Thermal Injury Decreases Hepatic Blood Flow and the Intrinsic Clearance of Indocyanine Green in the Rat

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The influence of severe thermal injury (full-thickness burns involving 50% of the body surface area) on hepatic blood flow in the rat was assessed using the tricarbocyanine dye indocyanine green (ICG). In a randomized crossover fashion, rats received sequential infusions of ICG through both the femoral vein and the portal vein, allowing the estimation of total hepatic plasma clearance and transhepatic extraction of the dye. These two parameters, along with the hematocrit, were used to calculate intrinsic hepatic clearance of ICG and hepatic blood flow. Animals were examined at 0 (control), 0.5, 12, or 24 hr following infliction of scald burns. Hepatic blood flow was decreased significantly by 0.5 hr postburn and remained approximately 20% below normal throughout the remainder of the study. The intrinsic efficiency of the liver in removing ICG from the systemic circulation was also decreased by thermal injury. The potential mechanisms involved in these two physiologic perturbations are discussed.

KEY WORDS: thermal injury; indocyanine green; hepatic blood flow; intrinsic hepatic clearance.

INTRODUCTION

Several recent investigations have indicated that thermal burns of the skin can perturb the hepatic elimination of xenobiotics. For example, the total body clearance of both diazepam (1) and lorazepam (2), two compounds eliminated predominantly by biotransformation in the liver, was found to be altered in thermally injured patients as compared to nonburned controls. Similarly, animal models of thermal injury have shown that cutaneous burns alter the rate of metabolism of several substrates (3–5).

The mechanisms underlying the apparent hepatic dysfunction in the immediate postburn period remain to be elucidated. However, one potential contributor to the observed perturbation is altered hepatic blood flow. The rate of perfusion of the liver is an important determinant of xenobiotic metabolism in two respects. First, for compounds that are extracted efficiently by the liver, hepatic clearance is limited by hepatic blood flow (6). Thus, any change in flow rate to the clearing organ will produce a parallel change in the systemic clearance of the compound. Second, the metabolism of compounds that are extracted inefficiently by the liver might be affected indirectly by altered hepatic blood flow. The hepatic clearance of this class of agents is determined primarily by the intrinsic efficiency of the clearing organ. Decreased delivery of oxygen and cofactors to the liver has been shown to reduce intrinsic hepatic clearance for xenobiotics with a low transhepatic extraction ratio (7).

Burn-induced perturbations in hemodynamics, including alterations in circulatory volume (8), cardiac output (8,9), and redistribution of blood flow between organs (10–12), are well documented. Although the influence of thermal injury on hepatic blood flow has not been studied extensively, several reports of decreased hepatic blood flow during the immediate postburn period in various animal models of thermal injury have been published (8,12–14). Hepatic blood flow at later time points has not been investigated in these animal models. However, two studies have suggested that hepatic flow in patients is increased at later times (1 to 2 weeks) following injury (15,16). These two sets of observations are not necessarily contradictory. It is possible that burn-induced changes in hepatic blood flow evidence a biphasic temporal pattern, with a decreased flow very early following injury and enhanced flow during recovery from trauma. The present study was undertaken to determine whether hepatic blood flow is altered in a rat model of thermal injury and to assess the time course of potential changes in hepatic flow during the early postburn period. A secondary goal of the study was to utilize the disposition kinetics of indocyanine green (ICG), a classic substrate for hepatic blood flow estimations (17), as a measure of hepatic excretory function in rats sustaining severe thermal injury.

METHODS

Animals. Male Sprague–Dawley rats (300 to 350 g), outbred from Hilltop Laboratory Animals (Scottdale, PA) stock, were housed individually in wire-mesh cages and maintained on a 12-hr light/dark cycle. Free access to food and water was allowed at all times. All procedures were in accordance with the Guidelines for the Care and Use of Laboratory Animals, as adopted by the National Institutes of Health, and approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

Thermal Injury Model. A standard rat scald burn model (18) was used to produce cutaneous burns covering 50% of the total body surface area. Rats were anesthetized with ether and the torso was shaved. Each animal was secured in a Plexiglas form exposing 17% of the body surface area through an adjustable aperture. The exposed skin was submerged in hot water (90°C) for 20 sec in order to produce full-thickness cutaneous burns without damage to underlying structures (19,20). The animal was repositioned in the form, and scalding was repeated twice to achieve coverage of approximately 50% of the body surface. Fluid resuscitation (Ringer's lactate, 1 ml/kg/% body surface area burned) was administered immediately and at 8 hr following injury. Control animals were anesthetized with ether and shaved; the duration of anesthesia in these animals was matched to that in the scalded rats.

Estimation of Hepatic Blood Flow. Hepatic blood flow was examined in control rats and at 30 min, 12 hr, or 24 hr postburn according to a previously published procedure (21). Briefly, the rat was anesthetized with urethane, body tem-

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perature was maintained at 37°C with a rectal temperature probe connected to a temperature controller and heating pad, and cannulas were inserted in the right jugular (silicone rubber) and left femoral (polyethylene, PE-10) veins. The portal vein was exposed through a midline abdominal incision, and a 30-G needle attached to PE-10 tubing was inserted into the vein. The tubing was anchored to the abdominal wall with tape, and the incision was covered with saline-moistened gauze to minimize evaporation of abdominal fluid. Anesthesia was maintained throughout the study period.

In a randomized crossover fashion, ICG was infused (0.185 mg/min/kg) via a syringe pump through either the femoral or the portal vein. Blood samples (0.1 ml, added to tubes containing 50 U heparin) were obtained through the jugular vein cannula immediately prior to administration of the dye and at 3, 6, 8, 10, and 12 min, at which time the infusion was terminated. Following a 30-min washout period, an additional blood sample was obtained and the infusion (with sampling) was repeated through the alternate vessel. The average hematocrit was determined from the first and last samples obtained. All samples, as well as the infusate solution, were protected from light during the course of the experiment and were analyzed for ICG content within 12 hr of collection. Following termination of the second infusion, the portal vein site was inspected to assure that leakage of infusate or blood had not occurred. The animal was sacrificed, and the liver was removed from the carcass, blotted dry, and weighed.

Analysis of ICG. Concentrations of ICG were determined in plasma and the infusate solution using a high-performance liquid chromatographic technique described previously (21).

Estimation of Hepatic Blood Flow. Steady-state conditions for ICG (i.e., the absence of a statistical trend in the concentration–time data) were achieved within 6 min for control or 8 min for burned animals; steady-state ICG concentrations were estimated by averaging the final three samples obtained during each infusion. Utilizing standard pharmacokinetic techniques (22), blood flow was estimated from the steady-state plasma concentrations of ICG produced during both femoral ($C_{SS,FV}$) and portal ($C_{SS,PV}$) infusions. ICG appears to be removed from the systemic circulation of the rat entirely by the liver based upon biliary excretion rate versus time data (23). Thus, hepatic plasma clearance (Cl_h) is approximated by total body clearance and could be estimated during infusion of the dye into the femoral vein:

$$Cl_h = \frac{k0}{C_{SS,FV}} \tag{1}$$

where $k0$ is the rate of infusion of ICG. Since ICG does not distribute into erythrocytes (24), hepatic blood clearance (Cl_H) was calculated from Cl_h and the hematocrit (HCT) as

$$Cl_H = \frac{Cl_h}{1 - \text{HCT}} \tag{2}$$

Extraction of ICG across the liver (E) was estimated in a model-independent fashion from the steady-state plasma concentrations of the dye produced by the two routes of infusion and was used in the subsequent calculation of hepatic blood flow (Q_H):

$$E = \frac{C_{SS,FV} - C_{SS,PV}}{C_{SS,FV}} \tag{3}$$

$$Q_H = \frac{Cl_H}{E} \tag{4}$$

To assess the ability of the liver to remove ICG from circulating blood, the intrinsic clearance (Cl_I) of ICG was calculated based upon both the well-stirred and the parallel tube models of hepatic elimination. For the well-stirred model, intrinsic clearance (Cl_I^{WS}) was calculated from steady-state concentrations achieved during infusion of the dye directly into the clearing organ (6):

$$Cl_I^{WS} = \frac{k0}{C_{SS,PV}} \tag{5}$$

For the parallel tube model, intrinsic clearance (Cl_I^{PT}) was estimated from Cl_H and Q_H (6):

$$Cl_I^{PT} = -Q_H * \ln\left[1 - \frac{Cl_H}{Q_H}\right] \tag{6}$$

Statistical Analysis. The influence of time postburn on the pharmacokinetics of ICG was determined using one-way analysis of variance. Comparisons of injured groups to the control group were performed by constructing the appropriate linear contrasts, with the level of statistical significance adjusted for the number of contrasts performed (25). Orthogonal linear regression was used to assess correlations between experimentally determined parameters.

RESULTS

Representative ICG concentration–time profiles for individual thermally injured and control animals are presented in Fig. 1. In all animals, steady-state was acheived within 8 min following initiation of the infusion. Control animals evidenced lower ICG concentrations during infusion through both the femoral vein (i.e., higher ICG hepatic clearance) and the portal vein (i.e., higher intrinsic hepatic clearance of

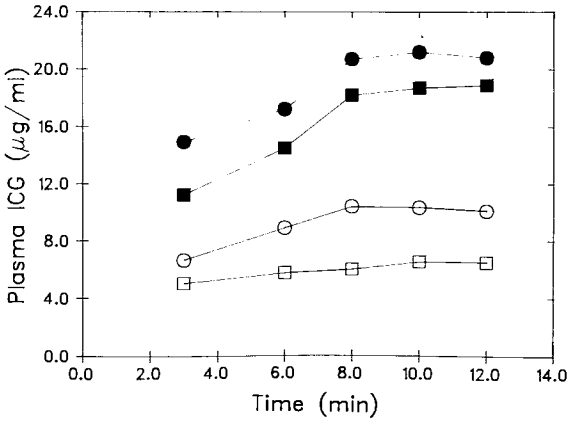

Fig. 1. Representative plasma concentration–time profiles during infusion of ICG into burned (filled symbols) or control (open symbols) rats via the femoral (circles) or portal (squares) veins.

ICG) than their injured counterparts. In addition, residual concentrations of the dye following the washout period between the two infusions were lower in controls than in burned animals. ICG concentrations during the second infusion were corrected, therefore, for residual ICG in all animals with detectable concentrations (>0.5 μg/ml) at the end of the washout interval. This correction was achieved by predicting residual ICG concentrations from the first infusion at each time point during the second infusion period, based upon the postwashout (from the first infusion) ICG concentration and the calculated elimination rate constant for a particular animal. These concentration estimates were subtracted from the total ICG concentration measured at each time point during the second infusion. This approach assumes that the disposition of ICG is linear within the observed concentration range. Preliminary dose-ranging experiments performed in this laboratory to determine the optimal ICG infusion rate for blood flow determinations in rats indicated that the disposition of the dye was linear at concentrations below 30 μg/ml (unpublished data). The ICG concentration at the end of the washout period was 5.95 ± 5.35% of the steady-state concentration achieved by the second infusion and did not vary between experimental groups. Thus, potential errors in estimating residual ICG would not influence pharmacokinetic calculations significantly.

The effect of thermal injury on hepatic blood flow and the disposition of ICG is summarized in Table I. The hepatic clearance of the dye was lower in each of the burned groups as compared to control. Moreover, the time postburn influenced the degree of inhibition of ICG clearance by thermal injury, with maximal inhibition observed 24 hr postburn. Similarly, the intrinsic clearance of ICG was decreased significantly following thermal injury, whether Cl_I was calculated based on the well-stirred or the parallel tube model; Cl_I also appeared to decrease progressively with time postburn. Hepatic blood flow was decreased significantly at 30 min postburn ($P < 0.05$) and remained lower than the control value for the duration of the experiment. However, differences were no longer significant at the 12-hr time point, because of the relatively large variability in blood flow in that group of injured animals.

The relationships between the hepatic clearance of ICG and either hepatic blood flow or the intrinsic clearance of the dye are presented in Figs. 2 and 3, respectively. As would be expected for a compound with a modest (<0.5) transhepatic extraction ratio, hepatic clearance was influenced significantly ($P < 0.001$) by both hepatic blood flow and intrinsic hepatic clearance. A stronger relationship was observed between hepatic and intrinsic clearances ($r^2 > 0.9$) than between hepatic clearance and hepatic blood flow ($r^2 = 0.637$). In addition, estimates of intrinsic clearance based upon the parallel tube model were more consistent with the observed hepatic clearance of ICG than estimates assuming a well-stirred hepatic compartment (Fig. 3).

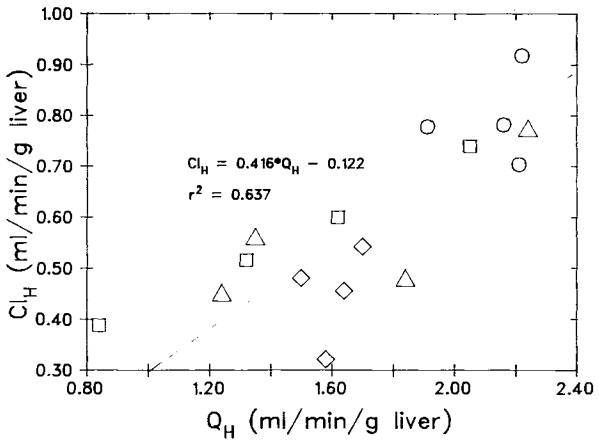

Fig. 2. Relationship between the hepatic blood clearance (Cl_H) of ICG and estimated hepatic blood flow (Q_H). The solid line indicates the results of orthogonal least-squares regression of the data. Symbols represent different groups of animals. Circles, control; squares, 0.5 hr; triangles, 12 hr; diamonds, 24 hr postburn. The correlation between the two parameters ($r^2 = 0.637$) was statistically significant ($P < 0.001$).

DISCUSSION

ICG cannot be classified as a high extraction substrate in the rat, since the transhepatic extraction ratio is approximately 0.4 (21). This is consistent with an extraction of 0.34 to 0.44 in control rats in the present investigation. The systemic clearance of the dye is therefore a function of both the intrinsic ability of the liver to extract ICG from the systemic circulation (i.e., the intrinsic hepatic clearance) and the rate of presentation of ICG to the liver (i.e., hepatic blood flow). The present investigation was designed to determine the relative importance of potential burn-induced changes in these two parameters. A previous experiment in this laboratory (26) indicated that the systemic clearance of ICG following bolus-dose administration of the dye was decreased significantly 24 hr following cutaneous burns. Since this initial ex-

Table I. Effect of Thermal Injury on the Disposition of ICG and Hepatic Blood Flow in Rats[a]

Time after injury (hr)	Liver wt (g)	(ml/min/g liver)[b]			
		Cl_H	Cl_I^{WS}	Cl_I^{PT}	Q_H
0	45.0 ± 3.2	0.796 ± 0.089	1.29 ± 0.22	1.00 ± 0.14	2.12 ± 0.15
0.5	40.4 ± 3.0*	0.561 ± 0.147*	0.921 ± 0.185*	0.710 ± 0.167*	1.46 ± 0.51*
12	28.9 ± 1.3*	0.562 ± 0.146*	0.866 ± 0.204*	0.690 ± 0.186*	1.67 ± 0.46
24	31.3 ± 1.3*	0.450 ± 0.093*	0.614 ± 0.158*	0.531 ± 0.125*	1.60 ± 0.08*

[a] Data presented as mean ± SD; $n = 4$ per group.
[b] Whole blood clearances and flow rates.
* Significantly different from time 0, $P < 0.05$.

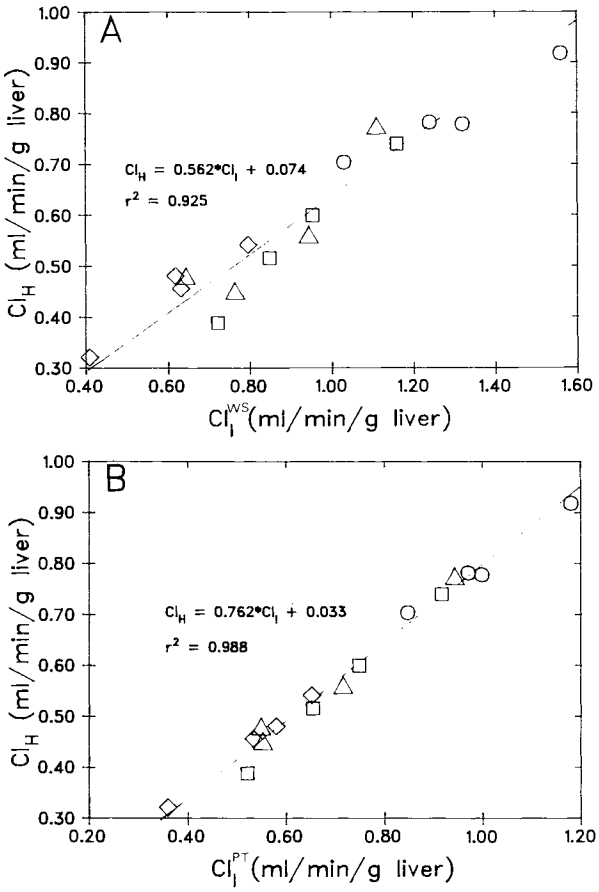

Fig. 3. Empirical relationship between the observed hepatic blood clearance (Cl_H) and intrinsic clearance (Cl_i) of ICG calculated according to the well-stirred (A) and parallel tube (B) models. The solid line indicates the results of orthogonal least-squares regression of the data. Symbols represent different groups of animals and are defined in the legend to Fig. 2. The correlations between hepatic and intrinsic clearances ($r^2 = 0.925$ for the well-stirred model; $r^2 = 0.988$ for the parallel tube model) were statistically significant ($P < 0.001$).

periment suggested that clearance of the marker was not different from control at 48 hr postburn, the present study was confined to the first 24 hr following injury.

As anticipated, hepatic blood flow was decreased secondary to thermal injury. Both circulatory volume and cardiac output are reduced rapidly following cutaneous burns (8,9), and blood flow is redistributed toward the site of injury at the expense of several vascular beds (including splanchnic) (10–12). These factors all suggest that total hepatic blood flow would be decreased at some time following thermal injury. A significant reduction in the delivery of oxygen to the liver immediately following injury, possibly due in part to severely restricted hepatic blood flow, is consistent with histological evidence of nonspecific anoxic damage of hepatic tissue in thermally injured patients (27).

The present data do not allow assessment of a precise time course of changes in hepatic blood flow. The burn-induced decrease in cardiac output is essentially instantaneous (28). If the changes in hepatic blood flow subsequent to thermal injury are due primarily to alterations in cardiac output, then the 30-min time point in the present study may represent a time at which hepatic flow is increasing from an earlier postburn nadir. Due to the surgical procedures involved, 30 min is the earliest time at which hepatic blood flow can be measured with the current technique.

Both hepatic blood flow and intrinsic hepatic clearance of ICG were decreased in response to thermal injury. However, the time course associated with alterations in each parameter differed. Hepatic blood flow decreased rapidly following thermal injury, with maximal change observed at the earliest time point examined. Some degree of recovery of flow appeared to occur over the remainder of the 24-hr postburn period. In contrast, intrinsic clearance appeared to decrease progressively with time postburn, regardless of the model of hepatic elimination employed. The parallel tube model appeared to describe the hepatic clearance of ICG more consistently than the well-stirred model, but further investigation is required to assess the validity of models of hepatic ICG elimination.

The mechanism(s) underlying the progressive decrease in the intrinsic hepatic clearance of ICG by the liver is not immediately apparent. The size of the organ was reduced 30 min following thermal injury, which is consistent with previous experiments (19), and continued to decrease throughout the remainder of the experiment. This change in liver weight roughly paralleled the change in intrinsic clearance of ICG. However, decreased organ size cannot explain the present data, since intrinsic clearance normalized for liver weight was decreased postburn.

ICG is transported into hepatocytes, and subsequently excreted into bile, via active, saturable processes (29). Such active transport systems could be inhibited by a number of physiologic perturbations, including a reduced energy supply, an increased concentration of endogenous substances competing for transport, destruction of the carrier system, or destruction of entire hepatocytes. Any one (or several) of these factors may be involved in the inhibition of hepatic clearance of ICG following thermal injury. For example, derangements in energy production and utilization by the liver, characterized by increased oxygen consumption, have been described following severe thermal trauma (30). Such alterations in hepatic energy requirements may affect energy-dependent transport processes within the liver. In addition, burn-induced increases in lipolysis result in increased circulating concentrations of free fatty acids (31), with eventual fatty infiltration of hepatocytes (19,32). These endogenous carboxylic acids may compete with ICG for hepatic anionic transport sites. Finally, histological analysis of hepatic tissue from burned animals and humans indicates that hepatocyte destruction occurs secondary to thermal injury (27,33), the mechanism of which is currently unknown. Loss of hepatocytes could result in a decrease in the number of available hepatic transport sites for ICG, thereby decreasing the elimination of the substrate.

The effect of thermal injury on the disposition of ICG may be associated with the mechanisms involved in postburn jaundice. Elevated serum concentrations of both unconjugated and conjugated bilirubin have been reported in burned patients (27). Increased circulating concentrations of unconjugated bilirubin occur immediately postburn and are likely due to hemolytic release of the pigment. A burn-induced decrease in glucuronidation of bilirubin may also

contribute to unconjugated hyperbilirubinemia, although this mechanism has not been explored. Increased systemic concentrations of conjugated bilirubin, however, occur later in the postburn course (27) and suggest impaired excretion of conjugated pigment by the liver. Disruption of the transport process for removal of conjugated bilirubin from the site of conjugation in the hepatocyte into bile would be expected to result in increased appearance of the conjugate in the systemic circulation. Since bilirubin, conjugated bilirubin, and ICG share common transport mechanisms (34), a decrease in the intrinsic hepatic clearance of ICG is consistent with impaired excretion of bilirubin and conjugated bilirubin following thermal injury. Furthermore, since bilirubin and/or its conjugate compete for transport sites with ICG, a potential mechanism for impaired ICG elimination is competitive inhibition by the endogenous pigment in its unconjugated or conjugated form.

An additional complication in assessing the effects of thermal injury on ICG disposition is binding of the dye to plasma proteins. ICG is bound significantly in plasma and appears to be associated with α_1-acid glycoprotein as well as albumin (35); the precise contribution of each protein to ICG binding in rat plasma is unknown. Changes in the plasma protein binding of ICG following thermal injury are therefore difficult to predict. Hypoalbuminemia is observed following severe burns (36), predominantly as a result of capillary leakage. Conversely, production of α_1-acid glycoprotein increases following thermal injury (37). Considering the moderate hepatic extraction ratio for ICG in the rat, alterations in unbound fraction subsequent to changes in the circulating concentrations of binding proteins would influence the calculated values of transhepatic extraction as well as hepatic and intrinsic clearances. However, the present approach to calculation of hepatic blood flow is independent of the degree of protein binding. Similarly, the correlations between Cl_H and Cl_I should be unaffected by changes in the unbound fraction in plasma. The contribution of burn-induced perturbations in ICG binding to the dispositional changes observed in the present investigation remain to be determined.

Although the hepatic clearance of ICG does not approximate hepatic blood flow in the rat, ICG clearance may be used to predict hepatic blood flow following thermal injury in a given animal, based upon the mathematical relationship described by the present data (Fig. 2). It is likely that similar relationships could be constructed for other disease states. Likewise, ICG may be used to examine the excretory function of the liver. In the present investigation, cutaneous burns involving 50% of the body surface area in rats were found to result in decreases in both hepatic blood flow and the intrinsic ability of the liver to remove ICG from the systemic circulation. The implications of such perturbations on the disposition of pharmacologic agents, as well as the mechanisms underlying these sequelae of thermal injury, require further investigation.

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