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Pharmacokinetics of indocyanine green in rats with chronic renal failure

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Indocyanine green (ICG) is commonly used to assess liver function in man and animals. This dye has proved useful in previous investigations of liver function in rats with acute renal failure (Bowmer et al 1982, 1983). Those studies showed that the hepatic uptake, plasma clearance and initial biliary excretion of ICG were all decreased in acutely uraemic rats. There is evidence that these aspects of liver function are also altered in rats with chronic renal failure (CRF), as Tse et al (1976) reported that the plasma disappearance and biliary excretion of rose bengal were both decreased in rats with CRF. We therefore set out to find out if the kinetics of ICG are also altered in CRF and to compare any changes with those in rats in acute renal failure.

Materials and methods

Male Wistar rats (100–150 g) were partially (five-sixths) nephrectomized; two thirds of the right kidney was removed at the first operation and one week later the left kidney was removed (Young et al 1973). Sham operations, where the kidneys were exposed and the capsule removed, were performed on a control group of rats. The animals were studied 28 days after the completion of surgery.

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.) and cannulae inserted into the trachea, left jugular vein and right carotid artery. ICG (Hynson, Wescott and Dunning Ltd., Baltimore) was injected via the jugular vein as an aqueous solution (7·5 mg kg^{-1}, 10 mg ml^{-1}). Blood samples (0·1 ml) were taken from the carotid artery 1, 3, 5, 7, 10, 15, 20, 30, 40, 50 and 60 min after dosing. The plasma concentration of ICG was measured spectrophotometrically at 800 nm (Iga et al 1980) and plasma urea concentrations were measured by reaction with diacetyl monoxime (Bowmer et al 1982).

Pharmacokinetic calculations were done on the basis of a two compartment model with elimination of ICG from the peripheral compartment (Bowmer et al 1982). Results are expressed as mean ± s.d. and statistical comparison was made by the non-paired Student's *t*-test.

Results

There was no significant difference in mean body weight between rats intended for sham operation (141 ± 12 g) and those about to undergo partial nephrectomy (135 ± 13 g). However, 28 days after surgery the partially nephrectomized rats had a significantly lower ($P < 0·01$) mean body weight than the controls (Table 1). By contrast, wet liver weight, as a fraction of body weight, was significantly greater ($P < 0·01$) in the partially nephrectomized rats (Table 1). This difference was probably related to the difference in body weights because mean liver weights when not expressed as a fraction of body weight were not significantly different between controls (12·85 ± 0·85 g; n = 7) and uraemics (12·06 ± 0·84 g; n = 7). The partially nephrectomized rats showed evidence of having developed chronic renal failure, namely increased plasma urea concentrations and a significantly decreased ($P < 0·001$) packed cell volume (PCV) (Table 1).

The effect of chronic renal failure on the plasma concentration-time data for ICG is shown in Fig. 1. Mean plasma concentrations of ICG in the period of 5 to 20 min after administration were significantly greater in the uraemic rats than in controls. The pharmacokinetic parameters obtained from these data showed a significantly prolonged α-phase half-life; significant decreases in the rate constants for the entry of ICG into the liver, k_{12}, and reflux from liver to plasma, k_{21}, and the plasma clearance, Clp, of ICG in the uraemic rats (Table 2). There was no statistical difference in the β-phase half-life; the elimination rate constant, k_{23}; the apparent volume of distribution, Vdss, and the apparent volume of the central compartment, Vc, between control and uraemic rats.

Discussion

Twenty-eight days after partial nephrectomy, the rats had developed a significant degree of chronic renal failure. In these animals there were substantial decreases in the rate constants for entry of ICG into the liver, k_{12}, and reflux from liver to plasma, k_{21}. The

Table 1. Body weight, liver weight, packed cell volume (PCV) and plasma urea concentration in rats with partial nephrectomy and sham-operated controls.†

	Sham-operated control rats n=7	Rats with partial nephrectomy n=7
Body weight (g)	341 ± 32	302 ± 22**
Liver weight (g/100g)	3·68 ± 0·27	4·00 ± 0·14**
PCV (%)	48 ± 3	42 ± 2***
Plasma urea (mg/100 ml)	49 ± 12	135 ± 26***

† Results are given as mean ± s.d.
** $P < 0·01$; *** $P < 0·001$ relative to control group.

* Correspondence.

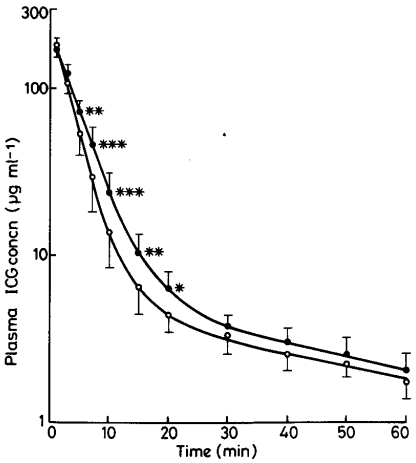

FIG. 1. Plasma concentrations of ICG (7·5 mg kg^{-1} i.v.) in male control (sham-operated) rats ○ and male rats with surgically-induced chronic renal failure ●. Each point is the mean ± s.d. of seven rats. *P <0·05; **P <0·01; ***P <0·001 relative to respective control value.

decrease in k_{12} suggests that the hepatic uptake of ICG is impaired in rats with CRF. As ICG is exclusively removed from plasma by the liver (Cherrick et al 1960; Leevy et al 1963) and there was no significant change in Vdss, the decrease in k_{12} was probably responsible for the reduced plasma clearance of ICG in the uraemic rats.

The results are similar to those obtained for ICG in rats with glycerol-induced acute renal failure (Bowmer et al 1982). In that model of acute renal failure k_{12}, k_{21} and the plasma clearance of ICG were all decreased in acutely uraemic rats. The elimination rate constant, k_{23}, was also decreased; but only the initial biliary excretion (during the first 10 min collection) was reduced, overall biliary excretion remained unchanged (Bowmer et al 1983). In rats with CRF k_{23} was not significantly altered and, together with observations on rats with acute renal failure, this suggests that the overall biliary excretion of ICG is unlikely to be affected in rats with CRF.

The altered kinetic behaviour of ICG is consistent with changes found in CRF for other dyes used to assess liver function. Tse et al (1976) found that the clearance of rose bengal from blood is decreased in chronically uraemic rats and the hepatic uptake of bromosulphophthalein is decreased in patients with CRF (Wernze & Spech 1971). This consistency of altered kinetic behaviour is not unexpected as these dyes inhibit each other's uptake into the liver (Hunton et al 1961; Scharschmidt et al 1975; Schwenk et al 1976), bind to similar hepatic cytoplasmic proteins (Levi et al 1969; Klassen 1976), and so may share common mechanisms for uptake into and storage within the hepatocyte.

Table 2 Effect of chronic renal failure on the pharmacokinetics of ICG (7·5 mg kg^{-1}) in male rats.†

Pharmacokinetic parameters	Control rats (n=7)	Uraemic rats (n=7)
$t_{0.5}\alpha$ (min)	2·1 ± 0·3	2·9 ± 0·3***
$t_{0.5}\beta$ (min)	34 ± 1	41 ± 12
k_{12} (min^{-1})	0·33 ± 0·05	0·24 ± 0·03***
k_{21} (min^{-1})	0·0069 ± 0·0009	0·0054 ± 0·0007***
k_{23} (min^{-1})	0·021 ± 0·003	0·018 ± 0·004
Vc (ml)	10 ± 1	9·9 ± 1·2
Vdss (ml)	128 ± 17·7	111 ± 21
Clp (ml min^{-1})/100g)	0·71 ± 0·09	0·60 ± 0·07**

† Results are given as mean ± s.d.
** P <0·01; *** P <0·001 relative to respective control groups.
k_{12} = rate constant for influx into the liver; k_{21} = rate constant for reflux from liver; k_{23} = rate constant for elimination from peripheral compartment; Vc = apparent volume of central compartment and Vdss = apparent volume of distribution at steady state; Clp = plasma clearance.

Our results provide little insight into the mechanism of CRF-induced changes of hepatic uptake. However, Wernze & Spech (1971) suggested that altered hepatic protein metabolism may be responsible. Renal failure can induce changes in protein metabolism (Knochel & Seldin 1976) and ICG, rose bengal and bromosulphophthalein bind avidly to hepatic cytoplasmic proteins (Levi et al 1969; Klassen 1976). Alteration in the intracellular concentration of these proteins could possibly alter the influx of their ligands into the hepatocyte.

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