^{99m}Tc Sulfur Colloid and ^{99m}Tc Mebrofenin Hepatobiliary Functional Liver Imaging in Normal and Diabetic Rats

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Key Words

Liver scan · Phagocytic system · Reticuloendothelial system · Hepatobiliary system · Diabetes · Rat

Abstract

Objectives: To use ^{99m}Tc sulfur colloid (^{99m}Tc -SC) and ^{99m}Tc mebrofenin (^{99m}Tc -BrIDA) to study liver function in normal and diabetic rats. **Materials and Methods:** Radionuclide imaging was performed on 2 groups of rats, using ^{99m}Tc -SC for one group and ^{99m}Tc -BrIDA for the other (20 rats per group) before and after induction of diabetes mellitus (DM) using streptozotocin administration (55 mg/kg i.p.). Dynamic acquisition was obtained for 1 h after the injection of 37 MBq of radiotracer. For the ^{99m}Tc -SC group, organ/tissue uptake was determined by drawing regions of interest (ROI) over the heart, liver, spleen and also the whole body (WB). The ratio of the ROI of each organ to the WB ROI was calculated. For ^{99m}Tc -BrIDA, ratios of cumulative count rates in liver, liver parenchyma, biliary tree and abdomen ROI to a WB ROI were also calculated. Statistical analysis was performed to compare the ratios of organ/tissue uptake to WB uptake before and after DM induction using the paired t test. **Results:** ^{99m}Tc -SC uptake ratios (means $\pm$ SD) showed a lower liver-to-WB uptake ratio (0.75 ± 0.05) in the rats after DM induction compared to baseline (0.81 ± 0.06), while the cardiac blood pool showed higher uptake ratios in the rats after DM induction ($p = 0.026$). For ^{99m}Tc -BrIDA, there was no significant difference in radiotracer uptake ratios obtained from the rats before and after DM induction ($p = 0.41$). **Conclusion:** Using

functional liver imaging, there was a statistically significant decrease in the liver phagocytic/reticuloendothelial system function after DM induction, as evidenced by decreased ^{99m}Tc -SC liver uptake and increased blood pool compared to prediabetes, while the hepatobiliary function remained unchanged after DM induction using ^{99m}Tc -BrIDA imaging.

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Introduction

Diabetes mellitus (DM) involves abnormalities of the metabolism of glucose in the body, leading to pathophysiologic complications in various organs. Aspects of such pathophysiologic complications in diabetes include a markedly increased mobilization of fats from storage areas, causing abnormal fat metabolism as well as the deposition of cholesterol in arterial walls and atherosclerosis [1]. Fatty infiltration of the liver has also been reported to be linked with diabetes and can be associated with elevated liver enzyme levels such as transaminases and high bilirubin levels [2–5]. In this study, we investigated the liver function of rat models of DM, using two types of radiopharmaceuticals: technetium-99m-labeled sulfur colloid (^{99m}Tc -SC) and technetium-99m-labeled mebrofenin (^{99m}Tc -BrIDA or commonly known as HIDA). ^{99m}Tc -SC is used routinely to study the function of the phagocytic/reticuloendothelial system, while ^{99m}Tc -BrIDA is also used routinely to study hepatobiliary function. There has been limited research involving both liver

phagocytic and hepatobiliary imaging in diabetic patients or even animal models of diabetes. Hence, the objective of this study was to use ^{99m}Tc -SC and ^{99m}Tc -BrIDA imaging to investigate liver functions in diabetic rats, and to check whether or not it could provide an experimental model for the pathophysiologic changes associated with this condition.

Materials and Methods

Radiopharmaceuticals

Mebrofenin (bromo iminodiacetic acid, BrIDA) and SC were purchased as commercial kits from Amersham International plc (Amersham GE, UK). ^{99m}Tc -SC and ^{99m}Tc -BrIDA were prepared according to the manufacturer's recommendations. All other chemicals used in this study were purchased and supplied by Sigma-Aldrich (UK).

Experimental Animals

Adult male Sprague-Dawley rats (250 ± 50 g body weight) bred at the animal facility of the Faculty of Medicine, Kuwait University, were used in this study ($n = 40$). The animals had free access to water and food and were handled in accordance with an established animal use protocol following recommendations of the Helsinki Declaration and Kuwait University's institutional animal care and use committee.

Induction of Diabetes in Rats and Blood Glucose Assay

DM was induced in the rats by intraperitoneal injection of 55 mg/kg body weight streptozotocin (STZ) freshly dissolved in 5 mmol/l citrate buffer at a pH of 4.5 [6, 7]. Blood was tested before the induction of diabetes for basal glucose determination, and 2 and 7 days after the induction of diabetes using the FreeStyle Freedom[®] Lite blood glucose monitoring system (Abbott Laboratories, Ill., USA). All rats showed elevated glucose levels equal to or higher than 11 mmol/l after having received the induction protocol.

Experimental Protocol

The 40 rats were divided into 2 equal groups for ^{99m}Tc -SC and ^{99m}Tc -BrIDA imaging. As a prediabetes baseline study, ^{99m}Tc -SC and ^{99m}Tc -BrIDA imaging were performed on all animals. At 1 week after induction of diabetes, both ^{99m}Tc -SC and ^{99m}Tc -BrIDA imaging was repeated using the same imaging protocol.

Imaging Protocol

The rats were anesthetized using an intraperitoneal injection of ketamine:xylazine (40 mg/kg:5 mg/kg body weight; Serumwerk Bernburg, Germany), and an intravenous line Butterfly 21G IV catheter (Medi Move Ltd., UK) was placed in the dorsal tail vein for radiopharmaceutical injection.

The rats were positioned face up, and an anterior view of whole body (WB) was taken using a large-field-of-view, 40×60 cm clinical gamma camera (Philips camera, Odyssey LX; Philips, UK) fitted with a low-energy, all-purpose, parallel-hole collimator connected to a data acquisition computer. Imaging involved 2 dynamic phases, a vascular phase of 1 s/frame for 1 min, followed by a parenchymal phase at 1 min/frame for 1 h after the injection of 37

MBq of ^{99m}Tc -SC or ^{99m}Tc -BrIDA. Images were acquired in a 64×64 pixel matrix, using a photopeak centered at 140 keV with a symmetric 20% window and zoom of 4. The pinhole collimator was not used because quantitative analysis could not be applied and the parallel hole collimator was suitable for this purpose.

Image Processing

On a 0- to 61-min composite image, the regional distribution of the radiotracer was determined by drawing regions of interest (ROI) over the heart, liver, spleen and WB for ^{99m}Tc -SC (fig. 1a), and over the liver, liver parenchyma, bile in the liver hilum, abdomen and WB for ^{99m}Tc -BrIDA (fig. 1b). The following ratios were obtained: heart, liver or spleen to WB for ^{99m}Tc -SC, and liver, biliary tree, liver parenchyma or abdominal cavity to WB for ^{99m}Tc -BrIDA for both baseline and diabetic rat scans. These ratios represent the percentage of radioactivity uptake in each organ compared to the WB counts accumulated over 60 min. In addition, a ratio before and after DM for each organ was calculated. The anterior view was adequate for imaging the organs of interest, i.e. the heart, liver and spleen.

Data Presentation and Statistical Analysis

All data, unless otherwise stated, are expressed as means $\pm$ SD. The paired t test was used to evaluate differences between baseline control and diabetic rats. Statistical analysis was performed using the SPSS software, version 13 (SPSS Inc., Chicago, Ill., USA).

Results

Induction of Diabetes and Blood Glucose Assays

The average blood glucose level was 5.7 ± 1.2 mmol/l at baseline, 15.7 ± 1.1 mmol/l at 48 h and 27.7 ± 1.4 mmol/l at 1 week after DM induction in all animals. In the diabetic rats, there was a significant loss of body weight from 250 ± 50 to 138 ± 40 g ($p < 0.05$).

Functional Imaging

The time activity curves from 0 to 61 min for a pre- and post-DM ^{99m}Tc -SC study of the heart, liver and spleen are given in figure 2. The cardiac time activity curve shows a rapid decline, while the liver and spleen show a fast uptake up to a peak at 5 min. A similar pattern was seen in DM rats. The uptake ratios for ^{99m}Tc -SC are shown in table 1 for both baseline (control) and DM rats, and the ratios before and after DM for each organ as prediabetic divided by diabetic (D/P) ratio. There is a significantly reduced cardiac blood pool clearance ($p = 0.026$) and significantly reduced hepatic SC uptake ($p = 0.026$) in diabetic rats in comparison to the baseline state. Besides, the D/P ratio was significant ($p = 0.026$) for the heart (1.27) and liver (0.92), but not significant ($p = 0.22$) for the spleen (1.17).

Fig. 1. Composite 0- to 61-min images and ROI used both before and after DM. **a** ^{99m}Tc -SC. **b** ^{99m}Tc -BrIDA.

Fig. 2. Time activity curves from 0 to 61 min for a pre- and post-DM ^{99m}Tc -SC study (**a**) and a pre- and post-DM ^{99m}Tc -BrIDA study (**b**). Hrtc = Heart control; Hrtd = heart diabetic; Lvrc = liver control; Lvrd = liver diabetic; Splnc = spleen control; Splnd = spleen diabetic; Biliaryc = biliary tree control; Biliaryd = biliary tree diabetic.

In figure 2b, images and time activity curves for a pre- and post-DM ^{99m}Tc -BrIDA study are displayed, showing the hepatic uptake and biliary excretion of radiotracer. The uptake ratios of liver, bile, liver parenchyma and abdomen to WB (mean $\pm$ SD) between prediabetic and diabetic rats, and the D/P ratio for each organ are shown in table 2. Using paired t test, the results do not show significant differences between before and after DM induction and the D/P ratio for each organ ($p = 0.41$).

Discussion

This study examined the use of ^{99m}Tc -SC and ^{99m}Tc -BrIDA in liver functional imaging in chemically induced diabetes in rats. The results of the ^{99m}Tc -SC studies showed a significantly delayed clearance of ^{99m}Tc -SC from the cardiac blood pool and significantly reduced hepatic SC uptake 1 week after diabetes induction. This suggests an impairment of phagocytic/reticuloendothelial

Table 1. Comparison of ^{99m}Tc -SC uptake ratios in rat models of DM and D/P ratios

Ratio to WB	Prediabetic	Diabetic	D/P
Heart	0.15 ± 0.03	$0.19 \pm 0.03^*$	1.27^*
Liver	0.81 ± 0.06	$0.75 \pm 0.05^*$	0.92^*
Spleen	0.06 ± 0.02	$0.07 \pm 0.02^{**}$	1.17^{**}

Values denote means $\pm$ SD. Difference between prediabetic and diabetic rats (n = 20). * p = 0.026, significant; ** p = 0.22, not significant.

system function in a similar way to that seen with early cirrhosis, portal hypertension or nonspecific liver damage [8–10]. The spleen uptake increased slightly after diabetes induction, but did not reach statistical significance in line with a mild colloid shift from liver to spleen.

In contrast, ^{99m}Tc -BrIDA uptake and the kinetics found in the diabetic rats in this study did not show significant differences compared to baseline prediabetic cases, especially in the liver-, bile-, liver-parenchyma- and abdomen-to-WB ratios. The implication of this finding is that early diabetes may not induce changes in hepatobiliary function in terms of uptake and secretion of the radiotracer. In one study in the literature, increased total hepatobiliary bile acid excretion was noted as an effect of STZ-induced diabetes compared to controls [2], which was also seen in our study (higher bile ratio, although not statistically significant). Our experimental setting did not allow for adequate evaluation of the biliary tree. It is not possible to accurately outline the biliary structures for analysis. However, measurement of the bile excreted in the bowel in the

Table 2. Comparison of ^{99m}Tc -BrIDA uptake ratios in rat models of DM before and after DM induction and their D/P ratios

Ratio to WB	Prediabetic	Diabetic	D/P
Liver	0.67 ± 0.07	0.69 ± 0.08	1.03^*
Bile	0.70 ± 0.08	0.72 ± 0.08	1.03^*
Liver parenchyma	0.13 ± 0.005	0.12 ± 0.006	0.92^*
Abdomen	1.10 ± 0.12	1.10 ± 0.13	1.00^*

Values denote means $\pm$ SD. Difference between prediabetic and diabetic rats (n = 20). * p = 0.41.

abdomen ROI and liver parenchyma did not show a significant difference between baseline and diabetes and the D/P ratio for each organ (p = 0.41) (table 2).

Conclusion

Our study showed a delayed cardiac blood pool clearance of ^{99m}Tc -SC by the liver, indicating a probable impairment of phagocytes. However, the clearance of concomitantly reduced hepatic ^{99m}Tc -BrIDA was not different in diabetic compared to prediabetic rats, possibly indicating that STZ did not adversely affect the hepatocytes.

Acknowledgments

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