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Abstract

^{99m}Tc -DTPA-galactosyl human serum albumin (Tc-GSA) is a new liver-imaging agent which binds specifically to hepatic binding protein. The purpose of this study was to evaluate the usefulness of Tc-GSA in quantitatively evaluating hepatic ischemia-reperfusion injury in the rat. Regional hepatic ischemia was induced by clamping the left hepatic artery and the left portal vein for 5 to 45 min. A hepatic accumulation index (t90) was obtained on the basis of the dynamic data. A significant difference of this index was observed between all ischemic groups and the control. In conclusion, ^{99m}Tc -GSA appears useful for evaluating the hepatic ischemia-reperfusion injury.

KEYWORDS: ^{99m}Tc -DTPA-galactosyl human serum albumin, liver scintigraphy, hepatic ischemia-reperfusion injury

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Quantitative Evaluation of ^{99m}Tc -GSA in the Rat Liver after Ischemia-Reperfusion Injury

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^{99m}Tc -DTPA-galactosyl human serum albumin (Tc-GSA) is a new liver-imaging agent which binds specifically to hepatic binding protein. The purpose of this study was to evaluate the usefulness of Tc-GSA in quantitatively evaluating hepatic ischemia-reperfusion injury in the rat. Regional hepatic ischemia was induced by clamping the left hepatic artery and the left portal vein for 5 to 45 min. A hepatic accumulation index (t_{90}) was obtained on the basis of the dynamic data. A significant difference of this index was observed between all ischemic groups and the control. In conclusion, ^{99m}Tc -GSA appears useful for evaluating the hepatic ischemia-reperfusion injury.

Key words: ^{99m}Tc -DTPA-galactosyl human serum albumin, liver scintigraphy, hepatic ischemia-reperfusion injury

Liver damage due to ischemia and reperfusion has been reported by several investigators (1, 2). The production of oxygen-derived free radicals by ischemic tissues has been suggested to contribute to tissue damage during organ preservation procedures (3). Recent advances in receptor biochemistry have led to the development of a new radiopharmaceutical marker. Galactosyl human serum albumin-diethylenetriaminepentaacetic acid (GSA-DTPA) is a synthetic ligand to a hepatocyte membrane receptor, hepatic binding protein (HBP) (4). Herein, hepatic ischemia-reperfusion injury was examined quantitatively by means of technetium- 99m GSA-DTPA (Tc-GSA) to determine its clinical usefulness.

Materials and Methods

Radiopharmaceutical preparation. GSA was synthesized by introducing galactose residues to human serum albumin (HSA). DTPA, a strong bifunc-

tional chelating agent, was attached to HSA to provide the stable binding of technetium. The molecular weight of GSA is approximately 76,000, containing 30-40 galactose residues. Previously synthesized GSA was labeled with technetium- 99m . The dose was standardized according to animal weight ($170\mu\text{g/kg}$). The injected radionuclide activity ranged from 3.7 to 7.4 Mbq.

Animal models. Male Wistar rats ($n = 34$) weighing 350-400g (15 weeks old), supplied by Shimizu Laboratory Supplies Co., Ltd., Kyoto, were used. Laparotomy was performed under sterile operative conditions under light ether anesthesia. Animals were divided into six groups, as described in Table 1. Regional hepatic ischemia was induced by clamping the left hepatic artery and the left portal vein. The blood supply to the right lobes was uninterrupted so that portal venous flow was maintained. There was no evidence of vascular congestion of the alimentary tract. The control group of rats were sham-operated, and ischemia was not induced.

Tc-GSA studies. After reperfusion, Tc-GSA was administered as a bolus injection via the inferior vena cava. The rats were anteriorly positioned under a gamma camera (DIAGNOST-C, PHILIPS) equipped with a pin-hole collimator. Digital images (64×64 pixels) were acquired at a rate of 4 frames per min. At 30 min after injection, the dynamic study was halted. Time-activity curves were generated from the computer (gamma PROCESSOR 673, PHILIPS) acquired images using regions of interest selected over the whole liver. Based on preliminary data and on a previous study by Woodle *et al.* (5), we selected t_{90} as an index of Tc-GSA hepatic uptake. This index represents the time required for the liver time-activity curve to reach 90 % of its peak, and t_{90} is assumed to correlate with the total number of functioning hepatocytes. At the same time, the maximal radioactivity level was also measured in each group, and the radioactivity ratio was defined as the maximal radioactivity

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ratio of the ischemic groups to the control group. Immediately before the rats were killed, blood samples were taken for biochemical analysis from the inferior vena cava. Serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were assayed with an RX40 (Japan Electron Optics Laboratory) by means of Karmen method and were reported as Karmen Units (KU). The liver biopsy specimens were obtained for histological examination. Mean values of the experimental groups and the control group were compared statistically

using analysis of variance (ANOVA).

Results

Reperfusion of ischemic liver tissue resulted in an appreciable increase in t_{90} (Table 1). A significant increase of t_{90} was noted in all ischemic groups compared with the controls. In groups II and III, the maximal radioactivity level was significantly lower than that of controls ($p < 0.01$), but no significant difference was

Table 1 Effects of ischemia-reperfusion on t_{90} ^a

Groups (n)	Ischemia ^b time (min)	Reperfusion time	t_{90} (min)	Radioactivity ratio ^c (ischemia/control)
Control (6)			3.76 ± 0.517	1.00
Ischemia (28)				
Group I (6)	5	15min	$4.99 \pm 1.03^*$	0.943
Group II (5)	10	15min	$5.08 \pm 0.418^{**}$	0.924
Group III (5)	45	15min	$14.0 \pm 1.48^{**}$	0.546
Group IV (6)	45	1week	$5.28 \pm 0.435^{**}$	
Group V (6)	45	2weeks	3.86 ± 0.649	

a: Liver uptake index (t_{90}) is the time when the liver time-activity curve reached 90 % of its peak. Values are the mean $\pm$ standard deviation. b: Hepatic ischemia was induced in rats by clamping the left hepatic artery and the left portal vein to evaluate the hepatic ischemia-reperfusion injury. c: The maximal radioactivity ratios of the ischemic group to the control group were measured. $^*p < 0.05$, $^{**}p < 0.01$, Significantly different from control group when measured by analysis of variance (ANOVA).

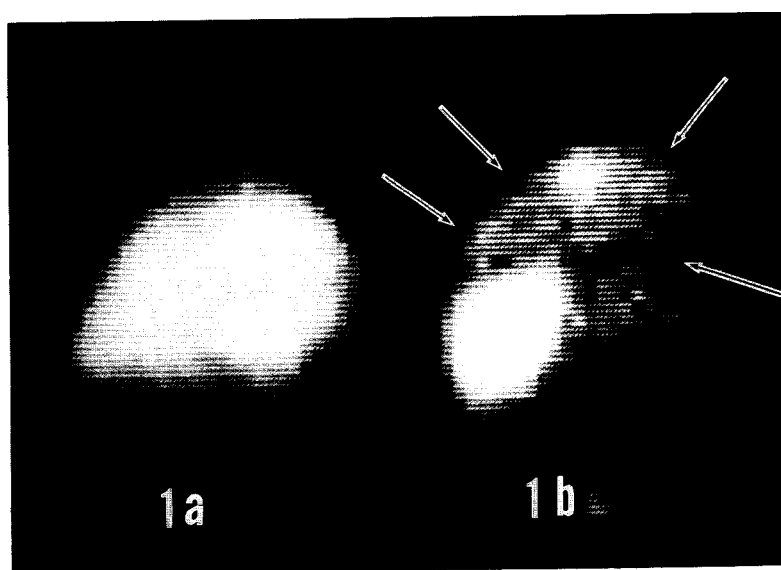


Fig. 1 Liver scintigrams of rats after the administration of Tc-GSA. (a); An excellent hepatic image was obtained in a control rat. (b); A focal area of decreased tracer accumulation (arrow) was noted in the left lobe in an animal in group III.

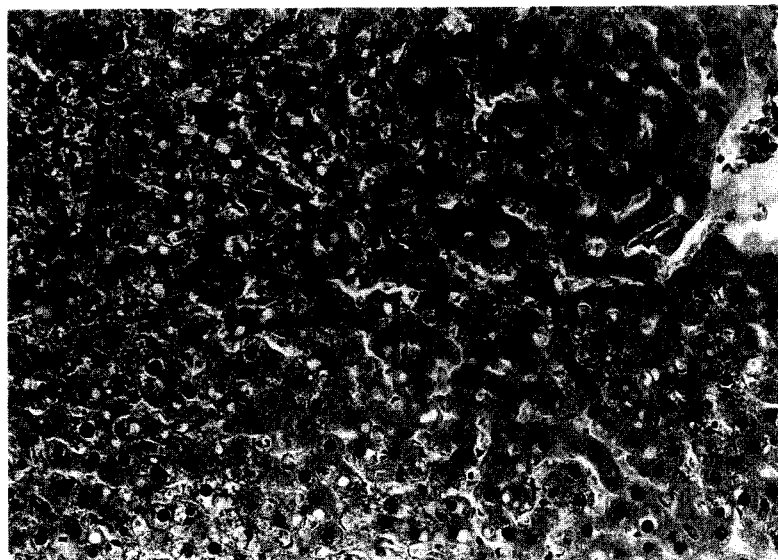


Fig. 2 Photomicrograph of a liver lobe subjected to 45 min of ischemia followed by 15 min of reperfusion. Compared with the controls, relatively severe fatty change in the cytoplasm was observed.

Table 2 Effects of ischemia-reperfusion on serum transaminases

Groups	GOT (KU) ^a	GPT (KU) ^a
Control	63.4 ± 2.88	34.2 ± 7.92
Ischemia		
Group I	72.8 ± 10.4	33.5 ± 8.43
Group II	85.8 ± 24.4	37.8 ± 8.32
Group III	477 ± 159**	327 ± 153**

^a: In each ischemic group (I-III) and the control group, GOT and GPT were assayed by means of Karmen method of which values were represented as Karmen Units (KU) (the mean ± standard deviation).

***p* < 0.01, significantly different from control group when measured by analysis of variance (ANOVA).

revealed in group I. Table 1 also shows the effects of reperfusion duration on t_{90} . The t_{90} was significantly higher in group IV compared with that of controls. However no significant difference in t_{90} was noted in group V. Table 2 shows the changes in serum transaminases. GOT and GPT values in group III were significantly higher than those observed in the controls. An excellent hepatic image was obtained in the control and a focal area of decreased tracer accumulation was noted in the left lobe in one animal in group III (Fig. 1).

Samples of the liver specimens obtained from the experimental groups (I-V) and the controls were evaluated light microscopically. The hepatic architecture remained almost intact in groups I and II, but compared with the other groups, relatively severe fatty change in the cytoplasm was observed in group III (Fig. 2).

Discussion

Prolonged ischemia and subsequent reperfusion are known to produce morphologic alterations such as endothelial cell swelling, interstitial edema and subsequent tissue necrosis (6) in a variety of organs.

The metabolic and histologic changes in the liver produced by temporary interruption of the hepatic circulation have been reported (7, 8). However, there is currently a need for better methods for evaluating hepatic anatomy and function. Ashwell *et al.* found that the asialoglycoproteins produced by removal of the terminal sialic acid from serum glycoproteins are recognized by a hepatic receptor, rapidly removed from circulation, and taken up by the hepatocytes (9). The serum asialoglycoprotein level showed an inverse relation to the number of hepatocyte receptors (10). Recent advances in receptor biochemistry have led to the development of a new

radiopharmaceutical imaging agent. Tc-GSA binds specifically to a membrane receptor which is found only on hepatocytes (4). Technetium-99m Sn colloid (Tc-Sc) particles are specifically phagocytosed by organs such as spleen, liver and bone marrow that contain reticuloendothelial cells. Compared with Tc-Sc, Tc-GSA is more specific and more effective for demonstrating common hepatic functional abnormalities, since Tc-Sc serves only as an indicator of Kupffer cell function. Also, Tc-GSA imaging, in contrast with Tc-Sc imaging, provides excellent hepatic images in case with severe hepatocellular dysfunction, since extrahepatic uptake does not occur. The use of Tc-GSA provides several advantages for evaluation of hepatic ischemia-reperfusion injury. A decrease in receptor concentration will produce a liver time-activity curve with a decreased slope, and a delay in the time at which the curve peaks. Imaging with Tc-GSA may also provide a means for assessing regional hepatic ischemia-reperfusion injury.

In this study, GOT and GPT values significantly increased after 45 min of ischemia followed by 15 min of reperfusion. However, these transaminases were not significantly altered by ischemia of 10 min or less. In this study t_{90} was significantly higher after 5 min of ischemia, and thus may be a more sensitive index of hepatic function. The values of t_{90} recovered to that of the controls after 2 weeks. A massive decrease in Tc-GSA uptake was seen in an animal after 45 min of ischemia.

In this study, we used the index t_{90} to evaluate hepatic ischemia-reperfusion injury, but a model method for analyzing labeled neoglycoalbumin has been reported using a three-compartment model (11) and a four-compartment model (12). This study indicates that Tc-GSA is useful for evaluating the hepatic ischemia-reperfusion injury. While Tc-GSA is specific to the liver, research is currently underway to increase the specificity of Tc-GSA regional intrahepatic analysis.

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