in control rats and rats with glycerol-induced acute renal failure*

	Control rats (N = 6)	Rats with glycerol-induced ARF (N = 7)
Body weight (g)	372 ± 47	367 ± 31
Liver weight (g/100 g body wt)	3.39 ± 0.24	3.41 ± 0.27
Plasma urea (mg/100 ml)	65 ± 11	397 ± 226‡
Bile flow rate† (ml/hr/kg)	3.45 ± 0.86	2.94 ± 0.63
% biliary recovery of ICG after 4 hr	83.0 ± 9.4	88.7 ± 3.6

* Results are given as means ± S.D.
† Calculated over the 4-hr collection period.
‡ $P < 0.01$ relative to respective control value.

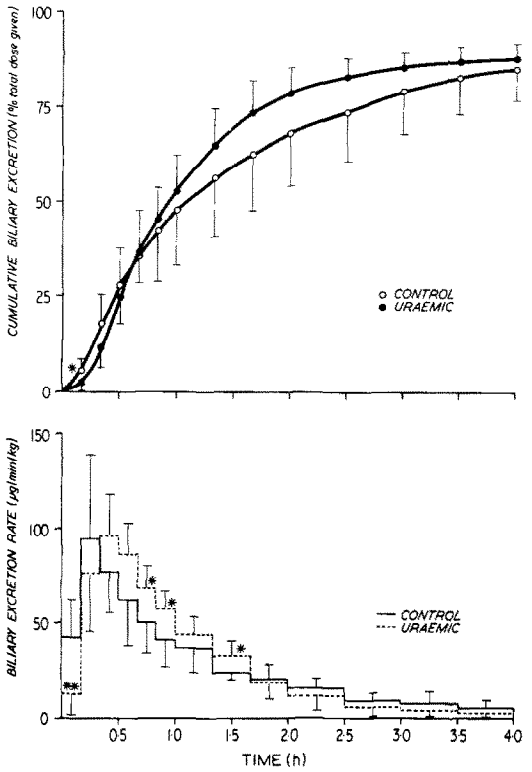

Fig. 1. Biliary excretion profile of ICG (7.5 mg/kg, i.v.) in six control rats and seven rats with glycerol-induced acute renal failure. Top panel: cumulative biliary excretion curves. Bottom panel: biliary excretion rate. Results are given as means ± S.D. Key: *$P < 0.05$, **$P < 0.01$, relative to respective control value.

rats [42.4 ± 20.3 μg/min/kg (N = 6)] for the first 10 min (Fig. 1 lower). By contrast, the excretion rate was significantly greater ($P < 0.05$) in the uraemic rats between 40 and 60 min and between 80 and 100 min (Fig. 1 lower). There was no significant difference in the bile flow rate between control and uraemic rats during any of the collection intervals.

The quantity of ICG delivered to the liver per unit time is dependent upon the plasma concentration of dye and the hepatic blood flow. Previous work has shown that after an i.v. dose of ICG (7.5 mg/kg) the plasma concentrations of dye were significantly greater in uraemic rats than in controls, over a 60-min period, even though the plasma volume was increased in the uraemic rats [1]. In addition, hepatic blood flow is increased by about 56% in uraemic rats 48 hr after the injection of glycerol [3]. It is possible that a change in the intrahepatic pool size for ICG was responsible for the delayed excretion. However, the apparent volume of distribution and the volume of the tissue compartment of ICG are not significantly different between control and uraemic rats [1]. It would seem unlikely that either a decrease in the load of dye presented to the liver or a change in the intrahepatic pool size is responsible for the decrease in the initial biliary excretion rate.

The bile flow rate is an important determinant of the rate of excretion of many compounds into bile [4, 5] and the former may be determined, at least in part, by the liver mass [6] and hepatic blood flow [7, 8]. The bile flow rate and liver weight were not significantly altered and hepatic blood flow is increased in uraemic rats [3]. Thus the delayed excretion of ICG cannot be accounted for by a decreased bile flow, liver weight or hepatic blood flow.

The most likely explanation for the initial decrease in the biliary excretion of ICG is a decrease in the rate of hepatic uptake, and the rate constant for the uptake process has been shown to be decreased in uraemic rats [1]. This would also account for the shift to the right in the excretion rate vs time curve (Fig. 1 lower) giving greater excretion rates in the uraemic rats between 40–60 and 80–100 min. If uptake is slowed then accumulation of ICG in liver cytosol will be retarded, resulting in an increase in the time required for maximum cytosol concentrations to be reached and hence a delay in the appearance of the maximum biliary excretion rate.

An attempt was made to study further the relationship between uptake and biliary excretion using constant-rate infusions of ICG. In contrast to a single bolus i.v. dose of ICG, infusions (1.1 and 0.44 mg/hr/100 g body wt) produced a progressive decrease in the bile flow and consequently steady-state plasma concentrations and maximal biliary excretion rates could not be obtained in either control or uraemic rats (Bowmer and Yates, unpublished observations). Klassen and Plaa [9] have also observed that infusions of ICG decrease the bile flow and suggested that this may be due to blockage of the biliary tract by aggregates of ICG.

Although the initial biliary excretion of ICG was decreased, overall its excretion into bile was not significantly altered in rats with ARF. We have obtained similar results with bromosulphophthalein [10], so it would seem that the biliary excretion of these substances is relatively unaffected in ARF.

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