Trease, G. E., Evans, W. C. (1972) in: Pharmacognosy, 10th Edition, Bailliere Tindall, London, p 444

Vane, J. R. (1971) Nature New Biol. 231: 232–235

Vinegar, R., Truax, J. F., Selph. J. L. (1976) Eur. J. Pharmacol. 37: 23–30

Walker, J. R., Smith, M. J. H., Ford-Hutchinson, A. W. (1976) Agents Actions 6: 602–606

Winter, C. A., Risley, E. A., Nuss, G. W. (1962) Proc. Soc. Exp. Biol. Med. 111: 544–550

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Hepatic clearance of indocyanine green during the course of glycerol-induced acute renal failure in the rat

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We have previously shown that in rats with glycerol-induced acute renal failure (ARF) there is a significant decrease in the plasma clearance of indocyanine green (ICG) and that this was probably due to decreased hepatic uptake of the dye (Bowmer et al 1982a). Similarly, the hepatic uptake of bromosulphophthalein is decreased in patients with chronic renal failure (Wernze & Spech 1971) and also in rats with glycerol-induced ARF (Bowmer et al 1982b). Furthermore, Tse et al (1976) found that the removal of rose bengal from plasma of rats with chronic renal failure was impaired. These studies provide evidence that both acute and chronic renal failure can affect the efficiency with which the liver removes these substances from plasma.

All these previous studies of hepatic function in renal failure have been done when either acute or chronic uraemia was well established. The purpose of this study was to determine how quickly the impairment of hepatic uptake of ICG occurs after the initiation of ARF and to see if this aspect of liver function is restored as renal function recovers. We have, therefore, studied the pharmacokinetics of ICG at various intervals after the induction of acute renal failure.

Materials and methods

Acute renal failure was produced by intramuscular injection of 50% v/v glycerol in 0·9% w/v sterile NaCl (saline), 10 ml kg^{-1} (Thiel et al 1967). Control rats were injected with saline, 10 ml kg^{-1}. Groups of rats were studied at 12, 24, 48 h and 7 days after injection of either glycerol or saline.

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.) and cannulae inserted into the trachea, left jugular vein and right carotid artery. Rectal temperature was maintained at 37 °C by means of a heating lamp. ICG (Hynson, Wescott and Dunning Ltd., Baltimore) was administered 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. After each sample was collected, blood was replaced by an equal volume of saline.

The concentration of ICG in plasma was measured spectrophotometrically at 800 nm (Iga et al 1980). Plasma urea concentrations were measured by reaction with diacetyl monoxime using the reagents and procedure contained in Sigma Technical Bulletin No 535 (Sigma Chemical Co.). The packed cell volume (PCV) was determined for blood samples taken before the injection of ICG.

The kinetics of ICG can be explained on the basis of a two compartment model with elimination of the dye from the peripheral compartment (Bowmer et al 1982a). Plasma concentration-time data were fitted to a biexponential equation by non-linear least squares regression analysis (Snedecor & Cochran 1967). The apparent volume of the central compartment (Vc) was calculated as:

$$Vc = \frac{Dose}{A + B}$$

where A and B are the intercept values at zero time for the individual α- and β-phases. The apparent volume of distribution at steady-state (Vdss) was calculated from the equation:

$$Vdss = Vc \frac{k_{12} + k_{21} + k_{23}}{k_{21} + k_{23}}$$

where k_{12} and k_{21} are the apparent first-order intercompartmental rate constants, and k_{23} is the apparent first-order rate constant for elimination from the peripheral compartment. These rate constants were calculated using the equations given by Gibaldi & Perrier (1975). The plasma clearance (Clp) of ICG was calculated from the equation:

$$Clp = \frac{Dose}{AUC_{0\rightarrow\infty}}$$

where $AUC_{0\rightarrow\infty}$ is the area under the concentration-time curve from zero to infinity. $AUC_{0\rightarrow\infty}$ is given by the equation:

$$AUC_{0\rightarrow\infty} = \frac{A}{\alpha} + \frac{B}{\beta}$$

Results are expressed as mean ± s.d. and statistical comparison of data at 12, 24 and 48 h were made by the

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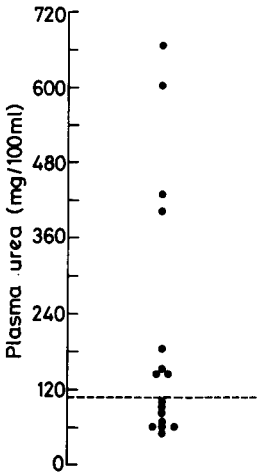

FIG. 1. The distribution of plasma urea levels in rats 7 days after the intramuscular injection of glycerol. The line is drawn at a value of 108 mg 100 ml^{-1} which is twice the mean plasma urea value in control rats. The rats with plasma urea levels above the line were termed 'uraemic' (group I) and those below 'recovering' (group II).

non-paired Student's *t*-test. The results at 7 days were analysed by one-way analysis of variance and means were compared by the method of Least Significant Difference (Snedecor & Cochran 1967).

Results

Up to 48 h no deaths occurred in the groups of uraemic rats, but four rats died out of a total of 20 which were allowed to survive for more than 48 h after the induction of ARF. This represents a mortality of 20% which is lower than that previously reported for 7 days after the induction of ARF: 31% (Hiley et al 1980) and 70% (Ayer et al 1971).

Plasma urea concentrations, indicating the degree of renal failure, rose quickly in the glycerol injected rats. At 12 h ARF was well established and at 48 h urea levels had reached a maximum (Table 1). However 7 days after the induction of ARF the uraemic rats could be broadly classified into two groups (Hiley et al 1980) with respect to plasma urea levels (Table 1; Fig. 1). Rats whose plasma urea concentrations were greater than twice the mean control value were termed 'uraemic' (group I) whilst animals with urea levels below this division were termed 'recovering' (group II). Group I consisted of those rats which were either moderately or severely uraemic whilst group II included rats which were mildly uraemic or had urea levels within the control range (Table 1).

The mean body weights of the rats intended for study at 7 days were not significantly different at the time of injection; but after 7 days had elapsed the rats in group I had a significantly lower mean body weight than controls (Table 1) and this weight loss may have contributed to the high plasma urea concentration of this group. There was no significant difference in wet liver weight at any time period but the PCV was decreased in uraemic rats at 12, 24 and 48 h (Table 1).

In all groups of uraemic rats the α-phase half-life was significantly prolonged (Table 2) and this was associated with a pronounced decrease in k_{12}, the rate constant for transfer of ICG from the central to peripheral compartment (Table 3). Furthermore, the rate constant for transfer from the peripheral to central compartment, k_{21}, was significantly decreased at all the times studied (Table 2), but the β-phase half-life and k_{23}, the rate constant for elimination of ICG from the peripheral compartment, were only significantly decreased at 24 and 48 h after the induction of ARF (Tables 2, 3). Few changes in apparent volume of distribution were found; but at 12 h both Vc and Vdss were significantly decreased in the uraemic rats whilst at 48 h Vc was significantly increased (Table 2). The plasma clearance of ICG was significantly decreased in all groups of uraemic rats (Fig. 2). The greatest decrease in Clp occurred at 24 h (36%) whilst at 7 days there were signs of a limited, but not significant, recovery of hepatic function with decreases in Clp of 17% (group I) and 21% (group II).

Discussion

The results clearly demonstrate that from as early as 12 h after the induction of ARF the plasma clearance of ICG was significantly decreased. The decrease was maximal at 24 h and persisted for up to 7 days even in rats whose plasma urea concentrations had almost returned to control levels. The principal cause of the reduction in Clp, at all time intervals, appears to be a significant decrease in k_{12}, the rate constant for the transfer of ICG from the central to peripheral compartment. As ICG has little extravascular distribution (Leevy et al 1963) and is eliminated almost entirely by the hepato-biliary system (Cherrick et al 1960; Leevy et al 1963), the decrease in k_{12} would suggest that the hepatic uptake of ICG is decreased in rats with ARF (Bowmer et al 1982a).

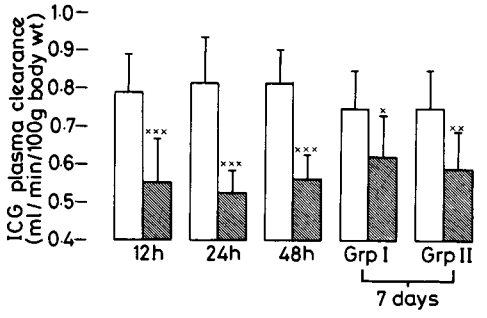

FIG. 2. Plasma clearance of ICG (7·5 mg kg^{-1}) in rats at various intervals after the i.m. injection of either saline or glycerol (shaded columns). Each value is the mean ± s.d. of eight rats. Key: $^{*}P < 0{\cdot}05$ $^{**}P < 0{\cdot}01$ $^{***}P < 0{\cdot}001$ relative to respective control value.

Table 1. Body weight, liver weight, packed cell volume (PCV) and plasma urea concentration in the rat at intervals after the induction of acute renal failure.†

	Body weight (g)	Liver weight (g/100 g body wt)	PCV (%)	Plasma urea (mg/100 ml)
12 h				
Control (6)	311 ± 28	3·89 ± 0·35	49 ± 1	51 ± 4
Uraemic (7)	294 ± 15	3·54 ± 0·31	47 ± 2*	226 ± 52***
24 h				
Control (9)	350 ± 43	3·95 ± 0·20	46 ± 2	49 ± 8
Uraemic (10)	321 ± 33	3·72 ± 0·34	43 ± 2**	295 ± 91***
48 h				
Control (8)	324 ± 30	3·75 ± 0·40	48 ± 2	32 ± 14
Uraemic (8)	331 ± 31	3·81 ± 0·39	44 ± 3**	347 ± 157***
7 days				
Control (8)	324 ± 49	3·75 ± 0·34	48 ± 2	54 ± 10
Group I (uraemic) (8)	258 ± 31***	3·58 ± 0·46	48 ± 3	341 ± 214***
Group II (recovering) (8)	302 ± 39	3·71 ± 0·50	46 ± 3	74 ± 19

† Results are given as mean ± s.d. and number of rats in parentheses.
*P <0·05; **P <0·01; ***P <0·001 relative to respective control group.

Table 2. Pharmacokinetic parameters for ICG (7·5 mg kg^{-1} i.v.) at various times after induction of acute renal failure.†

Group	$t_{0.5\alpha}$ (min)	$t_{0.5\beta}$ (min)	Vc (ml)	Vdss (ml)
12 h				
Control (6)	2·0 ± 0·2	39·7 ± 8·3	10·7 ± 1·9	152·1 ± 45·0
Uraemic (7)	2·6 ± 0·4**	37·7 ± 8·0	8·0 ± 0·8**	90·6 ± 18·6**
24 h				
Control (9)	1·9 ± 0·3	33·5 ± 3·7	10·8 ± 1·7	145·1 ± 35·5
Uraemic (10)	3·5 ± 0·6***	50·1 ± 14·3**	11·2 ± 1·5	129·4 ± 47·7
48 h				
Control (8)	1·6 ± 0·2	33·8 ± 8·7	8·4 ± 0·9	133·4 ± 34·6
Uraemic (8)	2·8 ± 0·4***	41·6 ± 4·3*	10·0 ± 1·1**	118·3 ± 20·6
7 days				
Control (8)	2·0 ± 0·4	35·1 ± 3·7	8·8 ± 1·2	117·1 ± 31·1
Group I (uraemic) (8)	2·7 ± 0·6*	37·1 ± 7·7	8·3 ± 1·1	92·0 ± 29·7
Group II (recovering) (8)	2·8 ± 0·8*	42·8 ± 13·5	9·6 ± 0·8	117·6 ± 49·1

† Results are given as mean ± s.d. and number of rats in parentheses.
*P <0·05; **P <0·01; ***P <0·001 relative to respective control group.

However, the clearance of ICG, at the dose used in this study (7·5 mg kg^{-1}), is partially dependent upon liver blood flow (McDevitt et al 1977) and Hiley et al (1980) have shown that following glycerol-induced ARF, liver blood flow is decreased at 12 h, unchanged at 24 h, increased at 48 h, and increased in both 'uraemic' (group I) and 'recovering' (group II) rats at 7 days.

In view of this, the decreased clearance of ICG at 12 h may be partly due to reduced liver blood flow. In addition, the early stages of glycerol-induced ARF are associated with a contraction of plasma volume (Thiel et al 1967), and it is interesting that both Vc and Vdss were significantly smaller at 12 h. Clearance is dependent upon the apparent volume of distribution, so the decrease in Vdss, along with decreases in k_{12} and liver blood flow, is likely to contribute to the reduction in ICG clearance at 12 h. At 24 h, 48 h and 7 days, liver blood flow is either unchanged or increased (Hiley et al 1980) and as Vc and Vdss remain unchanged, apart from a small increase in Vc at 48 h, it would seem unlikely that these variables contribute to the reduction in Clp observed at times later than 12 h.

The reason for the wide variation in plasma urea levels at 7 days after the induction of ARF is unclear, but other groups of investigators have also been able to divide rats similarly treated into 'uraemic' (group I) and 'recovering' (group II) (Ayer et al 1971; Hiley et al 1980). In addition to plasma urea concentrations, histological evidence and studies of renal blood flow also suggest that at 7 days kidney function is almost restored to normal in the 'recovering' group (Ayer et al 1971). In spite of the apparent recovery of renal function, both the clearance of ICG and k_{12} were still decreased and not significantly different from their respective values for the 'uraemic' group.

Table 3. Apparent first-order intercompartmental rate constants for ICG (7·5 mg kg^{-1} i.v.) at various times after the induction of acute renal failure.†

Group	k_{12} (min^{-1})	k_{21} (min^{-1})	k_{23} (min^{-1})
12 h			
Control (6)	0·3374 ± 0·0281	0·0078 ± 0·0006	0·0185 ± 0·0037
Uraemic (7)	0·2630 ± 0·0368**	0·0065 ± 0·0013*	0·0197 ± 0·0046
24 h			
Control (9)	0·3589 ± 0·0506	0·0076 ± 0·0004	0·0213 ± 0·0021
Uraemic (10)	0·1993 ± 0·0347***	0·0051 ± 0·0013***	0·0154 ± 0·0045**
48 h			
Control (8)	0·4331 ± 0·0652	0·0080 ± 0·0018	0·0219 ± 0·0046
Uraemic (8)	0·2467 ± 0·0352***	0·0056 ± 0·0010**	0·0172 ± 0·0017*
7 days			
Control (8)	0·3505 ± 0·0614	0·0087 ± 0·0016	0·0205 ± 0·0023
Group I (uraemic) (8)	0·2571 ± 0·0499**	0·0065 ± 0·0012**	0·0203 ± 0·0047
Group II (recovering) (8)	0·2597 ± 0·0658**	0·0067 ± 0·0015**	0·0178 ± 0·0044

† Results are given as mean ± s.d. and number of rats in parentheses.
*P <0·05; **P <0·01; ***P <0·001 relative to respective control group.

The reason for the defective hepatic uptake of dyes such as ICG in renal failure is not clear, but possible explanations include altered hepatic protein metabolism (Wernze & Spech 1971) and inhibition of uptake by endogenous metabolites retained in uraemic plasma (Liang et al 1978). Both mechanisms seem plausible as there is evidence to suggest that hepatic cytoplasmic proteins (Levi et al 1969) and carrier-mediated transport (Scharschmidt et al 1975) may be involved in the hepatic uptake of organic anions such as ICG. Whatever mechanism is responsible, it appears to develop rapidly and to persist even in rats whose renal function is recovering.

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REFERENCES

Ayer, G., Grandchamp, A., Wyler, T., Truniger, B. (1971) Circ. Res. 29: 128–135

Bowmer, C. J., Yates, M. S., Emmerson, J. (1982a) Biochem. Pharmacol. 31: 2531–2538

Bowmer, C. J., Hall, S. T., Yates, M. S. (1982b) Br. J. Pharmacol. 75: 110P

Cherrick, G. R., Stein, S. W., Leevy, C. M., Davidson, C. S. (1960) J. Clin. Invest. 39: 592–600

Gibaldi, M., Perrier, D. (1975) Pharmacokinetics. p 88, Marcel Dekker, New York

Hiley, C. R. Yates, M. S. Roberts, P. J., Bloom, A. E. (1980) Nephron 26: 244–248

Iga, T., Yokota, M., Sugiyama, Y., Awazu, S., Hanano, M. (1980) Biochem. Pharmacol. 29: 1291–1297

Leevy, C. M., Bender, J., Silverberg, M., Naylor, J. (1963) Ann. N.Y. Acad. Sci. 111: 161–174

Levi, A. J., Gatmaitan, Z., Arias, I. M. (1969) J. Clin. Invest. 48: 2156–2167

Liang, M. Y., Toporek, M., Schepartz, B. (1978) Nephron 22: 306–311

McDevitt, D. G., Nies, A. S., Wilkinson, G. R. (1977) Biochem. Pharmacol. 26: 1247–1250

Scharschmidt, B. F., Waggoner, J. G., Berk, P. D. (1975) J. Clin. Invest. 56: 1280–1292

Snedecor, G. W., Cochran, W. G. (1967) Statistical Methods. 6th edn, p 465, Iowa State University Press, Ames, Iowa

Thiel, G., Wilson, D. R., Arce, M. L. Oken, D. E. (1967) Nephron 4: 276–297

Tse, J. W., Wiebe, L. I. Ediss, C., Shysh, A. (1976) Int. J. Nucl. Med. Biol. 3: 134–137

Wernze, H., Spech, H. J. (1971) Klin. Wschr. 49: 1318–1322