Original Article

Indocyanine Green Clearance Test to Evaluate Liver Function in Rat Model of Extrahepatic Biliary Atresia

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Abstract

Background: Indocyanine green clearance test (ICG-K) has been shown as a sensitive marker of liver function in patients with cirrhosis. However, its role in the assessment of liver function in children with biliary atresia is not well established. The present study was undertaken to evaluate the ICG-K in an experimental model of cholangitis and partial biliary obstruction. **Materials and Methods:** Thirty albino rats were divided into 3 groups of 10 each. After exploration under anesthesia, a vial of OK-432 diluted in 0.2 ml of normal saline was injected into the common bile duct (CBD) in rats of Groups B and C. In the control Group A, only saline was injected. Re-exploration was done at 3 weeks in Groups A and B and at 6 weeks in Group C, and freshly prepared ICG was injected into the inferior vena cava. Blood samples were collected at periodic intervals, optical density of the serum was measured, and half-life of ICG and fractional clearance (K) were calculated. Blood and tissue samples were obtained for biochemical tests and histological examination. **Results:** The histological changes in CBD and liver were maximum in Group B; this correlated well with the K-value in this group, which was significantly delayed. In Group C, clearance was delayed than the control group with histological changes ranged from mild to moderate inflammation. The control group had normal histology of liver and CBD, and only four rats showed mild portal inflammation. **Conclusion:** ICG clearance rate is a reliable marker of liver function and can be utilized for evaluation of liver function in postoperative extrahepatic biliary atresia patients.

Keywords: Biliary atresia, indocyanine green clearance test, liver function, OK-432

Introduction

Liver functions in extrahepatic biliary obstruction have been customarily evaluated by the estimation of serum bilirubin and liver enzymes. However, these tests are not always reliable. Indocyanine green (ICG) is a tricarboxylic acid compound, and following intravenous injection, it is excreted solely by the liver alongside bile. Earlier studies have shown that the ability of the hepatocytes to take up ICG becomes rate limiting at very high concentrations, and ICG clearance (ICG-K) under these circumstances represents a sensitive measure of liver cell function.[1] The present study was conducted to evaluate the fractional clearance of ICG in an experimental rat model of cholangitis with partial biliary obstruction.

Materials and Methods

The study was approved by the institutional ethics committee. A total of 30 albino rats each weighing from 80 to 100 g were selected and randomly divided into 3 groups of 10 each. After inducing anesthesia by intraperitoneal ketamine injection (@ 50mg/kg), a midline laparotomy was performed. Common bile duct (CBD) was identified and 0.2 ml of diluted sclerosant (OK-432 in normal saline) was injected into the CBD with a tuberculin syringe in rats of Groups B and C [Figures 1 and 2]. Group A was taken as control and only normal saline was injected in these rats. The abdomen was closed. Each group of animals was housed separately in suitable environmental conditions (12 h daylight at 22°C temperature) with food and water "*ad libitum*." All the rats underwent re-explorations at 3, 3, and 6 weeks in Groups A, B, and C, respectively. Freshly prepared ICG (1 mg in 500 µl of 0.1 M phosphate-buffered saline, pH 7.4 of distilled water) was injected into the infrahepatic vena cava at the dose of

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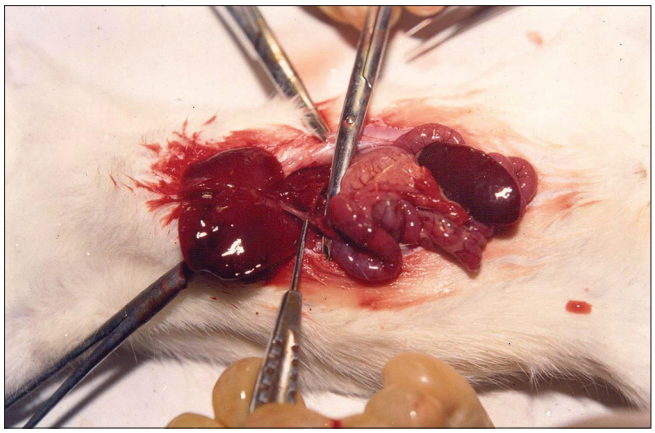

Figure 1: Photograph showing the liver, common bile duct, and duodenal loop; pointer showing the common bile duct

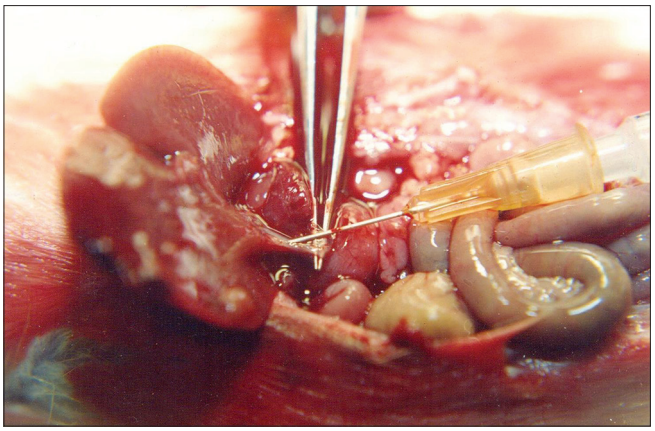

Figure 2: OK-432 being injected into the common bile duct

1 mg/kg body weight [Figure 3]. Blood samples (0.5 ml) were taken from abdominal aorta at 0,1,3,5, and 10 min [Figure 4]. Serum was separated and the optical density was measured in a spectrophotometer (SHIMADZU UV-160A) at wavelengths of 815 nm. A standard curve was obtained by adding freshly prepared ICG to rat sera with a concentration range of 2–12 μg of ICG [Chart 1]. Optical density was measured at wavelengths of 815 nm and the values were plotted on a graph. The amount of ICG in the test samples was calculated from the standard curve; half-life of ICG was calculated, and then, the fractional clearance (k) of ICG was determined after plotting these values on a graph. Blood samples were also taken for the estimation of serum bilirubin and liver enzymes. The specimens of the liver, bile duct, and duodenum were harvested in 10% formalin. Sections of the liver and CBD were embedded in paraffin wax, sectioned (5 μm), and stained with hematoxylin and eosin for evaluation. The parameters evaluated were as follows: the presence of inflammatory cells (cholangitis), portal fibrosis, and ductal proliferation.

Results

Of total 30 rats, four rats died due to unrelated causes and eight rats were taken from each group for study. Optical density of ICG in each serum sample was noted down, and serum concentration of the dye in μg was obtained from the standard curve [Chart 1]. In control group (Group A), clearance of the dye occurred in nearly exponential manner for 3–5 min, following which a deceleration of clearance occurred in all cases. Clearance of ICG was suppressed in rats of Groups B and C. Values were plotted on a graph with zero-time concentration taken as unity. The t/2 was calculated from the graph. The clearance curve of the groups was shown in the same plot for comparison [Chart 2]. The rats in the control group had a mean K-value of 0.239 ± 0.013; in Group B, it was 0.079 ± 0.009, and in Group C, the K-value was of 0.112 ± 0.005. Four of the eight rats in Group A had normal histology of the liver and porta. In the other four rats, there was mild portal inflammation. In Group B, four rats had mild-to-moderate inflammation and the other four had moderate-to-severe inflammatory changes in the portal structures. In Group C, four rats had mild and the other four had moderate-to-severe inflammatory changes. The degree of sinusoidal and portal inflammation was significantly higher in Groups B and C in comparison to Group A, where changes were mild ($P < 0.05$) [Table 1].

The mean serum alkaline phosphatase values were 99.3 ± 7.6 in Group A, 207 ± 133 in Group B, and 188 ± 72 in Group C [Table 2]. The serum bilirubin values were within normal limits in all rats.

K-values obtained in all the three groups were compared using Kruskal–Wallis test. Values obtained immediately after injection of dye/saline were comparable in all three groups and were found to be not significant ($P > 0.05$).

At 1 min after injection, Groups B and C were compared with Group A, and changes were significant in both the groups ($P < 0.05$).

At 3 min after injection, changes in Groups A and B and Groups A and C were highly significant ($P > 0.001$). However, changes between Groups B and C were less significant ($P < 0.05$).

At 5 min, changes between Groups A and B and Groups A and C were highly significant, whereas changes between Groups B and C were less significant ($P < 0.05$).

At 10 min, changes between Groups A and B and Groups A and C were significant ($P < 0.01$) and changes between Groups B and C were less significant ($P < 0.05$).

Discussion

An experimental rat model of partial biliary obstruction as observed in the early stages of extrahepatic biliary atresia (EHBA) was created by injecting OK-432 directly into the bile ducts. OK-432 is a lyophilized mixture of Group A *Streptococcus pyogenes* of human origin. When injected locally into tissues, it evokes inflammation and infiltration by macrophages and neutrophils with the release of cytokines, macrophages, natural killer cells, and lymphocytes.[2] Kuroiwa *et al*., in an experimental study, induced cholangitis by injecting

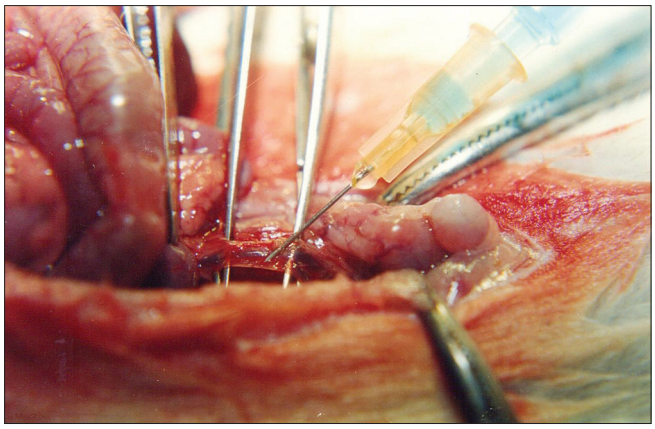

Figure 3: Indocyanine green being injected into the inferior vena cava

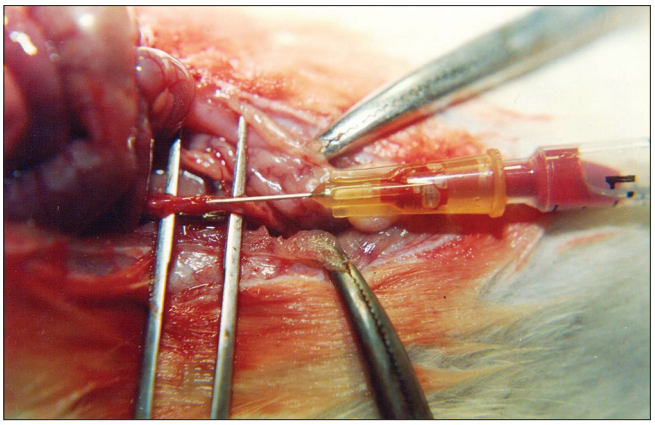

Figure 4: Blood sample collected from the abdominal aorta

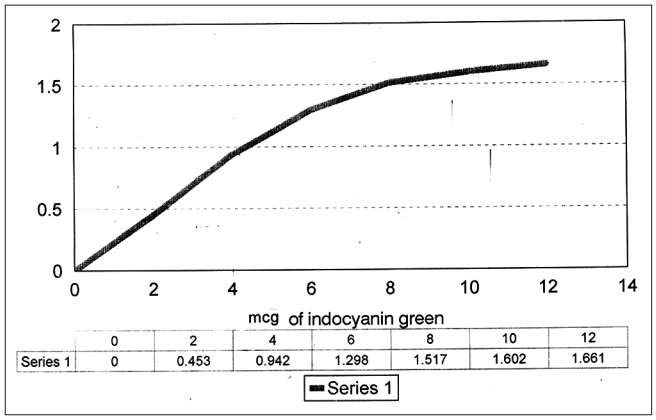

Chart 1: Standard curve showing optical density of ICG in isolated rat sera with concentration range from2 to 12 μg of indocyanine green

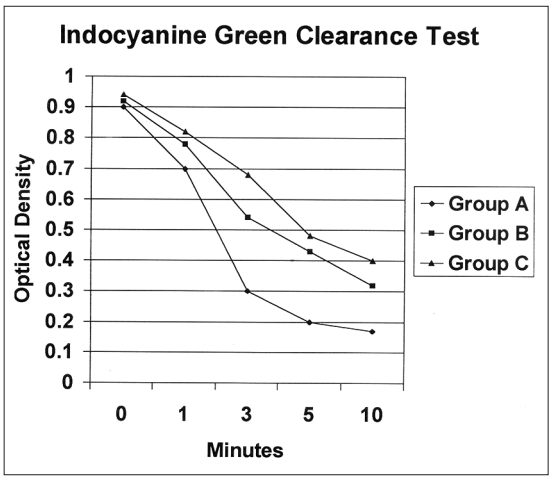

Chart 2: Fractional clearance of indocyanine green in various groups

Table 1: Histological changes and fractional clearance in various groups

Group	Histology (number of rats)				*K*
	Normal	Mild	Moderate	Severe	
A	4	4	-	-	0.239±0.13
B	-	4	1	3	0.079±0.009
C	0	4	3	1	0.112±0.005

Table 2: Liver function tests in the three groups

Parameter	Group A	Group B	Group C
Serum alkaline phosphatase	99.25±7.6	207.7±133.7	188.5±72.48
Serum bilirubin	0.6	0.59	0.53

OK-432 into hepatic duct of male guinea pigs and observed that acute inflammatory changes with edema and cellular infiltration in the proximal periportal area occurred as early as 24 h postinjection.[3] The changes observed in the present study were maximum after 3 weeks of OK-432 injection, and changes decreased gradually, so mild-to-moderate changes were noted after 6 weeks of injection. Serum bilirubin estimation in all the groups was within normal limits, which suggests that this is not a reliable marker of liver injury. Serum alkaline phosphatase levels were raised significantly only in those rats in whom the moderate-to-severe degrees of histological changes were observed. For mild-to-moderate histological changes, this parameter was not a very reliable marker. Four rats in the control group had mild inflammatory changes in the porta, which can be attributed to trauma caused by needle puncture.

The liver performs a variety of functions in human body. No single test can adequately assess all these functions. Various biochemical and imaging studies including the hepatic scintigraphy and elastography are now available. However, their clinical efficacy has not been established yet.[4] Hepatic clearance of ICG occurs by two major processes; by uptake across the sinusoidal plasma membrane with a high extraction ratio and by removal from the hepatocytes by cytoplasmic transport and exclusive biliary excretion (with neither intrahepatic conjugation nor enterohepatic circulation). After intravenous injection, ICG is rapidly and completely bound to plasma proteins, of which albumin is the principal carrier.[5] It is not cleared by the kidney, and the peripheral uptake of the dye is negligible.[6,7] The studies have further shown that

hepatic blood flow can be measured by calculating the rate of clearance in low dye concentration while the ICG clearance by hepatocytes at high dye concentrations represents a sensitive measure of hepatic cell function.[8,9] Hence, hepatic ICG clearance (both cytoplasmic transport and biliary excretion) has a close relationship with intrinsic liver cell function.[10-12] However, as yet this has not been applied in clinical use as a liver function test. Shinohara *et al*, in a study showed that direct measurement of hepatic ICG clearance can be a useful method for comprehensive evaluation of liver function in clinical practice.[9] In another study, Branch *et al*. showed that ICG provides qualitative assessment of two aspects of liver functions related to the uptake and the elimination of drugs.[13] Leevy *et al*. studied ICG clearance and its alterations in patients with liver disease and correlated these findings with liver histology and observed that serial study of ICG clearance reflects improvement or deterioration in liver histopathology and was invaluable in following the course of both acute and chronic liver injury.[14]

Sheng *et al*. investigated the correlation between the ICG clearance test and model for end-stage liver disease (MELD) score in patients with liver cirrhosis.[15] The authors opined that the ICG clearance test and MELD score were good parameters for evaluating liver function. As ICG clearance per unit time (K) and retention of dye at 15 min after injection (R 15) have significant correlations with MELD score, especially the K value, it may be used to evaluate the liver function of patients with significant liver disease. In another study, Gupta *et al*. correlated the ICG clearance test with MELD score in 40 patients with cirrhosis Class A and B (based on CP score) patients and concluded that ICG R15 has a higher sensitivity and specificity than MELD score in assessing the prognosis of patients with cirrhosis of the liver.[16]

Schneider studied the preoperative liver function in a group of Child–Pugh Class A patients using various tests and observed that no single test employed could adequately assess the liver function and the author suggested to complement these tests with 99mTc-galactosyl human serum albumin scintigraphy, which provides volumetric receptor data, as well as kinetic distribution curves.[17]

In a study to evaluate the ICG clearance test in postoperative liver function in 19 patients with EHBA who had undergone hepatic portoenterostomy, Kubota *et al*. found that ICG-K value failed to go up into a normal range or declined afterward in cases, who developed ascending cholangitis in the early postoperative period but remained anicteric and these patients later on developed portal hypertension.[18] The authors concluded that the estimation of ICG-K value was a reliable, comprehensive estimator of postoperative liver function. Hence, it was shown to be an important prognostic indicator as remaining of K-value below a normal range or declining below normal suggests clinical deterioration in the future. In the present study, ICG-K correlated well with different grades of liver injury as confirmed by histology. Variable grades of inflammatory changes were noted in the extrahepatic biliary ducts on histology. This confirms that OK-432 caused portal inflammation which caused changes in the liver as well. The grade of portal inflammation and sinusoidal infiltration with cells correlated well with the ICG-K value in different groups.

Conclusion

ICG clearance rate (ICG-K) is a reliable marker of liver cell function and can be utilized for evaluation of liver function in post-operative patients of extrahepatic biliary atresia.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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