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THE PLASMA CLEARANCE OF INDOCYANINE GREEN IN RATS WITH ACUTE RENAL FAILURE: EFFECT OF DOSE AND ROUTE OF ADMINISTRATION

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Abstract—The pharmacokinetics of various doses (1–7.5 mg/kg i.v.) of indocyanine green (ICG)† have been studied in control rats and rats with glycerol-induced acute renal failure (ARF). The pharmacokinetic changes seen at a dose of 1 mg/kg, after jugular vein administration, were significant decreases in uraemic rats in the rate of entry of ICG into the liver (k_{12}) and in the rate of movement of dye from liver to plasma (k_{21}). Greater and more numerous changes in pharmacokinetic parameters were recorded in experiments conducted using 4.0 and 7.5 mg/kg ICG. The results from these experiments showed that in addition to significant decreases in k_{12} and k_{21} there was a significant reduction in the rate constant for transfer of dye from liver to bile (k_{23}). These changes were accompanied by a significant decrease in plasma clearance.

In a separate series of experiments steps were taken to reduce the degree of uraemia produced by glycerol injection. The findings from these experiments showed no significant pharmacokinetic differences between control and mildly uraemic animals after administration of a dose of 7.5 mg/kg ICG. This suggests that the kinetic changes described above were a consequence of renal failure and not a direct hepato-toxic effect of glycerol.

In earlier work we reported that the hepatic uptake and plasma clearance of indocyanine green (ICG) were decreased in rats with glycerol-induced acute renal failure (ARF) [1, 2]. These studies were done at a single dose (7.5 mg/kg) of dye injected via the jugular vein. In the present study we have extended our investigation of the pharmacokinetics of ICG in ARF to include various doses of dye (1–30 mg/kg) and intra-portal administration. These experiments should allow us firstly, to determine whether the effects seen previously were dependent on the dose used and secondly, to calculate the hepatic extraction ratio for ICG in both control and uraemic rats [3].

One disadvantage of using glycerol-induced ARF to study hepatic function in uraemia is that glycerol itself could have a toxic effect on the liver [1]. In order to investigate this possibility we have examined the pharmacokinetics of ICG in rats in which an attempt was made to ameliorate the degree of ARF produced by injection of glycerol. These experiments were performed in rats which received saline as drinking fluid and had not undergone dehydration prior to the injection of glycerol. Both these procedures have been reported to either prevent or reduce the degree of ARF produced by glycerol [4, 5].

MATERIALS AND METHODS

Materials. ICG was purchased from Hynson, Wescott and Dunning Ltd., Baltimore. Reagents for the determination of urea were obtained from Sigma Chemical Co. All other reagents were available commercially and of analytical grade.

Induction of ARF. Methods for the production of ARF have been described in detail [1]. Groups of male Wistar albino rats were used and ARF was induced, after dehydration for 24 hr, by i.m. injection of glycerol. Uraemic and control rats (injected with saline) were studied 48 hr after the i.m. injection of glycerol or saline.

Prevention of ARF. Male Wistar albino rats were housed in groups of four and given saline (1.0% w/v NaCl) as drinking fluid for 14 days prior to i.m. injection of either glycerol or saline. In contrast to the induction of ARF, rats were not dehydrated for 24 hr before i.m. injection and they were maintained on saline as drinking fluid until ready for study 48 hr later.

Experimental protocol. Rats were anaesthetized with pentobarbitone (60 mg/kg, body wt., i.p.) and cannulae placed in the trachea, jugular vein and carotid artery. ICG was injected i.v. over 15–20 sec at five different doses, 1, 4, 7.5, 15 and 30 mg/kg. Heparinized arterial blood samples (0.1 ml) were taken at suitable times for 1 hr after injection of dye. In some experiments ICG (7.5 mg/kg) was administered over 15–20 sec via the hepatic portal vein. The shaft of a 23 gauge needle was attached to a cannula and the needle inserted into the portal vein for the duration of the experiment. Hepatic portal vein catheterization in this manner during pentobarbitone anaesthesia has been shown to have no effect on liver blood flow [6].

Methods for the assay of ICG have been described in detail [1]. The plasma concentration of ICG was determined by measuring its absorption at 800 nm. Urea was measured by reaction with diacetyl

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† Abbreviations used: ICG, indocyanine green; ARF, acute renal failure.

monoxime using the procedure contained in Sigma Technical Bulletin No. 535 (Sigma Chemical Co.).

Pharmacokinetic analysis. The disposition and elimination of ICG in the rat can be described by a two-compartment model with elimination from the peripheral compartment [1]. Rate constants for the uptake of ICG from plasma into the liver, k_{12}, for efflux from liver to plasma, k_{21}, and for transport from liver to bile, k_{23}, were calculated using the equations given by Gibaldi and Perrier [7]. Methods for calculation of the apparent volumes of the central and peripheral compartments (Vc and Vp respectively); the apparent volume of distribution at steady-state (Vdss) and the plasma clearance of ICG (Cl_p) have been described previously [1].

The apparent systemic availability (f) and the apparent hepatic extraction ratio (E_h) are given by

$$f = \frac{\text{AUC portal}}{\text{AUC jugular}}$$

$$E_h = 1 - \frac{\text{AUC portal}}{\text{AUC jugular}}$$

where AUC portal and AUC jugular are the mean areas under the plasma concentration–time curves after hepatic portal and jugular vein administration of ICG (7.5 mg/kg).

Results are expressed as mean ± S.D. and statistical analysis was performed using the non-paired Student's t-test.

RESULTS

In all the glycerol injected rats there was a significant increase in plasma urea concentration when compared to the corresponding controls (Table 1). In the rats which did not undergo dehydration prior to glycerol injection and which received saline as drinking fluid there was a significantly lower plasma urea concentration when compared to other uraemic groups. There was no significant difference in wet liver weight between control and uraemic animals in each group (Table 1).

In control rats k_{12}, k_{21} and k_{23} all decreased as the dose of indocyanine green increased and these changes were accompanied by a progressive decrease in plasma clearance (Table 2). The decreases in rate constants and clearance were most apparent when the dose was increased from 1 to 4 mg/kg. A comparison of pharmacokinetic parameters obtained from uraemic rats and their control counterparts are detailed below.

1 *mg/kg ICG experiments*

The plasma concentration–time data that were observed following the administration of ICG (1 mg/kg) to eight control rats and nine with glycerol-induced ARF are shown in Fig. 1. Mean plasma concentrations of ICG in uraemic rats were significantly higher between 1 and 5 min than in controls. As a result in uraemic animals the half-life of the α-phase was significantly increased (46%) and there were significant decreases in both k_{12} (31%) and k_{21} (29%) (Table 2); but there were no significant changes in any of the other pharmacokinetic parameters (Table 2).

4 *mg/kg ICG experiments*

Mean plasma concentrations of ICG in uraemic animals when compared to controls were, with exception of the 1 min sample, significantly higher at all sampling times (Fig. 2). In contrast to the experiments at 1 mg/kg there were numerous changes in the pharmacokinetic parameters (Table

Table 1. Body weight, liver weight and plasma urea concentration in rats with acute renal failure*

Group and dose of ICG	Body weight (g)	Liver weight (g/100 g body wt)	Plasma urea (mg/100 ml)
1 mg/kg			
Control	320 ± 58(8)	3.8 ± 0.4(8)	21 ± 5(8)
Uraemic	286 ± 29(9)	3.7 ± 0.2(9)	189 ± 93(9)‡
4 mg/kg			
Control	319 ± 63(9)	3.7 ± 0.3(9)	46 ± 6(9)
Uraemic	304 ± 39(8)	3.6 ± 0.2(8)	337 ± 165(8)‡
7.5 mg/kg†			
Control	321 ± 31(12)	3.8 ± 0.3(12)	31 ± 13(12)
Uraemic	338 ± 28(17)	3.6 ± 0.3(17)	218 ± 167(17)‡
7.5 mg/kg portal vein injection			
Control	316 ± 56(6)	3.6 ± 0.4(6)	51 ± 13(6)
Uraemic	340 ± 75(6)	3.5 ± 0.5(6)	194 ± 121(6)§
7.5 mg/kg in saline drinking rats			
Control	393 ± 39(6)	3.5 ± 0.2(6)	56 ± 4(6)
Uraemic	388 ± 31(6)	3.4 ± 0.2(6)	82 ± 4(6)‡‖

* Results are given as mean ± S.D. and number of rats in parentheses.
† Mean of combined results from two previous publications [1, 2].
‡ $P < 0.001$; § $P < 0.05$ relative to respective control group.
‖ $P < 0.05$ relative to other uraemic groups.

Table 2. Effect of glycerol-induced acute renal failure on the pharmacokinetics of ICG (1, 4 and 7.5 mg/kg i.v.) in male rats*

Pharmacokinetic parameters	1 mg/kg		4 mg/kg		7.5 mg/kg	
	Control rats ($N = 8$)	Uraemic rats ($N = 9$)	Control rats ($N = 9$)	Uraemic rats ($N = 8$)	Control rats ($N = 12$)	Uraemic rats ($N = 17$)
$t_{0.5}$ α (min)	0.89 ± 0.07	1.3 ± 0.3†	1.5 ± 0.2	2.2 ± 0.6†	1.6 ± 0.2	2.9 ± 0.5‡
$t_{0.5}$ β (min)	11 ± 4	8.9 ± 2.5	22 ± 6	30 ± 5†	33 ± 9	42 ± 9§
k_{12} (min^{-1})	0.73 ± 0.06	0.50 ± 0.10‡	0.47 ± 0.05	0.32 ± 0.06‡	0.43 ± 0.06	0.24 ± 0.04‡
k_{21} (min^{-1})	0.48 ± 0.011	0.034 ± 0.0108	0.014 ± 0.002	0.0089 ± 0.0023‡	0.0084 ± 0.0017	0.0055 ± 0.0011‡
k_{23} (min^{-1})	0.079 ± 0.027	0.090 ± 0.025	0.035 ± 0.009	0.024 ± 0.004†	0.023 ± 0.005	0.018 ± 0.004†
Vc (ml)	13 ± 2	12 ± 3	10 ± 2	9.8 ± 0.8	8.4 ± 1.4	11 ± 1‡
Vp (ml)	82 ± 37	51 ± 25	100 ± 38	97 ± 31	121 ± 33	113 ± 25
Vdss (ml)	95 ± 39	62 ± 27	113 ± 36	107 ± 31	130 ± 34	124 ± 26
Clp (ml/min per 100 g body wt)	1.8 ± 0.3	1.5 ± 0.4	1.1 ± 0.2	0.74 ± 0.17†	0.82 ± 0.16	0.57 ± 0.11‡

* Results are given as mean ± S.D. Those at 7.5 mg/kg are the combined results from two previous publications [1, 2].
† $P < 0.01$; ‡ $P < 0.001$; § $P < 0.05$ relative to respective control group.

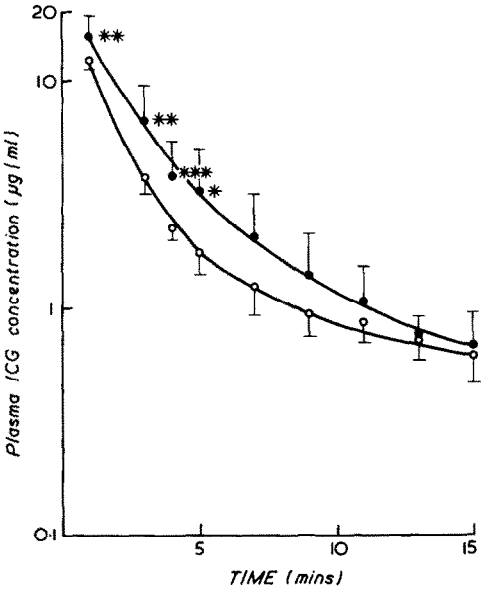

Fig. 1. Plasma concentrations of ICG (1.0 mg/kg) in control (saline injected) rats (○) and rats with glycerol-induced ARF (●). Each point is the mean ± S.D. of eight control rats and nine rats with glycerol-induced ARF. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ relative to respective control value.

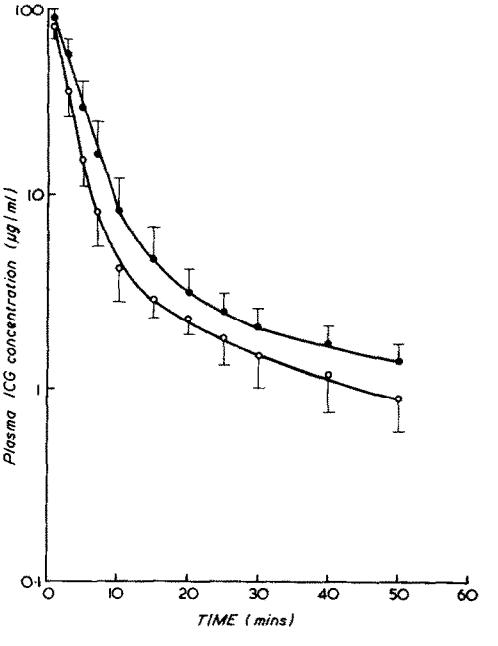

Fig. 2. Plasma concentrations of ICG (4.0 mg/kg) in control (saline injected) rats (○) and rats with glycerol-induced ARF (●). Each point is the mean ± S.D. of nine control rats and eight rats with glycerol-induced ARF. With the exception of the 1 min sample all concentrations in plasma from rats with ARF were significantly different from control values ($P < 0.05$).

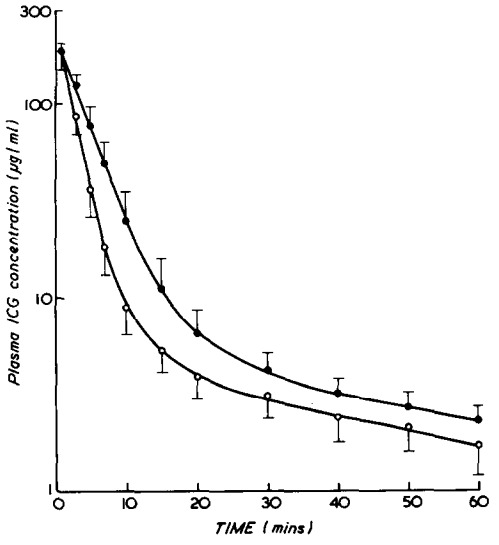

Fig. 3. Plasma concentrations of ICG (7.5 mg/kg) in control (saline injected) rats (○) and rats with glycerol-induced ARF (●). Each point is the mean ± S.D. of twelve control rats and seventeen rats with glycerol-induced ARF from the combined data from two previous publications [1, 2]. With the exception of the 1 min sample all concentrations in plasma from rats with ARF are significantly different from control values ($P < 0.01$).

2). There were significant increases in the half-lives of the α- and β-phases in uraemic rats (47% and 36% respectively) with significant decreases in k_{12} (32%), k_{21} (36%), k_{23} (31%) and plasma clearance (33%).

7.5 *mg/kg ICG experiments*

The results shown in Fig. 3 and Table 2 are the mean of combined results from two previous studies [1, 2]. Experiments performed at this dose level showed similar changes in mean plasma concentrations of ICG between uraemic and control rats to those observed in experiments at 4 mg/kg in that the concentrations in uraemic rats were significantly higher at all sampling times with the exception of the 1 min sample (Fig. 3). There were also similar changes in pharmacokinetic parameters such that the uraemic animals showed significant increase in the half-lives of both α- and β-phases (81% and 27% respectively) and significant decreases in k_{12} (44%), k_{21} (35%), k_{23} (22%) and plasma clearance (30%) (Table 2). In contrast to the results of experiments at 1 and 4 mg/kg ICG at a dose of 7.5 mg/kg uraemic animals showed a significant increase in the volume of the central compartment (31%).

7.5 *mg/kg ICG experiments, portal vein administration*

There was no significant difference in AUC between jugular and portal administration for either control or uraemic groups (Table 3). As a consequence, apparent systemic availability of ICG in both control and uraemic rats was slightly greater than unity which prevented the calculation of an apparent extraction ratio (Table 3). However, the AUC after portal vein administration was significantly greater in uraemic rats (Table 3) such that plasma clearance of ICG in uraemic animals (0.51 ± 0.16, $N = 6$) was 31% lower than in controls (0.74 ± 0.13, $N = 6$). This percentage reduction in clearance is very similar to that noted after jugular administration (Table 2).

7.5 *mg/kg ICG experiments in saline-drinking non-dehydrated rats*

The uraemic saline-drinking rats were found to have plasma concentrations of ICG which were not significantly different from controls. Furthermore the pharmacokinetic parameters showed no significant difference between control and uraemic rats (Table 4). The control pharmacokinetic parameters of the saline-drinking rats did show some differences from those obtained in water-drinking rats which received the same dose of ICG (Table 2). The saline-drinking rats showed significant increases in the half-life of the α-phase and volume of the central compartment and significant decreases in k_{12} and plasma clearance (Table 4).

These findings indicate that although saline-drinking and omission of the dehydration prior to glycerol injection can significantly reduce the degree of uraemia (Table 1) these protective measures themselves can perturb the pharmacokinetics of ICG.

15 *and* 30 *mg/kg ICG experiments*

A semilogarithmic plot of the decline of plasma ICG concentrations obtained from saline and

Table 3. Effect of glycerol-induced acute renal failure on the systemic availability and hepatic extraction ratio of ICG (7.5 mg/kg, i.v.) in male rats*

	Control rats	Uraemic rats
AUC (μg/ml.min)		
Jugular vein	936 ± 154(12)	1351 ± 253(17)†
Portal vein	1038 ± 230(6)	1610 ± 491(6)‡
Apparent systemic availability (f)	1.1	1.2
Apparent extraction ratio (E_h)	§	§

* Results are given as mean ± S.D. and number of rats in parentheses.
† $P < 0.001$; ‡ $P < 0.05$ relative to respective control group.
§ E_h could not be calculated.

Table 4. Effect of glycerol-induced acute renal failure on the pharmacokinetics of ICG (7.5 mg/kg, i.v.) in saline-drinking, non-dehydrated male rats*

Pharmacokinetic parameters	Control rats ($N = 6$)	Uraemic rats ($N = 6$)
$t_{0.5}$ α (min)	2.0 ± 0.4†	2.5 ± 0.5
$t_{0.5}$ β (min)	40 ± 8	48 ± 13
k_{12} (min^{-1})	0.35 ± 0.09†	0.28 ± 0.06
k_{21} (min^{-1})	0.0081 ± 0.0018	0.0068 ± 0.0013
k_{23} (min^{-1})	0.018 ± 0.003	0.016 ± 0.004
Vc (ml)	9.9 ± 1.3†	10 ± 1
Vp (ml)	132 ± 35	135 ± 26
Vdss (ml)	142 ± 35	146 ± 26
Clp (ml/min per 100 g body wt)	0.62 ± 0.12†	0.52 ± 0.05

* Results are given as mean ± S.D. There was no significant difference between control and uraemic rats.

† $P < 0.05$ relative to water-drinking, dehydrated rats given 7.5 mg/kg ICG (Table 2).

glycerol-injected rats which received either 15 or 30 mg/kg ICG had an initial convex shape indicative of zero order kinetics [8]. As a result of this complication experiments at these dose levels were discontinued.

DISCUSSION

The pharmacokinetic behaviour of ICG at all dose levels which were fully investigated was modified in rats with severe uraemia. The kinetic changes common to these experiments were significant decreases in k_{12} and k_{21}. These findings suggest an impaired movement of dye across the hepatocyte as previously discussed [1]. Although there was a reduction in the hepatic uptake of ICG at 1 mg/kg, there was no significant change in either k_{23} or plasma clearance in uraemic rats. By contrast, uraemic rats which received 4 and 7.5 mg/kg of ICG showed significant decreases in both plasma clearance and k_{23}. It might be anticipated that with a decrease in clearance and k_{23}, the biliary excretion of ICG at these higher doses would be impaired in uraemia. However, we have previously shown that in uraemic rats only the initial biliary excretion of ICG (7.5 mg/kg) is slowed [9].

The percentage decrease in plasma clearance in uraemic rats after hepatic portal administration of 7.5 mg/kg ICG was very similar to that noted after jugular injection of the same dose. A comparison of the data obtained from these two routes of administration gave an estimate of apparent systemic availability slightly greater than unity (Table 3) which indicates a negligible dependence of clearance on hepatic blood flow at this dose [3]. Similar estimates of apparent systemic availability for ICG in the rat have been noted by Iga and Klaassen [3] at doses of 1, 5 and 10 mg/kg. Iga and Klaassen [3] found that the plasma clearance of ICG was comparable at all doses (about 0.7 ml/min per 100 g) whereas in the present study plasma clearance of ICG was dose dependent ranging from 1.8 to 0.8 ml/min per 100 g in controls and from 1.5 to 0.6 ml/min per 100 g in uraemic rats over the dose range 1–7.5 mg/kg. These changes in clearance are probably related to the progressive decrease in the intercompartmental rate constants when the dose of ICG was increased, an observation previously noted by other workers [10]. The higher plasma clearance values for ICG at 1 mg/kg begin to approach the value for hepatic plasma flow (3.0 ml/min per 100 g) [3] which suggests that plasma clearance is more dependent on hepatic blood flow at 1 mg/kg than at 7.5 mg/kg.

This suggestion supports the finding of McDevitt *et al.* [11] who reported that liver blood flow is a more important determinant of the plasma clearance of ICG when administered at a dose of 1 mg/kg than at 10 mg/kg. Since liver blood flow is increased in rats 48 hr after glycerol injection [12] it is possible that at the low dose of 1 mg/kg the increased delivery of dye to the liver offsets the decrease in hepatic uptake (k_{12}) resulting in no significant decrease in clearance. Unfortunately an attempt to further investigation the effect of dose on the kinetics of ICG in ARF by increasing the dose to 15 and 30 mg/kg resulted in the appearance of zero order kinetics which renders pharmacokinetic analysis very difficult [8]. The occurrence of zero order kinetics with ICG has been noted in rats by other workers using a dose of 25 mg/kg [8] whereas no such observation was made by another group of investigators after administration of a dose of 50 mg/kg to rats [11].

The administration of saline as drinking fluid and the omission of dehydration prior to glycerol injection resulted in animals with plasma urea levels significantly lower than water-drinking rats which were dehydrated for 24 hr before injection (Table 1). This effect has been noted previously by other workers [4, 5]. Although these two manoeuvres by themselves produced some kinetic changes, the reduction in the degree of uraemia resulted in an absence of significant kinetic changes between control and mildly uraemic rats (Table 4). These findings provide evidence that the alteration of ICG pharmacokinetics in glycerol-induced ARF is a result of renal failure and not due to a direct hepato-toxic effect of glycerol. This is supported by our previous observation that a reduction in the hepatic uptake of ICG occurs in rats with ARF produced by ureter ligation [1] and furthermore, in a recent study we have noted a decrease in the hepatic uptake and plasma clearance of ICG in rats with chronic renal failure produced by partial nephrectomy [13].

In conclusion, the changes in the pharmacokinetics of ICG in glycerol-induced ARF appear to be dose dependent with greater changes occurring at the higher doses of 4.0 and 7.5 mg/kg than at 1.0 mg/kg. These kinetic changes appear to be a consequence of renal failure and not a direct hepato-toxic effect of glycerol.

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