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Kupffer cell and hepatocyte function in rat transplanted liver

G. Svensson[3], M. Fjälling[1], J. Gretarsdottir[2], L. Jacobsson[2], and S. B. Holmberg[3]

Departments of Nuclear Medicine[1], Radiation Physics[2], and Transplantation Surgery[3] Sahlgrenska Hospital, Göteborg, Sweden

Abstract. The liver consists essentially of two compartments, parenchymal cells (PC) and non parenchymal cells (NPC) i. e. Kupffer cells, endothelial cells, fat storing cells and pit cells. PC remain after transplantation but NPC are eventually exchanged with host cells. Dynamic liver scintigraphy with albumin colloid, extracted by NPC, and IODIDA, extracted by PC, were tested to evaluate function as determined by clearance rates in these two cellular compartments. Experimental liver transplantation was performed in 15 syngeneic rats. Following transplantation, we performed dynamic liver scintigraphy with 0.5 ml 5 MBq ^{99m}Tc-Nanocoll and 0.5 ml 20 MBq ^{99m}Tc-IODIDA, 10 s per frame, 30 min for each examination. Percentage clearance rate, per minute was calculated from uptake curves over the liver. Uptake curves were nearly exponential and clearance rates could be estimated from a logarithmic plot of uptake versus time. The clearance rate was 25 ± 4 % per min (mean ± SD) for NPC and 32 ± 15 % per min for PC in controls. After liver transplantation it was 31 ± 7 % per min for NPC and 30 ± 15 % per min for PC. Dynamic liver scintigraphy with ^{99m}Tc-Nanocoll and ^{99m}Tc-IODIDA alloweds a separate assessment of the function of PC and NPC after experimental liver transplantation in rats.

Key words: Liver transplantation – Liver scintigraphy – Kupffer cells

The liver can be divided into two functional compartments. The parenchymal cells (PC) including hepatocytes and bile ducts are responsible for many metabolic functions and the production of bile. The non parenchymal cells (NPC) or sinusoidal cells include Kupffer cells (liver macrophages), endothelial cells, fat storing cells and pit cells (NK cells). The NPC have not only a metabolic role but also an important immunologic and surveillance function, since the liver sinusoids with fenestrated endothelial cells and Kupffer cells act as a "sieve", establishing contact with particulate matter including cells in the blood stream [5, 16]. According to analyses of liver biopsies post-transplantation NPC are replaced by host cells from the recipient bone marrow after liver tranplantation [13, 14]. This procedure of host cell replacement of NPC seems to influence graft rejection or tolerance [11, 13].

^{99m}Tc-Nanocoll, an albumin colloid with a diameter of 50 nm, is used mainly for bone marrow imaging. Nanocoll is phagocytosed by macrophages and 70 % is accumulated in the liver by Kupffer cells. Dynamic liver scintigraphy and calculation of the clearance rate of ^{99m}Tc-Nanocoll has been used to measure RES macrophage phagocytic function [3, 5]. ^{99m}Tc IODIDA (N 2,6 diethyl 3-iodophenyl carbamoyl iminodiacetic acid) is an iminodiacetic acid derivative used to assess hepatocyte function and visualize the hepato-biliary system (HBS). It is excreted by the HBS and allows examination even with high bilirubinemia [2]. IODIDA is protein bound in the blood and transported to the space of Disse in the liver sinusoids by albumin. IODIDA enters the hepatocyte by a carrier-mediated non-sodium-dependant membrane transport mechanism similar to bilirurbin [8]. Dynamic registration and calculation of the clearance rate from the blood of ^{99m}Tc IODIDA is used to measure liver PC uptake function in humans [1].

The aim of this study was to develop a method, in vivo, using dynamic liver scintigraphy for examining the function in these two liver compartments after experimental liver tranplantation.

Materials and methods

Animals. We used 8 control and 15 procedure syngeneic Wistar-FU rats weighing about 250 g in this study. They were fed water and pellets ad libitum and maintained on a normal day and night cycle.

Surgical procedure. Liver transplantation was performed according to Kamada and Sun-li on 15 normal Wistar/FU rats weighing about 250 grams. Atropine (0.1 mg/100 g body weight) was given for premedication and Buprenorphin (Reckitt & Colman, Hull, UK) was given as a postoperative analgesic. Ether was used for induction and

Offprint requests to: Stig B Holmberg, Dept of Surgery, Sahlgrenska Hospital, 413 45 Göteborg, Sweden

Table 1. Clearance rate, % per minute, (mean ± SD) by liver parenchymal cells (IODIDA) and liver non parenchymal cells (Nanocoll)

	Parenchymal cell	Non Parenchymal cell	*n*
Normal rats	32 ± 15	25 ± 4	8
Liver transplantation	30 ± 15	31 ± 7*	15

* $P = 0.02$ compared with normal rats

maintenance of anaesthesia in donor and recipient animals. No other durgs were used. Postoperatively, the rats maintained normal weight and feeding habits. Serum bilirubin and liver transaminases were within normal limits.

The technique of orthotopic liver transplantation in the rat without rearterialization (reanastomosis of the hepatic artery) was first described by Lee [9] and was performed in this study using a modified technique [10, 15]. In this rat model the vascular anastomosis is performed with a running suture technique and without using any cuffs. This model has proved reliable and acceptable for all types of biochemical and immunological studies of orthotopic liver transplantation [6, 7, 17].

Dynamic liver scintigraphy. The animals were anaesthetized with nembutal 60 mg/kg 2–60 days after liver transplantation. The rats were placed on a gamma camera after canulation of the jugular vein. Dynamic liver scintigraphy was performed after the injection of 0.5 ml 5 MBq ^{99m}Tc-Nanocoll i. v. and followed 30 min later by 0.5 ml 20 MBq ^{99m}Tc-IODIDA. Aquisition time was 10 s per frame for 30 min for each examination. Uptake curves were constructed by the Region Of Interest (ROI) over the liver on the gamma camera images. Calculation of clearance rate (k) was done using the formula:

$$\ln [1-U(t)/U_{final}] \text{ versus } t$$

where k is the slope in a least square fit of the plot. Q_0 = injected activity; t = time, U = uptake counts; U_{final} = final liver uptake

The clearance rate was calculated as the percentage uptake per minute. Uptake counts were measured in the ROI over the liver for the ^{99m}Tc-Nanocoll but the ROI was expanded to the include the intestines for the ^{99m}Tc IODIDA uptake count, since there was excretion into the intestines during this examination.

Results

The clearance rate in control rats was 25 ± 4% per min (mean ± SD) for Nanocoll albumin colloid and 32 ± 15% for IODIDA. After liver tranplantion the Nanocoll clearance rate was 31 ± 7% and IODIDA clearance rate was 30 ± 15% per min (Table 1). The clearance rate for Nanocoll was significantly elevated after liver transplantation ($P = 0.02$)

Discussion

There are more than 500 metabolic functions performed by the liver and liver function can be tested in many ways. Physiologic and anatomic studies suggest two main liver compartments, one essentially metabolic consisting of hepatocytes and bile ducts around the bile canaliculi and the other endothelial with sieving and immunologic functions around the liver sinusoids [16]. These two compartments fare differently after liver transplantation since liver biopsies after liver tranplantation have revealed that sinusoidal donor cells have been replaced by host bone marrow cells while parenchymal donor cells remain intact. These studies have used monoclonal antibodies directed at specific donor and recipient HLA-antigens. This exchange of donor cells with recipient cells might influence outcome after liver tranplantation or even initiate a host-versus-graft disease. Steinhoff et al. have shown that a complicated course post liver transplantion in humans is associated with an early exchange of donor sinusoidal cells [11–14].

Clearance studies of injected substanses with established affinity for different liver cells offer a physiologic in vivo model to study different liver functions. In vivo models allow for repeated examination during postoperative follow-up and are therefore suitable for examining processes in the liver that evolve over a period of weeks or months [1, 2, 8]. IODIDA is completely eliminated from the bloodstream by the liver and is one of several iminodiacetic acid substances used to examine the hepatic biliary system. IODIDA is highly protein bound and lipophilic and thus has almost no renal excretion. Hepatocyte uptake is competitive with bilirubin but there are additional uptake mechanisms. IODIDA can be used with a high bilirubinemia [1, 2, 8], in competion with spleen and bone marrow macrophages.

Due to Nanocoll's relative small colloid size, 50 nm, and large particle amount given, liver extraction is not complete on a first passage. Clearance rate is not wholly dependant on liver blood flow [3, 5]. This study used the total clearance rate for IODIDA and Nanocoll. A liver clearance rate (k_{liver}) can be calculated according to the formula

$$k_{liver} = k \times U_{final}/Q_0$$

Q_0 = injected activity; U_{final} = final liver uptake
k_{liver} for IODIDA would be identical since U_{final} is completely (100%) in the liver but k_{liver} for Nanocoll would only be about 70% of the total clearance rate or about 18% per min. The results in normal rats showed essentially the same total clearance rates but the liver clearance rate would be 40% lower for Nanocoll. This supported different elimination mechanisms from the blood for the two test substances.

After liver tranplantation between syngeneic rats, with a short period of cold ischemia, no change was noted in the parenchymal function of the transplanted liver. There was a small but significant elevation of the non parenchymal cell clearance rate of Nanocoll. This might signify an activation of sinusoidal cells after transplantation [11]. In studies of clearance rates in normal and liver macrophage stimulated rats, a similar rise in the clearance rate of Nanocoll is noted [4]. Such macrophage activation is then correlated with cytotoxic ability against liver tumour growth.

Future studies will be aimed at studying more complicated experimental transplantation procedures such as longer periods of cold ischemia and possible reperfusion injuries or transplant rejection.

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