Hepatic Blood Flow Measurements and Indocyanine Green Kinetics in a Chronic Dog Model

Andrej Skerjanec,[1] Darryl W. O'Brien,[2] and Yun K. Tam[1,3]

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The objective of this study was to compare hepatic blood flow measurements using ultrasonic flow probes and ICG in a conscious dog model and to evaluate whether ICG can be used to estimate relative change in hepatic blood flow. Seven mongrel dogs (3 M, 4 F, BW = 21 ± 1.8 kg, Hct = 0.39 ± 0.05) were used in the study. Catheters were surgically inserted into carotid artery and portal, hepatic and jugular vein. Transit-time ultrasonic flow probes were implanted around the portal vein and hepatic artery. After two weeks of recovery, a single i.v. bolus dose of ICG (0.5 mg/kg) was administered to each dog. The disposition profiles for ICG in the four catheters were measured for 15 minutes and the hepatic blood flow reading from the probes recorded. Jugular vein ICG blood clearance (Cl = 5.9 ± 1.1 ml/min/kg) was low compared to the electronically measured hepatic blood flow rate (Q = 27.8 ± 9.1 ml/min/kg). Extraction ratios (E = 0.15 ± 0.05) estimated using data from the inlet and the outlet of the liver were consistent with the clearance values, suggesting that ICG is not highly extracted by dog livers. Three dogs were used in experiments where liver blood flow was increased by food intake. Consistent with characteristics of low extraction ratio drugs, ICG was insensitive to blood flow changes while there was an overall increase in electronically measured liver blood flow of 30%. Therefore, ICG is a poor indicator of hepatic blood flow and the present dog model permits continuous and reliable measurements of hepatic blood flow and can be a useful tool in studying the effects of hepatic hemodynamics on pharmacokinetics.

KEY WORDS: indocyanine green; hepatic blood flow; transit-time ultrasonic flowmeter; pharmacokinetics.

INTRODUCTION

Rat liver perfusion has proved to be a useful tool in studying kinetic and metabolic processes of various drugs. However, this approach has limited application in studying disposition of drugs which alter liver hemodynamics. To overcome this limitation, a chronic, conscious dog model has recently been developed in our laboratory (1) to study hepatic hemodynamics and its effect on pharmacokinetics and liver transport of drugs with high metabolic liver extraction. In order to continuously measure liver blood flow and drug disposition in the organ, dogs were implanted with four blood sampling catheters placed in the portal, hepatic and right external jugular veins and carotid artery, and two flow probes: one each on the portal vein and hepatic artery. With simultaneous intermittent blood sampling from all four catheters and continuous measurement of hepatic blood flow, the rate of drug removal by the liver can be measured in a conscious animal. Long-term blood flow measurement was achieved using an implanted transit-time ultrasonic probe (2), which provides signals for accurate and direct measurement of volume flow that is independent of vessel dimension or flow velocity profile. Since its introduction into clinical research, transit-time ultrasonic flow device has been successfully used to measure hepatic blood flow in humans and animals (3).

Preliminary studies were focused on the validation of the model to establish reliable and accurate recordings of liver blood flow and to examine long-term effects of surgical instrumentation on the physiology of the dog. Although there are a number of techniques such as ultrasonic, electromagnetic, Doppler ultrasonography that are used for blood flow measurements in animal species and humans, we chose ICG because it is easy to use and is widely accepted tool for hepatic blood flow measurement in the past 30 years (4,5). The use of ICG, a tricarbocyanine dye, an anionic compound, is based on the assumptions that it is highly extracted by the liver and systemic clearance is equivalent to liver blood flow. Although the use of ICG as a hepatic blood flow marker in humans (6) and rats (7) was questioned recently, it is common to see its continuous application both in human and animal research. The objective of the present study was to examine the disposition characteristics of ICG in dogs and compare its clearance values with electronically measured blood flows.

MATERIALS AND METHODS

Chemicals and Instrumentation

Indocyanine Green (Cardio-Green®) was purchased from Becton Dickinson, USA. For blood flow measurements, transit-time ultrasonic probes and flow meter (Model T201, Transonic Systems, Ithaca, NY,) were applied. Blood flow was recorded on an IBM-compatible PC using the P-Option software (Transonic Systems).

Indocyanine Green Studies

Surgical protocol was approved by Animal Ethics Committee at the University of Alberta and the procedure has been published in detail recently (1). Random source, mixed breed dogs were selected for the study. Physiological parameters such as body weight, hematocrit, blood and liver biochemistry were monitored starting from arrival to our facilities and throughout all investigations for all dogs. Seven dogs (3m, 4f, BW = 21 ± 1.8 kg, Hct = 0.39 ± 0.05) have been studied in total. Three dogs underwent the ICG study prior to surgery to examine potential effects of implanted catheters on the kinetics of ICG. Three dogs were used (crossover study) to study the effect of increased hepatic blood flow on ICG kinetics. Standard dog ration (1 can of Dr. Ballard, 630g) was given to induce liver blood flow increase. Plateau effect was reached after 30 minutes and remained stable throughout the experiment.

[1] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8.
[2] Surgical Medical Research Institute, University of Alberta, Edmonton, Alberta, Canada T6G 2N8.
[3] To whom correspondence should be addressed.

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After an overnight fast, the dog was brought to the laboratory at 9 a.m. on the day of experiment. The animal was placed in a sling frame that provided gentle restrain and support. All dogs were accustomed to this procedure during the recovery period and catheter maintenance. In all experiments, 0.5mg/kg of ICG was administered as a bolus injection into the left cephalic vein, and simultaneous blood samples were collected from the four catheters at 0, 2.5, 5, 7.5, 10, 12.5 and 15 minutes post-injection. Fed dogs received the bolus dose 30 minutes after food intake. Fed and fasted experiments were separated by a week.

Blood Flow and ICG Measurements

Flow probes were factory tested and precalibrated. We also performed our bench testing before surgery and after the probes were recovered from the dogs. Calibration range encompassed 0–400 ml/min and 0–2000 ml/min interval for hepatic artery and portal vein, respectively. All tests gave results within 10% of the reading from a timed collection method. For experimental blood flow measurements, we utilized n-averaging smoothing filter to eliminate undesirable noise. Moving average of 10 sec (n = 10) was used to filter blood flow measurements.

An aliquot of the ICG i.v. solution was stabilized with blank dog plasma and used for standard curve preparation. ICG standards and plasma samples were measured spectrophotometrically at 805nm, according to the manufacturer's procedure. ICG in dog plasma was measured immediately after each experiment.

Data Analysis

ICG plasma versus time data were subjected to nonlinear regression analysis using PCNONLIN (8). One compartment open model was adequate to describe all data sets. Only the data collected from the jugular vein were subjected to conventional kinetic analysis; standard parameters such as CL_B, $T_{1/2}$ and V_d were calculated using conventional methods (9). The conversion from CL_p to CL_B was achieved by multiplying the former by 1/(1-Hct). This calculation was based on the assumption that ICG does not distribute into RBC (10). ICG hepatic extraction ratio was calculated on a point-by point basis using the following equation:

$$E_H = \frac{flux_{in} - flux_{out}}{flux_{in}} \quad (1)$$

where fluxes were computed using equations 2 and 3:

$$flux_{in} = \frac{Q_{HA} * C_{CA} + Q_{PV} * C_{PV}}{(1 - Hct)} \quad (2)$$

$$flux_{out} = \frac{(Q_{HA} + Q_{PV}) * C_{HV}}{(1 - Hct)} \quad (3)$$

where Q_{HA} and Q_{PV} are blood flow rate of hepatic artery and portal vein, and C_{CA}, C_{PV}, C_{HV} are carotid artery, portal vein and hepatic vein plasma ICG concentrations, respectively. It should be pointed out that E_H values, calculated using plasma and blood data are the same. ICG lung extraction was estimated on a point-by-point basis as follows:

$$E_L = \frac{C_{JV} - C_{CA}}{C_{JV}} \quad (4)$$

where C_{JV} and C_{CA} are jugular vein and carotid artery ICG plasma concentrations. Individual values of E_H (Eq. 4) and E_L (Eq. 7) were pooled and averaged over the 15 min period. Blood flow based on ICG kinetics was calculated according to Eq. (5):

$$Q_{ICG} = \frac{CL_B}{E_H} \quad (5)$$

For statistical analysis, Student's paired t-test at the significance level of $p = 0.05$ was used for evaluating potential differences. All values are reported as mean ± SD, except for electronically measured blood flows, where the values are reported as mean ± SEM.

RESULTS

Our results show that surgical instrumentation has no effect on ICG disposition kinetics (Table I). Table II summarizes pharmacokinetic parameters of ICG and electronically measured blood flow values from chronically instrumented dogs that underwent ICG treatment. Plasma ICG concentration followed a monoexponential decline during the first 15 min (Fig. 1A) with a 8.9 ± 1.6 min half-life. Estimated volume of distribution closely approximated plasma volume in the dog (V_d = 45.7 ± 5.9 ml/kg). Systemic blood clearance was low compared to the electronically measured hepatic blood flow rate (CL_B = 5.9 ± 1.1 ml/min/kg vs. Q_B = 27.8 ± 9.1 ml/min/kg) and extraction ratios estimated using ICG fluxes from the inlet and the outlet of the liver were consistent with the clearance values, suggesting that ICG is not highly extracted by dog livers (E_H = 0.15 ± 0.05). Extrahe-

Table I. Comparison of pharmacokinetic parameters before and after surgical instrumentation

	Noninstrumented			Instrumented		
DOG	CL_P (ml/min/kg)	$T_{1/2}$ (min)	V_d (ml/kg)	CL_P (ml/min/kg)	$T_{1/2}$ (min)	V_d (ml/kg)
#3	3.5	8.8	43.9	3.5	7.9	39.9
#4	3.4	11.2	55.1	2.8	11.3	46.4
#6	3.9	7.3	40.3	4.3	6.9	42.6
AVG	3.6	9.1	46.4	3.5	8.7	42.9
STD	0.3	1.9	7.7	0.7	2.3	3.7

Table II. Pharmacokinetic parameters for chronically instrumented dogs that underwent ICG treatment. Electronically measured blood flows in hepatic artery (Q_{HA}), portal vein (Q_{PV}), sum of arterial and portal blood flow (Q_B), ICG estimation of hepatic blood flow (Q_{ICG}) and ratio between ICG and electronically measured hepatic blood flows

DOG	CL_B (ml/min/kg)	$T_{1/2}$ (min)	V_d (ml/kg)	E_H	E_L	Q_{HA} (ml/min/kg)	Q_{PV} (ml/min/kg)	Q_B (ml/min/kg)	Q_{ICG} (ml/min/kg)	Q_{ICG}/Q_B
#1	6.6	7.4	46.2	0.14	0.07	18	21	39	46.7	1.19
#2	7.2	8.4	51.2	0.14	0.03	5.1	35	40	51.9	1.30
#3	5.5	8.8	43.9	0.25	0.04	2.8	15	18	22.4	1.26
#4	6.6	11.2	55.1	0.11	0.02	4.8	20	25	58.6	2.34
#5	3.7	11.1	38.3	0.14	0.05	1.8	16	18	26.7	1.50
#6	6.1	7.3	40.3	0.20	0.00	4.5	20	25	30.9	1.25
#7	5.7	8.4	44.9	0.09	0.18	8.5	22	30	62.2	2.07
AVG	5.9	8.9	45.7	0.15	0.06	6.6	22	28	42.8	1.56
STD	1.1	1.6	5.9	0.05	0.06	2.1*	2.5*	9.1*	16.0	0.46

* SEM.

patic uptake of ICG, as demonstrated by extraction across the lungs, was substantially lower than that of the liver (E_L = 0.06 ± 0.06) and the values varied among dogs. Electronically measured blood flow values in individual blood vessels during experiments were 6.6 ± 2.1 ml/min/kg in hepatic artery and 21.2 ± 2.5 ml/min/kg in portal vein (Fig. 1B). Blood flow, estimated from ICG systemic blood clearance and liver extraction ratio was 42.8 ± 16 ml/min/kg; this value was approximately 56% higher than that measured electronically.

Results from fasted and fed studies are summarized in Table III. Food intake did not influence the pharmacokinetics of ICG and all measured kinetic and ICG based blood flow parameters remained unchanged between the two experiments. However, food intake resulted in an average increase in electronically measured liver blood flow by approximately 30%.

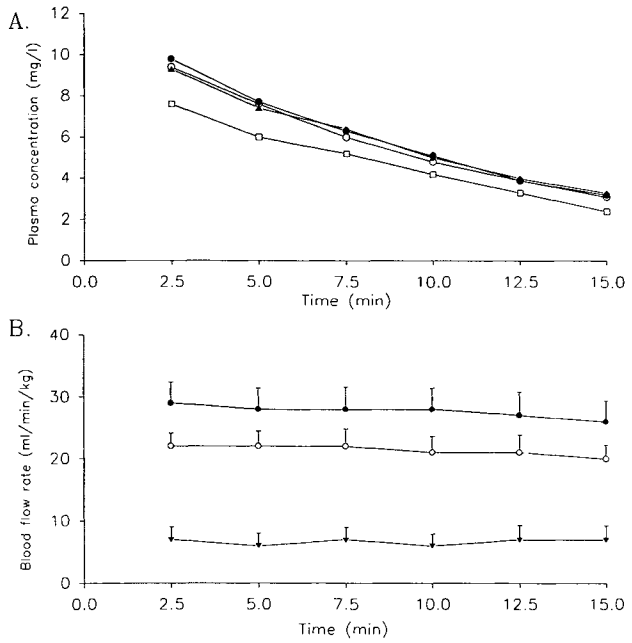

Fig. 1. (A) ICG plasma concentration vs. time profile in a representative dog: jugular vein (●), carotid artery (○), portal vein (▲), hepatic vein (□). (B) Electronically measured blood flow rates during the ICG experiments in dogs; total liver flow (●), portal vein flow (○), hepatic artery flow (▼). Each data point represents the mean (±SEM) of seven dogs.

DISCUSSION

We have developed a conscious dog preparation in which the rate of drug removal can be measured directly across the liver chronically. This model has proven to be a useful method to study transhepatic insulin balance in normal and pancreatic islet cell-transplanted dogs (11) and the interactions between food and drugs that undergo extensive first pass metabolism (12). In the present study we examined the disposition characteristics of ICG, which is used as a non invasive marker of liver blood flow and as a test of hepatic function in humans and animals. We monitored ICG plasma time course in systemic circulation and extraction across the liver without subjecting the dog to an anesthetic agent, thus maintaining an experimentally "clean" system. Indeed, several studies demonstrated that sodium pentobarbital (13) and halothane (14) interfered with liver cell ability to absorb and excrete the dye in experimental dogs receiving these anesthetic agents.

We showed that complex surgical instrumentation did not compromise liver function in our experimental dogs as indicated by the unaltered ICG disposition parameters after surgery (Table 1). Moreover, results from liver chemistry tests, before and after instrumentation, remained stable and were within normal limits throughout the course of investigation.

Studies in instrumented dogs showed that ICG has a low systemic blood clearance and this observation is consistent with the low liver extraction measured across the liver. Therefore, the assumption that ICG systemic clearance reflects hepatic blood flow in dogs is invalid. Moreover, the dog has one of the lowest dye extraction among animal species studied so far; extraction ratios in rats (7) and cats (15) were reported to be 0.30 and 0.26, respectively. Extractions close to 100% have never been observed. In human, an extraction ratio of 0.6–0.8 was measured (4,16), but its application in clinical research has been seriously questioned by several investigators (6,17). Our dog data are consistent with the data reported by others (18); however, the fact that ICG has a low extraction ratio did not step researchers from using it to monitor liver hemodynamics in dogs.

Table III. Food effect on liver blood flow (Q_B) and ICG disposition characteristics

	Fasted						Fed					
DOG	CL_B (ml/min/kg)	$T_{1/2}$ (min)	V_d (ml/kg)	E_H	Q_B* (ml/min/kg)	Q_{ICG} (ml/min/kg)	CL_B (ml/min/kg)	$T_{1/2}$ (min)	V_d (ml/kg)	E_H	Q_B (ml/min/kg)	Q_{ICG} (ml/min/kg)
#4	6.5	11.2	55.1	0.11	25.0	58.4	5.1	11.6	48.1	0.09	33.7	54.8
#6	6.0	7.3	40.3	0.20	24.7	30.5	5.6	8.6	42.7	0.11	34.9	51.2
#7	5.7	8.4	44.9	0.09	30.0	61.9	4.4	10.0	41.1	0.09	35.1	51.1
AVG	6.1	9.0	46.8	0.13	26.6	50.2	5.0	10.1	43.9	0.10	34.6	52.4
STD	0.4	2.0	7.6	0.06	3.0	17.2	0.6	1.5	3.7	0.01	0.8	2.1

* Significantly different from the fed state ($p < 0.05$).

The accuracy of the electronically measured hepatic blood flow is of prime importance in this model. Every set of flow probes was factory calibrated and tested for required accuracy. Our own calibrations were consistent with manufacturer's data. Moreover, the accuracy of *in vivo* flow measurements has been verified in sheep using microsphere technique (19). Direct *in-situ* calibration of flow probes has been performed in lactating cattle (20); the transit time ultrasonic flow meter provided accurate measurements of mammary arterial blood flow. Therefore, we have sufficient evidence to support that this method provides an accurate and reliable system for chronic measurements of hepatic blood flow in dogs.

ICG based blood flow consistently overestimated electronically measured hepatic blood flow. According to eq. 5, systemic blood clearance and extraction ratio of ICG are required to calculate the liver blood flow. Since it is assumed that systemic blood clearance is equal to hepatic clearance, it is clear that the presence of a significant extra-hepatic removal route would lead to an overestimation of hepatic blood flow. Furthermore, nonlinear ICG hepatic uptake would give rise to lower extraction ratio than under linear conditions, resulting in overestimated blood flow. Last, but not least, variability of ICG extraction ratio within measured time interval could also contribute to erroneous estimates. Extraction ratio is likely the most sensitive parameter due to its low value in dogs. Our data show that ICG lung extraction ratio values amount to 40% of that of the liver's. Considering that lungs receive total cardiac output, lung elimination of ICG is an important disposition process. Interestingly, the kidney was found to be a major organ responsible for extrahepatic uptake of ICG in cats (15), another potential source of overestimation of ICG hepatic blood clearance. An overestimation of ICG hepatic clearance could also be a result of incomplete sample collection. If one allows for longer sampling, the plasma concentration decline can be described by a sum of two exponentials. We extended the sampling time up to 60 minutes in two dogs. Surprisingly, biexponential decline, with the second phase starting at around 30 minutes was apparent in one dog only; the last phase accounted for 20% of the total AUC. We certainly cannot extrapolate these observations to the remaining five dogs, but in view of kinetic complexities of ICG, we believe that this degree of underestimation is insignificant in the present context and longer sampling times are unwarranted. We minimized the variability due to blood sampling procedure by rapid and simultaneous withdrawal of samples. Results from individual dogs showed that liver extraction varied up to 40%, but there was no obvious trend pointing toward nonlinear uptake and consequently to reduced extraction. It appears that ICG kinetics in dogs precludes accurate determination of hepatic extraction which could lead to erroneous estimation of blood flows. Our results showed that ICG clearance cannot be used to measure hepatic blood flow accurately. Our second objective was to evaluate whether ICG can be used to estimate relative change in hepatic blood flow. Consistent with a low extraction drug, the systemic clearance remained unchanged when hepatic blood flow was induced with food, whereas electronically measured liver blood flow registered a 30% increase.

In conclusion, ICG is a poor indicator of hepatic blood flow in dogs and its use for such purpose is not recommended. The present dog model permits continuous and reliable measurements of hepatic blood flow and proved to be an indispensable tool for studying the effects of hepatic hemodynamics on pharmacokinetics.

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