Indocyanine Green elimination in patients with liver disease and in normal subjects

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(Received 23 March/7 August 1990; accepted 14 September 1990)

SUMMARY
1. The validity of a two-compartment pharmacokinetic model for the estimation of the hepatic extraction ratio of Indocyanine Green was tested in six patients with cirrhosis of the liver.
2. No agreement was found between the value of the hepatic extraction ratio measured directly and that calculated using the two-compartment model.
3. To investigate the reasons for the failure of the model, an extended sampling period was used to define the time course of Indocyanine Green in plasma in six healthy subjects and in six patients with cirrhosis of the liver after a bolus injection of the dye.
4. Indocyanine Green was measurable in the plasma for up to 10 h after injection in healthy subjects, and up to 48 h after injection in the patients. The plasma elimination curve in both groups was best described by a tri-exponential function.
5. The clearance of Indocyanine Green calculated using data collected in the first 20 min after injection overestimated that calculated using data collected for as long as Indocyanine Green was measurable in the plasma. In the patients with cirrhosis the mean overestimate was 87%.
6. Thus, a two-compartment pharmacokinetic model was inappropriate for the description of the disposition of Indocyanine Green and estimates of the hepatic extraction ratio obtained using this model in patients with cirrhosis were inaccurate.

Key words: Indocyanine Green, liver disease, pharmacokinetics.

Abbreviations: AUC, area under plasma dye concentration–time curve from time 0 to infinity; $E_H$, hepatic extraction ratio; ICG, Indocyanine Green.

INTRODUCTION
Indocyanine Green (ICG) is commonly used to measure liver blood flow [1–5] as it possesses many of the properties of an ideal indicator for use with clearance techniques. Thus, it is considered to have a high hepatic extraction ratio ($E_H$), it does not undergo extrahepatic elimination, its volume of distribution approximates to plasma volume, and it is not subject to enterohepatic recirculation [1–3, 6]. The original description of the use of the dye for the measurement of liver blood flow involved intravenous infusion to steady state and simultaneous sampling from the hepatic vein and a peripheral vein (transhepatic sampling) to allow the application of the Fick principle [7]. The assumption is made that the concentration of dye in the portal vein and hepatic artery is the same as that in peripheral venous blood. Subsequently, the clearance of the dye has been equated with liver blood flow, assuming an $E_H$ of 1 [8–10]. However, as estimates of the $E_H$ of ICG in healthy man vary from 0.5 to 0.9 [1–3, 11, 12], such assumptions will result in a variable overestimate of liver blood flow. In liver disease, when the $E_H$ of ICG may be much lower [1, 11], it is not valid to equate ICG clearance directly with liver blood flow.

Direct measurement of the $E_H$ of ICG is highly invasive, requiring hepatic venous catheterization. However, a relatively non-invasive method has been described and has been validated in healthy man and in the baboon by Grainger et al. [12]. The method is based on the application of a two-compartment pharmacokinetic model and uses the exponents and intercepts of a biexponential function describing the dye plasma concentration–time curve constructed by sampling for 22 min after a single intravenous bolus dose of ICG. These values allow calculation of the rate constants describing the distribution of the dye between plasma and liver compartments. $E_H$ is calculated using the rate constants [12]. Thus, neither prolonged intravenous infusion nor hepatic venous catheterization is required.
The use of this method in patients with liver disease has produced contradictory findings. Navasa et al. [13] found close agreement between the estimates of \( E_H \) when measured directly by transhepatic sampling and indirectly by peripheral venous sampling from a single site. In a further study in patients with liver disease, Clements et al. [14] observed no such relationship. Furthermore, in both of these studies ICG was assayed by a non-specific and relatively insensitive spectrophotometric method with sampling periods of up to 60 min.

We have demonstrated previously that the two-compartment model fails to estimate accurately the \( E_H \) of ICG in the rat [15]. In the present experiment we have compared estimates of the \( E_H \) of ICG in patients with cirrhosis of the liver using both direct transhepatic sampling and the two-compartment pharmacokinetic model. The application of the two-compartment pharmacokinetic model depends upon the accurate definition of a biexponential elimination curve, and therefore a second experiment was performed in order to characterize the time course of the elimination of ICG from the plasma. This involved giving an intravenous bolus dose of ICG to healthy subjects and to patients with liver disease and collecting venous blood samples for up to 48 h.

METHODS

Experiment 1: estimation of the \( E_H \) of ICG and liver blood flow in patients with cirrhosis

Subjects. Six patients (three male, three female) with clinically stable biopsy-proven liver cirrhosis gave written informed consent to the study, which had the approval of the local Hospital Ethics Committee. All these patients were undergoing hepatic venous catheterization as part of the routine investigation of their portal venous blood pressure.

Direct transhepatic measurement of the \( E_H \) of ICG. An ICG infusion (0.25 mg/min) was administered via a peripheral vein immediately after an intravenous bolus of 5 mg of ICG to allow the earlier attainment of steady state. The hepatic vein was catheterized via the brachial vein and the inferior vena cava under fluoroscopic control without any premedication. After wedging of the catheter to allow assessment of the wedged hepatic venous pressure, the catheter was withdrawn to an unwedged position in the hepatic vein and its position was checked radiologically. At steady state (defined as a variation of no more than 10% in the mean ICG plasma concentration measured over a 30 min sampling period) simultaneous blood samples were collected from the hepatic vein and from a peripheral vein the the contralateral arm to that used for the ICG infusion. Three paired samples were collected at 10 min intervals.

Estimation of the \( E_H \) of ICG using the pharmacokinetic model. Two to fourteen days after the infusion experiment, an intravenous bolus of ICG (0.5 mg/kg) was given and blood samples were collected from the contralateral arm for 2 h. The sampling times were as follows: 0, 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105 and 120 min.

Experiment 2: extended description of the plasma ICG concentration–time curve

Subjects. Six healthy subjects and six patients with biopsy-proven cirrhosis gave written informed consent to the study, which had the approval of the local Hospital Ethics Committee. The patients were not the same subjects who participated in experiment 1. After an intravenous bolus dose of ICG (0.5 mg/kg), serial blood samples were collected from a peripheral vein in the contralateral arm for 48 h. The sample times were as follows: 0, 2, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 150 and 180 min, and 4, 5, 6, 8, 10, 12, 14, 22, 24, 28, 32, 36 and 48 h.

Data analysis

Experiment 1. Infusion of ICG. The clearance of ICG was calculated by dividing the infusion rate by the plasma ICG concentration at steady state. Whole-blood clearance was calculated from the plasma clearance and the packed cell volume. \( E_H \) was calculated using the following equation:

\[
E_H = \frac{(C_p - C_s)}{C_s}
\]

where \( C_p \) is the concentration of ICG in the peripheral vein and \( C_s \) is the concentration of ICG in the hepatic vein. Hepatic blood flow was estimated by dividing whole-blood clearance by \( E_H \).

Intravenous bolus of ICG. The plasma clearance of ICG was calculated from dose/AUC, where AUC is the area under the plasma dye concentration–time curve from time 0 to infinity. The latter was calculated as the sum of AUC(0,\( t_i \)), calculated by the linear trapezoidal rule (where \( t_i \) is the time at which the terminal log linear portion of the plasma dye concentration–time curve commenced), and AUC(\( t_i \), infinity), calculated by dividing the concentration of ICG at \( t_i \) by the terminal elimination rate constant derived by least squares regression analysis. This method of calculating the AUC does not use the values of the exponents and intercepts of the elimination curve. Whole-blood clearance was calculated from the plasma clearance and the packed cell volume.

Compartmental rate constants were calculated from the exponents and intercepts of the biexponential function describing the plasma dye concentration time curve fitted by a non-linear least squares regression computer program (NONLIN) [15]. \( E_H \) was calculated using the following equation, as derived by Matthews [16] and Grainger et al. [12].

\[
E_H = k_{20}/(k_{30} + k_{21})
\]

where \( k_{30} \) is the first-order rate constant describing the transfer of ICG from the liver to the plasma and \( k_{20} \) is the first-order rate constant describing elimination of ICG from the liver into the bile. Hepatic blood flow was again estimated by dividing the whole-blood clearance by \( E_H \).
**Experiment 2.** The plasma concentration-time data obtained for as long as ICG remained measurable (to time \( t \)) was fitted by bi- and tri-exponential functions using non-linear least squares regression with weighting of the data by \( 1/y^2 \) using NONLIN [15].

The plasma clearance of ICG was calculated using data collected in the first 20, 60 or \( t \) min after injection of the dye. For each time period the clearance was calculated by dividing the ICG dose by the area under the plasma dye concentration–time curve extrapolated to infinity. The latter was calculated as the sum of AUC\((0,t_i)\), calculated by the linear trapezoidal rule (where \( t_i \) is the time of the start of the second exponential), and AUC\((t_i,\infty)\), calculated by dividing the concentration of ICG at \( t_i \) by the terminal elimination rate constant derived by least squares regression analysis. This method of calculating the AUC does not use the values of the exponents and intercepts of the elimination curve.

**Statistical analysis**

'Goodness of fit' of the bi- and tri-exponential functions to the experimental data was assessed by comparison of the residual sums of squares. From this comparison an \( F \)-ratio was derived. Comparisons between the clearance values calculated in the different sampling periods were made by analysis of variance.

**ICG assay**

Plasma ICG concentrations were measured using a highly sensitive and specific h.p.l.c. assay based on the method described by Burns et al. [17] with some minor modifications. In contrast to spectrophotometric assays, this method allows the chromatographic resolution of native ICG from an impurity or metabolite of the compound [18, 19].

Blood samples were centrifuged immediately and 0.5 ml of plasma was collected. Acetonitrile (0.5 ml) was added and the mixture was vortexed for 45 s; it was then centrifuged at 3000 rev./min for 10 min to sediment the protein. After decanting the supernatant, ammonium sulphate crystals were added to aid separation of the aqueous and organic phases. The sample was mixed again by vortexing for 45 s and centrifuged again at 3000 rev./min for a further 10 min. The organic phase containing ICG was aspirated and mixed with an equal volume of phosphate buffer (0.05 mol/l \( \text{KH}_2\text{PO}_4 \) mixed with 0.05 mol/l \( \text{K}_2\text{HPO}_4 \) to pH 6) immediately before injection into the chromatograph.

Chromatography was performed as described previously [17]. A fresh calibration curve was constructed using each subject's plasma for each batch of assays. The calibration graphs were linear and passed through the origin. The limit of measurement of the assay was 5 ng/ml, at which concentration the coefficient of variation was 6% (\( n = 6 \)).

**RESULTS**

**Experiment 1**

There was no agreement between the two estimates of the \( E_H \) of ICG, nor between the two estimates of liver blood flow (Table 1).

The clearance of ICG was consistently underestimated from the intravenous bolus kinetics of ICG compared with the value obtained after intravenous infusion (Fig. 1).

**Experiment 2**

Prolonged sampling after ICG bolus: healthy subjects.

ICG was measurable for up to 10 h after injection. Fig. 2 shows a representative plasma ICG concentration–time curve together with the curves generated by fitting the data with bi- and tri-exponential functions using NONLIN [15]. For each subject the tri-exponential function gave the better fit, and this was significantly so in five of the six subjects (\( F \)-ratios > 8.84; \( P < 0.01 \) in five of the subjects).
Fig. 2. Plasma concentrations of ICG in a representative healthy subject after an intravenous bolus injection of 0.5 mg of ICG/kg. •, Experimental data; ◦, fit of triexponential function; ●, fit of biexponential function.

Values of plasma ICG clearance calculated using data collected in the first 20, 60 or t min after injection of the dye are shown in Table 2. The mean clearance value calculated using data from the first 20 min overestimated that calculated using data collected to time t by 13% \((791 \text{ ml/min} \pm 438)\) versus 702 ml/min \((331)\). The time t varied from 3 to 10 h.

Prolonged sampling after ICG bolus: patients with liver cirrhosis. ICG was measurable in plasma for up to 48 h after injection. Fig. 3 shows a representative plasma dye concentration–time curve together with the curves generated by fitting the data with tri- and bi-exponential functions using NONLIN [15]. The fit of the triexponential function to the experimental data was significantly better in four of the six patients \((F\text{-ratios} > 8.23; P < 0.01\) in four of the patients).

Table 3 shows the values of the plasma clearance of ICG calculated using data collected in the first 20, 60 or t min after injection of the dye. The mean clearance value calculated using data collected in the first 20 min overestimated that calculated using data to time t by 87% \((227 \text{ ml/min} \pm 92)\) versus 127 ml/min \((48)\); \(P < 0.05\). The time t varied from 6 to 48 h.

**DISCUSSION**

The purpose of this study was to determine whether the two-compartment pharmacokinetic model for the estimation of the \(E_{ih}\) of ICG, proposed and validated in healthy subjects by Grainger et al. [12], could be applied to patients with cirrhosis of the liver. In patients with cirrhosis this two-compartment pharmacokinetic model failed to allow the accurate estimation of the \(E_{ih}\) of ICG. Hence, estimates of liver blood flow were also in error.

Two previous studies have examined the use of this compartmental model for the estimation of the \(E_{ih}\) of ICG in patients with liver disease [13, 14]. Navasa et al. [13] employed a 36 min sampling period with an intravenous bolus dose of ICG of 0.25 mg/kg and found agreement between \(E_{ih}\) measured directly and that calculated using the model. This study was reported in abstract form only and experimental details are not available. However, on the basis of this report, the pharmacokinetic model has been used to estimate liver blood flow in patients with cirrhosis [20].

Clements et al. [14] using a 60 min sampling period after an intravenous bolus dose of 0.25 mg/kg, found no agreement between the directly estimated value of \(E_{ih}\) and that calculated using the model. However, a relatively insensitive and non-specific spectrophotometric assay was used and poor definition of the second phase of ICG elimination may have influenced their findings. Indeed, in five of the 20 patients they studied no second phase of ICG elimination was found, and thus, in these patients, the application of a two-compartment pharmacokinetic model was clearly invalid.

A requirement for the use of the two-compartment model is that the elimination of ICG occurs as a first-order process. The ICG plasma clearance values obtained after infusion of the dye were consistently higher than those calculated after an intravenous bolus, indicating that saturation of ICG elimination occurred after the bolus dose. The elimination of ICG has been described by Michaelis–Menten kinetics [21], but it has previously been assumed that at the dose used in this study the elimination of the dye is first-order [22-24]. However, in patients with liver disease this may not be so. The use of a smaller dose of ICG may have avoided the appearance of saturation kinetics; however, Clements et al. [14], using a bolus dose of 0.25 mg of ICG/kg, also found greater ICG clearance values after infusion compared with bolus injection of the dye in patients with liver disease, suggesting that at this lower dose saturation of hepatic uptake or elimination mechanisms also occurred. This was so in spite of the methodological problems associated with the use of a short sampling period and a relatively insensitive
assay, which tend to result in an underestimate of the AUC and therefore an overestimate of clearance after the bolus dose.

It is possible that the use of a lower dose of ICG than has previously been practical may allow the avoidance of saturation kinetics. Such studies may be feasible using a highly sensitive ICG assay. However, the appearance of non-linear kinetics with doses of ICG commonly employed in cirrhotic patients precludes the use of the simple two-compartment model.

A further reason for the failure of the two-compartment pharmacokinetic model is suggested by the results of experiment 2, which demonstrate that the elimination of ICG is better described by a tri- than by a bi-exponential function.

The physiological identity of the third component of ICG elimination is not known. ICG may be taken up by an extrahepatic tissue and subsequently released slowly into the plasma; temporary distribution of ICG into the kidneys of heptatectomized cats has been demonstrated (although urinary excretion has not been found) [25]. Another possibility is that ICG is sequestered in the liver before elimination.

The clearance of ICG is often calculated from data collected in the first 20 min after injection of the dye [3, 8–10, 23, 24]. However, we have shown that the elimination of ICG is prolonged, both in healthy subjects and in patients with cirrhosis of the liver. In the healthy subjects, the use of the data from the 20 min sampling period overestimated the clearance by 13% when compared with the value obtained using data collected for as long as ICG remained measurable, but this difference was not significant. However, in the patients with liver disease the short sampling period resulted in an 87% overestimate of clearance ($P<0.05$). The method described by Grainger et al. [12] for non-invasive estimation of liver blood flow used an $E_H$ derived from the pharmacokinetic model and a clearance calculated using a 22 min sampling period after a bolus injection of ICG. The results of the present study show that the accurate assessment of ICG clearance in patients with liver disease after an intravenous bolus of the dye requires a prolonged sampling period.

Conventionally, a procedure employing infusion of ICG to steady state and transhepatic sampling has been considered to be the optimal method of measuring its hepatic extraction and deriving an estimate of liver blood flow both in subjects with and without liver disease, provided that the $E_H$ of ICG was not prohibitively low. The present study highlights two potential problems with the use of this method. First, the prolonged terminal elimination phase of ICG suggests that the attainment of true steady state may require extended infusion of the dye, and secondly, the possibility of extrahepatic uptake or elimination questions the assumption that the infusion rate of ICG at steady state is equal to hepatic elimination. However, the magnitude of error introduced by either of these factors is small. Thus, the results of this study do not preclude the use of ICG to measure liver blood flow in patients with cirrhosis provided that intravenous infusion to steady-state and transhepatic sampling are employed.

**ACKNOWLEDGMENTS**

We are grateful to the Hallamshire Therapeutics Research Trust for financial support, and to Dr P. R. Jackson for statistical advice.

**REFERENCES**


