

# Experimental study of liver dysfunction evaluated by direct indocyanine green clearance using near infrared spectroscopy

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**Background:** Blood clearance of indocyanine green (ICG) is an objective test of liver function. Hepatic ICG clearance can now be measured directly using near infrared spectroscopy (NIRS). The aim of this study was to evaluate measurement of hepatic ICG clearance by NIRS in an animal model of acute hepatic dysfunction.

**Methods:** New Zealand white rabbits ( $n = 36$ ) underwent laparotomy for liver exposure. Hepatic blood flow and microcirculation were measured along with hepatic ICG concentration by NIRS. Hepatic ICG clearance was measured in groups of six animals after reduction of the hepatic blood flow by hepatic artery occlusion and portal vein partial occlusion, lobar ischaemia and reperfusion (I/R), colchicine administration and bile duct ligation. Hepatic ICG uptake and excretion rates were calculated by a non-linear least square curve fitting method from the ICG concentration–time curve.

**Results:** There was a significant positive correlation between hepatic ICG rate of uptake and both hepatic blood flow and microcirculation ( $r = 0.79$ ,  $P = 0.0001$ ;  $r = 0.59$ ,  $P = 0.005$  respectively). I/R resulted in a significant reduction of both the rates of ICG uptake (mean(s.d.)  $0.85(0.59) \text{ min}^{-1}$ ;  $P = 0.0002$  versus control) and ICG excretion ( $0.020(0.006) \text{ min}^{-1}$ ;  $P = 0.02$  versus control). Colchicine decreased the rate of hepatic ICG excretion ( $0.030(0.010) \text{ min}^{-1}$ ;  $P = 0.02$  versus control) as did bile duct ligation ( $0.002(0.001) \text{ min}^{-1}$ ;  $P = 0.01$  versus control).

**Conclusion:** Measurement of hepatic ICG clearance by NIRS is a promising technique for assessing hepatic parenchymal dysfunction and may have application in liver surgery and transplantation.

Presented to the Third World Congress of the International Hepato–Pancreato–Biliary Association in Madrid, Spain, May 1998 and to the Surgical Research Society in Dublin, Ireland, July 1998

Paper accepted 10 April 1999

British Journal of Surgery 1999, 86, 1005–1011

## Introduction

Indocyanine green (ICG) is a synthetic dye that has been used for many years to measure hepatic blood flow and as a test of liver function<sup>1–3</sup>. In liver transplantation ICG clearance has been used for evaluation of the donor graft<sup>4</sup> as well as for postoperative assessment of liver graft function<sup>5</sup>.

Conventionally, ICG handling by the liver is predicted from its blood clearance curve<sup>2</sup>. However, Ott *et al.*<sup>6</sup> have suggested that the ICG plasma clearance curve contains no information about liver–bile interaction. Direct measurement of hepatic ICG concentration could provide an accurate way to study ICG kinetics in the liver including its uptake and excretion.

Near infrared spectroscopy (NIRS) is a light-based non-invasive technique originally developed for monitoring tissue oxygenation. It has been used for measurement of cerebral tissue oxygenation<sup>7</sup> and more recently for hepatic tissue oxygenation<sup>8,9</sup>.

ICG has a characteristic absorption peak in the near infrared light region allowing its direct measurement in the tissue. The application of NIRS for measurement of hepatic ICG concentration was first reported by Shinohara *et al.*<sup>10</sup> in 1996, who studied the handling of ICG by the rabbit liver. They demonstrated that NIRS was sufficiently sensitive to measure the effect of competitive inhibitors of ICG handling and the alterations to ICG clearance in ischaemia and reperfusion (I/R) injury.

The aim of this study was to extend the preliminary studies of Shinohara *et al.*<sup>10</sup> to other causes of acute hepatic

dysfunction that could be evaluated by measuring ICG clearance by NIRS, and to correlate these data with hepatic blood flow and parenchymal perfusion.

## Materials and methods

### Animal preparation and surgical procedure

The study was conducted under a licence granted by the Home Office in accordance with the Animals (Scientific Procedures) Act, 1986. New Zealand white rabbits (mean(s.d.) 2.9(0.3) kg,  $n = 36$ ) were used. Anaesthesia was induced using Hypnorm (Janssen-Cilag, High Wycombe, UK) (fentanyl-fluanisone) 0.5 ml kg<sup>-1</sup> and intramuscular diazepam 2.5 mg kg<sup>-1</sup>, and maintained by Kalothane (May and Baher, Dagenham, UK) via a standard anaesthetic circuit. Body temperature was maintained by a warming blanket at 37–38.5°C (Homeothermic blanket control unit; Harvard, Massachusetts, USA). The arterial oxygen saturation and heart rate were monitored continuously by pulse oximetry (Ohmeda Biox 3740-pulse oximeter; Ohmeda, Louisville, Colorado, USA). Catheters were inserted into the right femoral artery and vein for mean arterial blood pressure monitoring and fluid administration. Normal saline solution was given intravenously during the experiment at a rate of 10 ml kg<sup>-1</sup> h<sup>-1</sup> to compensate for intra-operative fluid loss. For measurement of hepatic ICG clearance, a bolus of ICG (Cardiogreen, 90 per cent dye content; Sigma Chemical Company, Poole, UK) 0.5 mg kg<sup>-1</sup> was given. ICG was dissolved in sterile water (1 mg ml<sup>-1</sup>) and given via the marginal ear vein over 20 s.

Laparotomy was performed through a midline incision. The ligamentous attachments from the liver to the diaphragm were divided and the liver was exposed. The hepatic artery and portal vein were dissected and exposed for the application of the flowmeter probes. For continuous measurement of total hepatic blood flow, an ultrasonic flowmeter system (HT207; Transonic Medical System, Ithaca, New York, USA) was used with perivascular flow probes of 1 and 4 mm in diameter for the hepatic artery and portal vein respectively<sup>11</sup>. Flow in the hepatic microcirculation was measured by a surface laser Doppler flowmeter (DRT4; Moor Instruments, Axminster, UK) in flux units<sup>12</sup>.

### Near infrared spectroscopy

NIRS depends on the light absorption properties of key biochemical components in the tissues and can be used to measure concentration changes of oxyhaemoglobin (HbO<sub>2</sub>) and deoxyhaemoglobin (Hb) as well as synthetic dyes, such as ICG. The change in the concentration of these chromophores can be quantified using a modified Beer Lambert law<sup>13,14</sup>.

The NIRS instrument (NIRO-500; Hamamatsu Photonics, Hamamatsu, Japan) used in this study produced light at four wavelengths which was transmitted in sequential pulses via a bundle of optical fibres (probes) to the liver. Photons emerging from the liver were collected by the second bundle of optical fibres and detected by a photomultiplier tube. The difference between transmitted and received light intensity at each wavelength was used to determine the optical density changes at each wavelength. A modified computer program was used to measure continuously the changes in hepatic HbO<sub>2</sub>, Hb and ICG concentrations based on the absorption coefficients of these chromophores<sup>9</sup>. For continuous monitoring of hepatic tissue oxygenation and ICG concentration NIRS probes were positioned flat on the liver surface with a 20-mm separation.

### Analysis of hepatic indocyanine green concentration curve

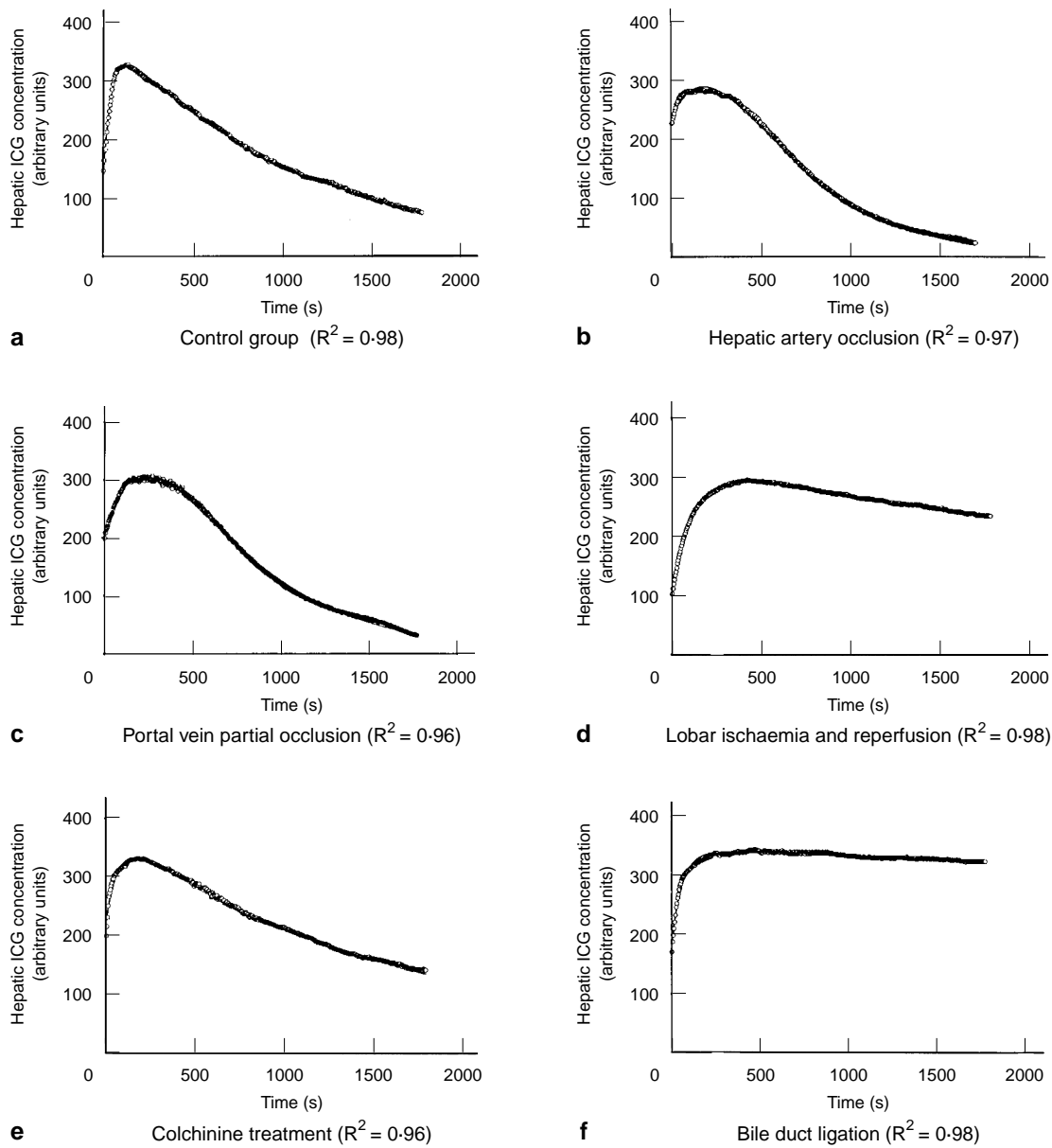
Continuous measurement of hepatic ICG by NIRS produces a concentration–time curve. This curve was analysed to produce two exponential rate constants, representing hepatic ICG uptake from the plasma to the hepatocytes ( $\alpha$ ) and hepatic ICG excretion from the liver by cytoplasmic transport and biliary excretion ( $\beta$ ). These rate constants were calculated by fitting the ICG concentration–time curve to a two-compartment mathematical model as defined by the sum of two exponential equations, as reported previously by Shinohara *et al.*<sup>10</sup>:

$$\text{ICG}(t) = -A \times \exp(-\alpha \times t) + B \times \exp(-\beta \times t),$$

where ICG( $t$ ) is the hepatic concentration of ICG at any time ( $t$ ), and  $\alpha$  and  $\beta$  (per minute) are the rate constants for hepatic ICG uptake and excretion respectively.  $A$  and  $B$  are zero time intercepts, both theoretically equal to the initial hepatic concentration. The assumption is that  $\alpha > \beta$  and  $A \approx B$ . The ICG concentration–time curves were fitted by a non-linear least square regression model and the goodness of fit was evaluated by the  $R^2$  value.

### Experimental groups

There were six experimental groups ( $n = 6$  per group). (1) Hepatic ICG clearance was measured without reduction of hepatic blood flow or induction of hepatic damage (control group). (2) Hepatic blood flow was reduced by hepatic artery occlusion 30 min before measuring hepatic ICG clearance. (3) Hepatic blood flow was reduced by partial (50 per cent) occlusion of the portal vein 30 min before measuring hepatic ICG clearance. (4) Lobar I/R was



**Fig. 1** Typical examples of indocyanine green (ICG) concentration–time curves and their fitted curves.

achieved by clamping the vascular pedicles of the median and left lateral lobes of the liver using a microvascular clip for 60 min followed by reperfusion for 60 min; this is an established model of hepatic I/R<sup>15</sup>. Hepatic ICG clearance was measured at the end of the reperfusion period. (5) Impairment of ICG excretion was achieved using colchicine, a potent microtubule toxin previously shown

to reduce ICG excretion from the liver<sup>16</sup>. Colchicine 2 mg kg<sup>-1</sup> (95 per cent; Sigma Chemical Company) was dissolved in 2 ml saline before direct injection into the portal vein using a 27-G needle. Hepatic ICG clearance was measured 2 h after colchicine administration. (6) The bile duct was ligated for 60 min before measuring hepatic ICG clearance.

**Table 1** Hepatic haemodynamic and oxygenation parameters with reduction of liver blood flow

	Hepatic artery occlusion		Portal vein partial occlusion	
	Baseline	After occlusion	Baseline	After occlusion
Hepatic artery blood flow (ml min <sup>-1</sup> )	11(7)	0	15(5)	20(7)†
Portal vein blood flow (ml min <sup>-1</sup> )	84(11)	88(9)	90(9)	42(6)†
Total hepatic blood flow (ml min <sup>-1</sup> )	95(10)	88(9)*	105(8)	62(8)†
Hepatic microcirculation (flux)	239(13)	216(16)*	229(22)	176(19)†
HbO <sub>2</sub> (μmol l <sup>-1</sup> )	0	-12.7(6.6)†	0	-26.9(9.2)†
Hb (μmol l <sup>-1</sup> )	0	9.7(8.5)†	0	20.8(11.2)†

Values are mean(s.d.) of six animals in each group. Values are 1-min means before occlusion (baseline) and at the end of the occlusion period. HbO<sub>2</sub>, oxyhaemoglobin; Hb, deoxyhaemoglobin. \**P* < 0.01, †*P* < 0.001 versus baseline (Student's *t* test)

**Table 2** Hepatic indocyanine green uptake and excretion rates

Group	α (min <sup>-1</sup> )	β (min <sup>-1</sup> )
Control	1.85(0.51)	0.100(0.060)
Hepatic artery occlusion	0.37(0.09)*	0.080(0.030)
Portal vein partial occlusion	0.27(0.14)*	0.120(0.080)
Lobar I/R	0.85(0.59)*	0.020(0.006)†
Colchicine	1.74(0.40)	0.030(0.010)†
Bile duct ligation	1.82(0.77)	0.002(0.001)†

Values are mean(s.d.) of six animals in each group. α, Hepatic indocyanine green (ICG) uptake rate; β, hepatic ICG excretion rate; I/R, ischaemia and reperfusion. \**P* < 0.001, †*P* < 0.05 versus control (Student's *t* test)

### Data collection and statistical analysis

In the groups with reduction of hepatic blood flow, the transonic flowmeter, laser Doppler flowmeter and NIRS measurements were calculated by taking 1-min means at the start (baseline) and at the end of the occlusion interval. In the I/R group these measurements were calculated as 1-min means before ischaemia (baseline), at the end of ischaemia and following reperfusion. Values were expressed as mean(s.d.). For statistical analysis Student's *t* test was used with Bonferroni adjustment for multiple comparisons. *P* < 0.05 was considered significant. The relationships between hepatic ICG uptake rate and hepatic blood flow and microcirculation were tested using the Pearson correlation coefficient.

### Results

Typical examples of ICG clearance in the control group and with alteration of liver blood flow and function are shown in Fig. 1.

#### Reduction of hepatic blood flow

Hepatic artery occlusion significantly reduced the total hepatic blood flow and hepatic microcirculation with no significant effect on portal venous blood flow (Table 1).

**Table 3** Hepatic haemodynamic and oxygenation parameters with lobar ischaemia and reperfusion

	Baseline	Ischaemia	Reperfusion
Hepatic microcirculation (flux)	241(19)	43(9)*	195(11)*
HbO <sub>2</sub> (μmol l <sup>-1</sup> )	0	-274(38)*	-78(13)*
Hb (μmol l <sup>-1</sup> )	0	182(37)*	43(16)*

Values are mean(s.d.) of six animals in each group. Values are 1-min means before ischaemia (baseline) and at the end of the ischaemia and reperfusion period. HbO<sub>2</sub>, oxyhaemoglobin; Hb, deoxyhaemoglobin. \**P* < 0.001 versus baseline (Student's *t* test)

Hepatic tissue oxygenation, measured by NIRS, showed a significant decrease in HbO<sub>2</sub> with a simultaneous increase in Hb (Table 1). Hepatic artery occlusion resulted in a significant decrease in ICG uptake rate (α) from a control value of 1.85(0.51) to 0.37(0.09) min<sup>-1</sup> (*P* = 0.0001) with no significant change in ICG excretion rate (β) (Table 2).

Portal vein partial occlusion resulted in a significant decrease in portal venous blood flow with a simultaneous increase in hepatic artery blood flow (Table 1). Despite this increase in hepatic artery blood flow there was a significant reduction in total hepatic blood flow and hepatic microcirculation (Table 1). This was associated with a significant decrease in hepatic HbO<sub>2</sub> and a simultaneous increase in Hb (Table 1). Portal vein partial occlusion resulted in a significant decrease in ICG uptake rate from the control value of 1.85(0.51) to 0.27(0.14) min<sup>-1</sup> (*P* = 0.0002) with no significant change in ICG excretion rate (Table 2).

In controls and after hepatic artery occlusion and portal vein occlusion, total hepatic blood flow was correlated with ICG uptake rate (*r* = 0.79, *P* = 0.0001).

#### Ischaemia-reperfusion injury

At the end of the ischaemic interval the hepatic microcirculation was significantly reduced and this did not

recover completely following reperfusion (*Table 3*). Hepatic tissue HbO<sub>2</sub> was significantly reduced at the end of the ischaemic period and the Hb level increased simultaneously. Following reperfusion there remained an impairment of tissue oxygenation with a significant decrease in HbO<sub>2</sub> and an increase in Hb (*Table 3*). After I/R there was a significant reduction in ICG uptake rate from the control value of 1.85(0.51) to 0.85(0.59) min<sup>-1</sup> ( $P=0.0002$ ). There was also a reduction in ICG excretion rate from the control value of 0.100(0.060) to 0.020(0.006) min<sup>-1</sup> ( $P=0.02$ ) (*Table 2*).

The correlation between hepatic microcirculation and ICG uptake rate was investigated in control, hepatic artery occlusion, portal vein occlusion and I/R groups. A positive correlation was found between the two parameters ( $r=0.59$ ,  $P=0.005$ ).

### Colchicine treatment

The use of colchicine resulted in a significant decrease in ICG excretion rate from the control value of 0.100(0.060) to 0.030(0.010) min<sup>-1</sup> ( $P=0.02$ ) with no significant change in ICG uptake rate (*Table 2*).

### Bile duct ligation

With bile duct ligation there was a significant decrease in ICG excretion rate from the control value of 0.100(0.060) to 0.002(0.001) min<sup>-1</sup> ( $P=0.01$ ) with no significant change in ICG uptake rate (*Table 2*).

### Discussion

The hepatobiliary transport of ICG is affected by various factors such as hepatic blood flow, binding to plasma proteins, influx across the sinusoidal plasma membrane, intracellular transport and transport across the biliary canalicular membrane, and bile flow<sup>17,18</sup>.

Conventionally ICG handling by the liver is predicted from its plasma clearance curve<sup>2</sup>. There has been controversy regarding the use of the plasma concentration decay curve of ICG as an index of hepatic excretion<sup>2,7</sup>. Direct measurement of hepatic ICG could provide a more accurate index of ICG kinetics, including its uptake and excretion. NIRS was used to measure directly the hepatic ICG concentration. From its concentration-time curve the ICG uptake and excretion rates were calculated under different experimental conditions that are known to affect ICG uptake and excretion, and could be encountered in a clinical context.

Reduction of the total hepatic blood flow, by hepatic artery occlusion or portal vein partial occlusion, was

associated with a similar reduction of hepatic ICG uptake with no change in ICG excretion. These results are in agreement with those of other studies<sup>19-21</sup> demonstrating that the plasma clearance of dyes is determined mainly by hepatic blood flow. There is controversy over the contribution of hepatic arterial and portal venous blood flow to ICG clearance from plasma. Some experimental observations suggested that plasma clearance of dyes was principally influenced by arterial flow to the liver<sup>19</sup>, while others suggested that the change in clearance was secondary to a decreased total hepatic blood flow from either occlusion of the hepatic artery or shunting of portal vein flow<sup>20,21</sup>. The present results accorded with the second view, that the reduction in hepatic ICG uptake reflected a decrease in the total hepatic blood flow, irrespective of whether this was caused by reduction of hepatic arterial or portal vein inflow.

With lobar I/R there was a reduction in the hepatic microcirculation and tissue oxygenation with a decrease in both the hepatic ICG uptake and excretion rates. I/R injury results in progressive microcirculatory obstruction<sup>22</sup> with the subsequent reduction of hepatic tissue oxygenation<sup>23</sup> and ICG uptake<sup>24</sup>. Possible mechanisms for this microcirculatory obstruction include cellular oedema with subsequent capillary plugging<sup>25</sup>, and leucocyte accumulation and adherence in both liver sinusoids and postsinusoidal venules<sup>22</sup>. The reduced ICG excretion with I/R could be explained by the reduced cellular adenosine 5'-triphosphate (ATP) production<sup>26</sup> resulting in impairment of bile excretion<sup>27</sup>. Several mechanisms have been suggested for the cellular injury and dysfunction after I/R injury, including hypoxic depletion of ATP<sup>28</sup> with incomplete recovery of the hepatocyte ATP level after reperfusion<sup>26</sup>. The direct measurement of hepatic ICG concentration by NIRS in I/R injury could differentiate between the reduction in hepatic ICG uptake owing to microcirculatory impairment and the reduction of ICG excretion due to hepatocellular injury.

Colchicine, via its toxic effect on cellular microtubules, inhibited ICG cytoplasmic transport with reduction of its biliary excretion and plasma clearance<sup>16</sup>. In the present study there was a decrease in hepatic ICG excretion after colchicine administration which confirmed the importance of intact cellular microtubules to ICG excretion from the liver and indicated that the ICG excretion rate reflected hepatocellular function.

Ligation of the common bile duct caused a dramatic reduction in ICG excretion rate. The absence of bile flow resulted in ICG accumulation in the liver, slowed ICG efflux across the bile canalicular membrane and led to its retention in the hepatocytes<sup>29</sup>. Unlike sulphobromophthalein, ICG does not regurgitate into the hepatic lymph with biliary obstruction<sup>29</sup>. This study quantified by NIRS the

reduction in ICG excretion resulting from biliary obstruction. Further studies are required to define whether the excretion curve could be used to differentiate between intrahepatic cholestasis and extrahepatic obstruction.

NIRS allowed direct and continuous monitoring of hepatic ICG concentration for evaluation of the hepatic microcirculation and function. This technique could have important application in liver transplantation for evaluation of graft circulation and function.

### Acknowledgements

This work was supported by the Royal Free Hospital Special Trustees who purchased the dual Transonic Medical Flowmeter system. The Wellcome Trust and Hamamatsu Photonics funded the calibration equipment for liver near infrared spectroscopy. The authors thank the Egyptian Government for the sponsorship of Dr A. El-Desoky. Dr R. Morris, Senior Lecturer in Medical Statistics, Academic Department of Public Health and Primary Care at the Royal Free Hospital Medical School advised on statistical methods. Dr J. Hebden, Wellcome Senior Research Fellow, Department of Medical Physics and Bioengineering, University College London, helped perform 'time of flight' measurement for the liver.

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