The Value of Tc-99m Mibi for Differential Diagnosis of Biliary Atresia and Hepatitis in Rats

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Abstract

Cholestatic diseases in neonates which characterized by deterioration of bile passage to intestine. We investigated the usability of Tc-99m MIBI as a hepatobiliary scintigraphy radiopharmaceutical for diferential diagnosis of biliary atresia and hepatitis in experimental model. A total of 20 males Wistar albino rats who had weights ranging from 200-350 g were included in this study. Rats were randomly divided into 4 groups. Control group, sham operated group, biliary atresia group and chemical hepatitis group (with carbon tetrachloride) were created, respectively. Blood flow phases, consantration and early excreation phases was performed after an intravenous injection through the internal jugular vein of 37 MBq Tc-99m MIBI using a gamma camera. Dual late static images were obtained at the same position at 15, 30, 60, 90 and 120 min after injection. In control group, radiopharmaceutical passage into the small intestine was seen between 45 and 60 min and evidently seen between 120 and 150 min as large hyperactive focus on midline or non-linear (snaky) radiopharmaceutical accumulation at the level of kidney. In biliary atresia group, radiopharmaceutical concentration was seen normal in liver but passage into the small intestine was not seen all rats. In carbon tetrachloride group, blood flow, concentration and early excretion scintigraphic images did not differ from control group and radiopharmaceutical passage into the small intestine was seen between 45 and 60 min. Although radiopharmaceuticals passage into the intestine was not shown in all rats of biliary atresia group. We suggest that Tc-99m MIBI as a radiopharmaceutical of hepatobiliary scintigraphy would be the contribution of the differential diagnosis of biliary atresia and hepatitis in humans.

Keywords: Tc-99m MIBI, hepatobiliary scintigraphy, biliary atresia, carbon tetrachloride, hepatitis, rat

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Introduction

Cholestatic diseases in neonates which characterized by deterioration of bile passage to intestine is show in the first 3 months after birth whom have increased conjugated bilirubin and jaundice [1]. The first 14 days after birth, there was jaundice in 2.4-15% of infants and indirect hyperbilirubinemia has been identified in most of them [2]. Cholestatic jaundice is observed in the live 1/2500 births [3]. Several infectious, metabolic or genetic diseases can lead to neonatal hepatitis. While biliary atresia is approximately 25% of neonatal hepatitis, idiopathic neonatal cholestasis is 15% of them. Twenty percent of them are intrahepatic cholestatic syndromes such as Alagille syndrome and progressive familial intrahepatic cholestasis [4].

Iminodiacetic acid derivatives, especially Tc-99m mebrofenin, are widely used for evaluation of liver and gallbladder function due to excreted into bile [5,6]. Generally, it is accepted that concentration and excreation of Tc-99m IDA derivatives in the liver was depended to mass of hepatocytes and presence or absence of liver damage.

We investigated the usability of Tc-99m MIBI as a hepatobiliary scintigraphy radiopharmaceutical for diferential diagnosis of biliary atresia and hepatitis in experimental model.

Materials and Methods

A total of 20 males Wistar albino rats, ranging in weight from 200 to 350 g, were included in this study. Control group (group 1), sham oparated group (group 2), biliary atresia group (group 3) and chemical hepatitis group (group 4) were constituted randomly. Before all operation and hepatobiliary scintigraphy, animal nutrition has been stopped 6 hour ago and anesthesia was performed with intraperitoneal injection of 40/2 mg/kg ketamine/xylazine (Ketalar 50mg/ml flacon, Phizer, İstanbul, Turkey/ Xylazin Bio 2% flacon, BIoveta Plc. Ivanovice na Hane, Czech Republic) [7]. Spontaneous respirations of rats were monitored throughout the experiment. In control groups, scintigraphy was performed directly following injection of radiopharmaceutical injection. In sham operated groups, we had entered abdomen through a midline incision and closed with prolin. Because of wound healing, it was expected 14 days and scintigraphy was performed. In biliary atresia groups, we had entered abdomen

through a midline incision, suspended its deudenum, found main bile duct and cutted after both site ligation. Scintigraphy was performed in 14th day. In chemical hepatitis group, Carbon tetrachloride (CCl_4, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) injection was performed intraperitoneally every third day for 14 days (1.0 ml/ kg, 1: 1 in pure pure olive oil) [8, 9, 10, 11]. Scintigraphy was performed in 28th day.

Preparation of Radiopharmaceuticals

Freshly milked 40 mCi Tc-99m pertechnetate was added into kit for the preparation of Tc-99m MIBI vial (Kit for the preparation of Tc-99m MIBI, Polatom, I Odwock, Poland) and was boiled in boiling water (100 °C) for 10 minutes. The resulting pH of the solution was 5.5. Quaity control test was performed according to the manufacturer's description. The sum of contaminations of unbound pertechnetate Tc-99mO_4^- and a reduced form Tc-99m was not higher than 5% of the total activity.

Hepatobiliary scintigraphy

Anesthesized rats were fixed in the supine position. Neck region was wiped 1% povidone-iodine. Right supraclavicular incision was performed and jugular vein was observed. Tc-99m MIBI (1±0.2 mCi/ 0.2 mL) was given into the jugular vein. Twenty images from 3 seconds (blood flow phases) and subsequently 20 images from 30 seconds (consantration and early excreation phases) dual dynamic imaging of the abdomen in anterior and posterior projection was obatined after an intravenous bolus injection through the internal jugular vein of Tc-99m MIBI (1±0.2 mCi/ 0.2 mL) using a gamma camera (Vertex V60, Adac, Milpitas, CA, USA). Additionally, four-minute anterior ve posterior dual statics images were obtained at 15, 30, 60 and 90 min. Low-energy high-resolution, parallel-hole collimator was used. Energy spectrum was adjusted 140 KeV and 20% window. All images were evaluated visually and quantitatively. For quantitative evaluation, 10x10 pixels square region of interest were drawn on liver, heart, kidney and bladder on tenth images of "concentration and early excretion

phase". Using average count values, "Liver / Heart Rate", "Liver / Kidney Rate" and "Bladder / Liver ratio" were calculated and used statistical evaluation (Figure 1).

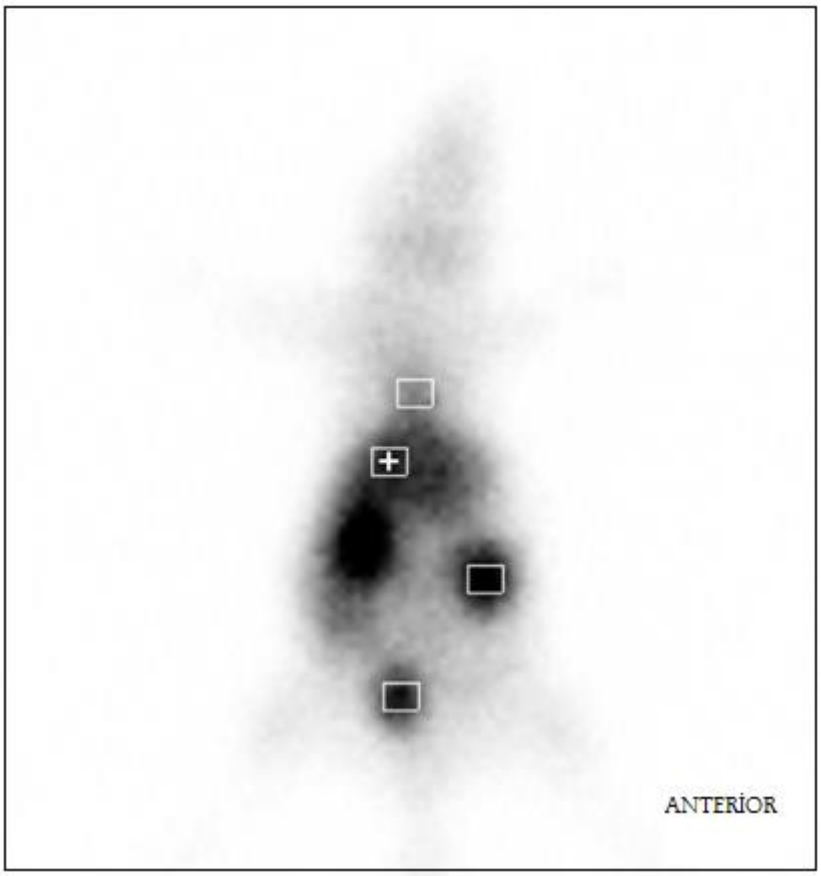

Figure 1. Used region of interests on anterior images.

Statistical evaluation

Ethics Committee of School of Medicine in Inonu University was approved this study. To assess differences among groups, Kruskal-Wallis test and Mann-Whitney U test was used. The differences between the fourth and tenth images in each group were compared with Wilcoxon signed rank test. $p < 0.05$ was considered as significant. Data were analyzed using the statistical package for the social sciences (SPSS) version 15.0 (SPSS Inc.; Chicago, IL, USA).

Results

"SNM Practice Guideline for Hepatobiliary Scintigraphy 4.0" was accepted as a basic reference for image acquisition and interpretation.

After the kidney, liver blood flow was evidently shown from the second image in all groups. The liver was filling in a wide area (approximately one third of the abdomen) and was chosen in the following areas than the kidney. While radioactivity passage into the intestine was seen unclear between kidneys at 15 and 30 min in the first two groups, it was seen evidently on focal or snaky areas between kidneys at 45 and 60 min. The best radiopharmaceuticals passages into the intestine were shown at 120 and 150 min (Figure 2 and 3).

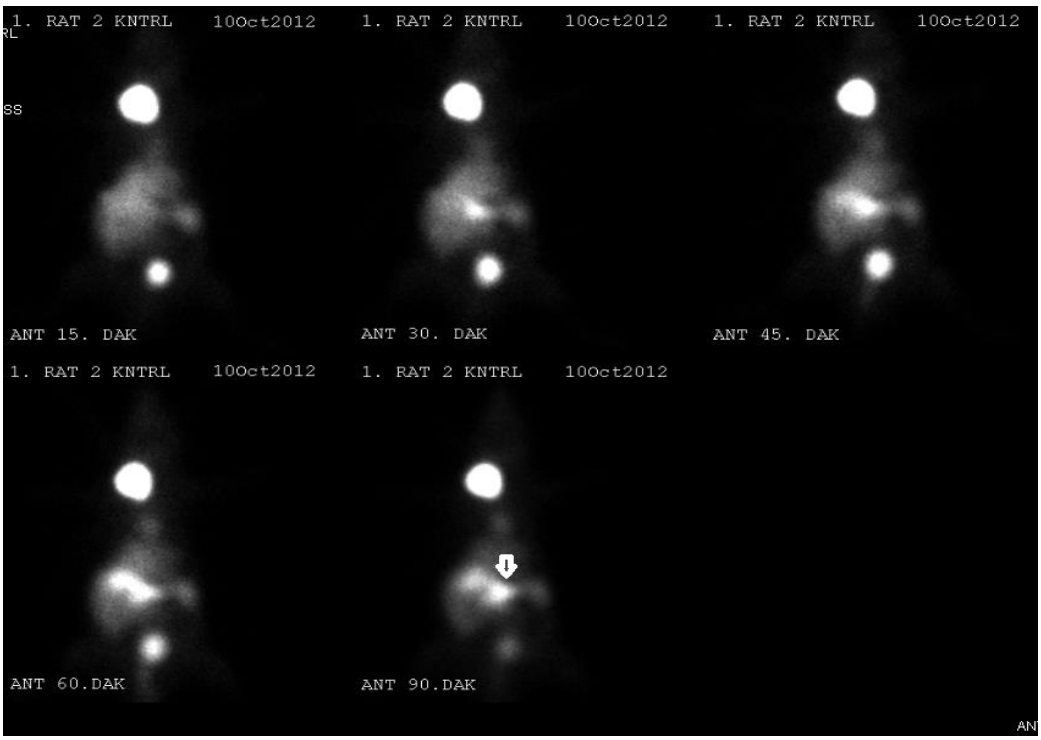

Figure 2. Focal radiopharmaceuticals passages into the intestine (Anterior images)

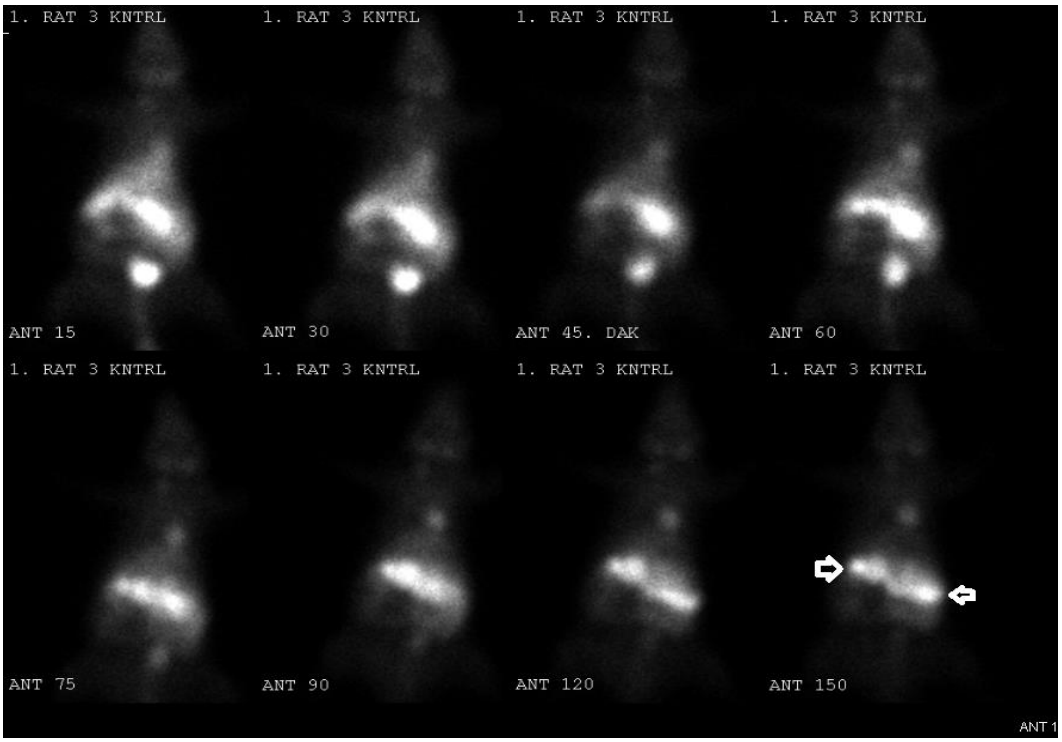

Figure 3. Snaky radiopharmaceuticals passages into the intestine (Anterior images)

Obvious jaundice was shown but radiopharmaceuticals passage into the intestine was not shown in all rats of biliary atresia group (Figure 4).

Tc-99m MIBI passage into the intestine was shown at 30 and 60 min in all rats of chemical hepatitis group with CCl_4 (Figure 5).

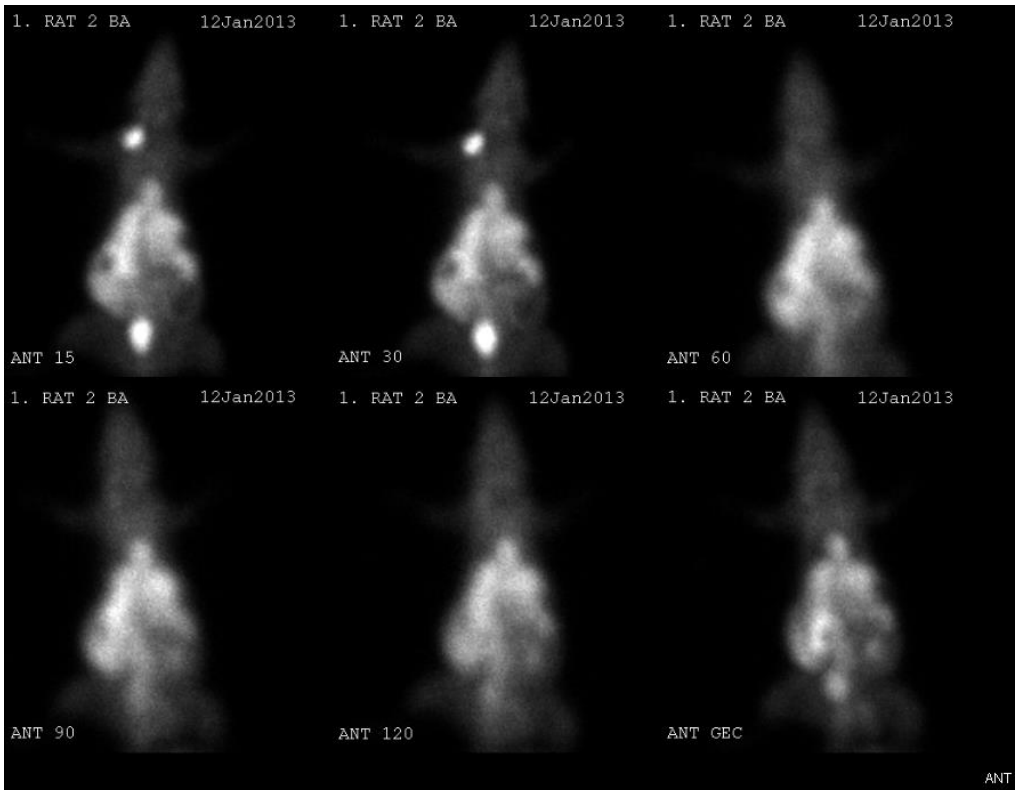

Figure 4. Radiopharmaceuticals passage into the intestine was not shown in biliary atresia group

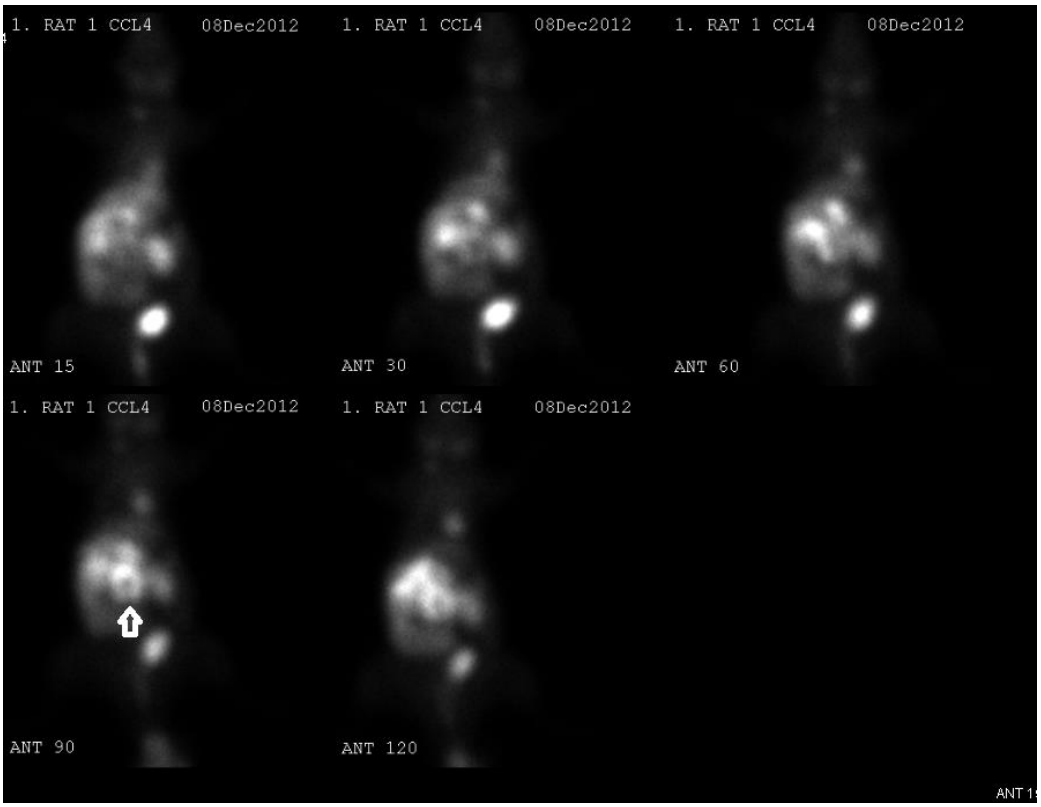

Figure 5. Tc-99m MIBI passage into the intestine in chemical hepatitis group with CCl_4

Using average count values, "Liver / Heart Rate", "Liver / Kidney Rate" and "Bladder / Liver ratio" were calculated and used statistical evaluation on tenth images of "concentration and early excretion phase" but significant differences were not found statistically among groups ($p>0.05$) (Table 1).

Tablo 1. Calculated "Liver / Heart Rate", "Liver / Kidney Rate" and "Bladder / Liver ratio" on tenth images of "concentration and early excretion phase" and statistical analysis.

	N	Grup 1	Grup 2	Grup 3	Grup 4	
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	p value
Liver / Heart Rate	5	2.053 ± 0.137	1.662 ± 0.190	1.342 ± 0.195	1.596 ± 0.132	0.063
Liver / Kidney Rate	5	0.920 ± 0.132	0.784 ± 0.052	0.922 ± 0.139	0.776 ± 0.112	0.914
Bladder / Liver ratio	5	1.031 ± 0.219	1.089 ± 0.251	0.559 ± 0.124	0.892 ± 0.173	0.293

Discussion

Many exams are referenced in the differential diagnosis of biliary atresia and neonatal hepatitis. One of them is the hepatobiliary scintigraphy with Tc-99m iminodiacetic acid derivatives. It is accepted that concentration and excreation of Tc-99m IDA derivatives in the liver is decreased with liver demage. Hepatic inflammation, cellular injury and fibrosis are disrupt carriers of Tc-99m IDA derivatives [12]. Except in patients without gallbladder dysfunction or biliary atresia, especially in liver cirrhosis and hepatitis, cause of Tc-99m IDA derivatives' (such as Tc-99m mebrofenin) retention is not known.

The ischemia and reperfusion studies reported that release of cytokines in Kupffer cells (especially interleukin-6 and tumor necrosis factor-α) and liver regeneration can be affect expression of different organic anion-transporting polypeptide (OATP) isoforms independently from each others. There are a positive correlation between the level different isoforms of OATP in the liver and uptake of Tc-99m mebrofenin. Initial liver Tc-99m mebrofenin uptake is independent from sodium and is only affected by OATP and OATP inhibitors such as rifamycin SV and glycyrrhizic acid. Previous studies had shown that Tc-99m mebrofenin is substrates for subtypes of OATP1B1 and OATP1B3 [13, 15].

de Graaf et al found that both TNF-alpha and IL-6 levels had increased in 15-30 minutes ischemia-reperfusion performed rats after 30% partial hepatectomy, though messenger

ribonucleic acid expression of all OATP isoforms had decreased belonging to hepatocellular damage. This decline might be related with cytokine release. They has suggested that reduction of the uptake of Tc-99m mebrofenin in the hepatitis can be attributed to reduction of OATP isoforms' expression which depends to cytokines [16]. Previous studies reported that canalicular excretion of Tc-99m mebrofenin was reduced by inhibitors of Multidrug Resistance-Associated Protein 2 (MRP2) in rats [15].

Tc-99m MIBI, substrate of P-glycoprotein, is a cationic lipophilic isonitrile and is widely used in myocardial perfusion scintigraphy. Tc-99m MIBI, depending on the electrical potential, passes the cell wall and membrane of mitochondria with passive diffusion and is fixed on the mitochondria due to electrostatic charge. Similarly, Tc-99m MIBI passes the cell wall of hepatocyte with passive diffusion and is secreted into bile duct. Tc-99m MIBI is commonly used in routine because of low dose cost and readily available. Because IDA kits had not been obtained in our country for a long time, we want to investigate which Tc-99m MIBI can be used in the differential diagnosis biliary atresia and hepatitis.

Tc-99m MIBI is also used as a showing sensor for P-glycoprotein and MRP2. Initial liver Tc-99m MIBI uptake is independent from sodium and is affected by OATP inhibitors. Tc-99m MIBI uptake of sodium is independent of the initial liver is affected by OATP inhibitors. Biliary excretion of Tc-99m MIBI is not affected in the absence of MRP2 [15].

In biliary atresia groups, we had entered abdomen, found main bile duct and cutted after both site ligation. Previous study showed that induced liver damage by this operation shows similar findings with damage after biliary obstruction in human [17]. Sadegi et al had taken both Tc-99m MIBI and Tc-99m Mebrofenin scintigraphy in 20 infant with neonatal hepatitis and biliary atesia (half of them with neonatal hepatitis). While radioactivity passage into the intestine with Tc-99m MIBI was seen in all infant, it was seen only in 5 infants with neonatal hepatitis. They suggested that Tc-99m MIBI can passage into intestine by another route in biliary atresia and it has limited value in the differential diagnosis of neonatal hepatitis [18]. We thougt that precise diagnosis of the biliary atresia could not been proved in Sadegi's study. In our study, obvious jaundice was shown but radiopharmaceuticals passage into the intestine was not shown in all rats of biliary atresia group. We believe that it can be useable in the differential diagnosis of cholestasis.

CCl_4 is converted to toxic intermediates products (CCl_3 and Cl which are highly reactive free radicals) by cytochrome P450 enzymes and these free radicals make peroxidative liver damage. Serum ALT and AST levels increase 5-200 fold. These two reactive free radicals interact with various proteins and long chain saturated fatty acids and initiate chain reactions. Level of lipid peroxide rises. They cause fatty degeneration and cell death around cetrilobular necrosis in liver. While clinical short exposure to carbon tetrachloride cause acute liver injury, continuous exposure to this toxin cause cirrhosis, portal hypertension and death by progressive liver demage and fibrosis [19, 20, 21, 14]. Another study showed that liver degeneration and number of the necrosis by CCL_4 peaks is 12-24 hours, but it did not disappear even after 72 hours [22]. Effect of various cytochrome P450 enzyme inhibitors in reducing carbon tetrachloride damage has been shown clinically [14].

Sogano et al reported that the total phospholipid content of liver after administration of CCl_4 decrease to 86% and 70% and after 6 and 20 hours, respectively [23]. Carbon tetrachloride increases degradation of membrane phospholipids by regulated with phospholipase C and catalyzes from phosphatidylcholine to phosphorylcholine and phosphatidic acid [24, 25]. Released phosphorylcholine can reusable to synthesis of phosphatidyl choline because phosphorylcholine are directly involved in the regulation of phosphatidyl choline. Remaining phospholipids can be reused in the regeneration of liver cells and increased phospholipase activity in the liver increases release of phosphoryl choline from phosphotidyl choline. Therefore, additional choline come from the outside of the damaged liver is not needed [24].

Recent study showed that TNF-α, organic anion transporter polypeptides 1 and 2 in sodium-taurocholate cotransporter polypeptide release reduce in mRNA levels with CCl_4 taken rats [21]. While levels of the TNF-α, organic anion transporter polypeptide 2 and MRP2 and MRP3 affects in mRNA levels, levels of organic anion transporter polypeptide 1 and the bile salt export pump level do not affect in mouse [23]. Joseph et al reported that not only TNF impaire Tc-99m mebrofenin excretion but also interleukin-6 inhibits this excretion [20]. Joseph et al reported from another study that Tc-99m mebrofenin excretion was inhibited in rats even treated with 0.25 ml / kg CCl_4, minimal liver damage in this dose of CCl_4 is rapidly improved but abnormalities of Tc-99m mebrofenin excretion can persist in a long time. They suggested that this situation may be responsible from elongation of activation in Kupffer cell or other mechanisms [8].

Decreased concentration functions in chemical hepatitis group induced with carbon tetrachloride were waited for us due to liver demage but it was not seen in our rats. We though that uptake of Tc-99m MIBI in liver cell and excretion into bile might have been different than Tc-99m Mebrofenin.

These can be considered that selection of rat in hepatobiliary scintigraphy is not a good choice because it does not have a gallbladder and its duodenum and jejunum are localized behind of liver. However, it can be an advantage because it may not affected by fasting and gallbladder dysfunction.

Conclusions

Although radiopharmaceuticals passage into the intestine was not shown in all rats of biliary atresia group, it was shown at 30 and 60 min in all rats of chemical hepatitis group with CCl_4. In carbon tetrachloride group, radiopharmaceutical passage into the small intestine was clearly seen at 150 min in one rat while it was seen at 60 min in others.

Using average count values, "Liver / Heart Rate", "Liver / Kidney Rate" and "Bladder / Liver ratio" were calculated and used statistical evaluation on tenth images of "concentration and early excretion phase" but significant differences were not found statistically among groups.

We suggest that Tc-99m MIBI as a radiopharmaceutical of hepatobiliary scintigraphy would be the contribution of the differential diagnosis of biliary atresia and hepatitis in humans.

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Conflict of interests

The authors have no conflicts of interest.

References

1. Batres LA, Maller ES. Laboratory Assesments of Liver Function and Injury in Chidren. In: Suchy FJ, Sokol RJ, Balistreri WF, eds, Liver Disease in Children. 2nd edition. Philadelphia: Lippincott, Williams&Wilkins. 2001:155-69.

2. Kelly DA, Stanton A. Jaundice in babies: Implications for community screening for biliary atresia. BMJ. 1995;310(6988):1172-3.

3. Balistreri WF. Neonatal cholestasis. J Pediatr. 1985;106(2):171-84.

4. Suchy FJ. Neonatal cholestasis. Pediatr Rev. 2004;25(11):388-96.

5. Daniel GB, Bahr A, Dykes JA, DeNovo R, Young K, Smith GT. Hepatic extraction efficiency and excretion rate of technetiun-99m-mebrofenin in dogs. J Nucl Med. 1996;37(11):1846–9.

6. Koruk M, Ozkilic S, Savas MC, Celen Z, Kadayifci A, Ozkilic C. Evaluation of hepatic functions and biliary dynamics in patients with liver cirrhosis by quantitative scintigraphy. Hepatogastroenterology. 2003;50(54):1803-5.

7. Demirag K, Cankayali I, Eris O, Moral AR, Pehlivan M. The comparison of therapeutic effects of atropine and pralidoxime on cardiac signs in rats with experimental organophosphate poisoning. Adv Ther. 2005;22(2):79-86.

8. Joseph B, Bhargava KK, Tronco GG, Palestro CJ, Gupta S. Systemic and local release of inflammatory cytokines regulates hepatobiliary excretion of ^{99m}Tc-mebrofenin. Nucl Med Commun. 2008;29(4):336-44.

9. Surendran S, Eswaran MB, Vijayakumar M, Rao CV. In vitro and in vivo hepatoprotective activity of Cissampelos pareira against carbon-tetrachloride induced hepatic damage. Indian J Exp Biol. 2011;49(12):939-45.

10. Xu T, Wang X, Chen G, He Y, Bie P. Autologous bone marrow stem cell transplantation attenuates hepatocyte apoptosis in a rat model of ex vivo liver resection and liver autotransplantation. J Surg Res. 2013;184(2):1102-8.

11. Mukherjee S, Sur A, Maiti BR. Hepatoprotective effect of Swertia chirata on rat. Indian J Exp Biol. 1997;35(4):384-8.

12. Jansen PLM, Sturm E. Genetic cholestasis, causes and consequences for hepatobiliary transport. Liver Int. 2003;23(5):315–22.

13. Tanaka Y, Chen C, Maher JM, Klaassen CD. Kupffer cell-mediated down-regulation of hepatic transporter expression in rat hepatic ischemia-reperfusion. Transplantation. 2006;82(2):258.

14. Kim MK, Song BJ, Seidel J, Soh Y, Jeong KS, Kim IS, Kobayashi H, Green MV, Carrasquillo JA, Paik CH. Use of ^{99m}Tc-mercaptoacetyltriglycine (MAG3)-biocytin hepatobiliary scintigraphy to study the protective effect of a synthetic enzyme inhibitor on acute hepatotoxicity in mice. Nucl Med Biol. 1998;25(6):561-8.

15. Swift B, Yue W, Brouwer KL. Evaluation of (99m) technetium-mebrofenin and (99m)technetium-sesta MIBI as specific probes for hepatic transport protein function in rat and human hepatocytes. Pharm Res. 2010;27(9):1987-98.

16. de Graaf W, Heger M, Spruijt O, Maas A, de Bruin K, Hoekstra R, Bennink RJ, van Gulik TM. Quantitative assessment of liver function after ischemia-reperfusion injury and partial hepatectomy in rats. J Surg Res. 2012;172(1):85-94.

17. Cantürk NZ, Canturk Z, Utkan NZ, Yenisey C, Ozbilim G, Yildirir C, Yalman Y. Cytoprotective effect of alphatocopherol against liver injury induced by extrahepatic biliary obstruction. East African Medical Journal. 1998;75(2):77-80.

18. Sadeghi R, Kianifar HR, Kakhki VR, Zakavi SR, Ansari K. 99mTc sesta MIBI imaging-can it be a useful substitute for hepatobiliary scintigraphy in infantile jaundice? Nuklearmedizin. 2009;48(3):100-3.

19. Gagandeep S, Rajvanshi P, Sokhi RP, Slehria S, Palestro CJ, Bhargava KK, Gupta S. Transplanted hepatocytes engraft, survive and proliferate in the liver of rats with carbon tetrachloride-induced cirrhosis. J Pathol. 2000;191(1):78–85.

20. Joseph B, Bhargava KK, Tronco GG, Kumaran V, Palestro CJ, Gupta S. Regulation of hepatobiliary transport activity and noninvasive identification of cytokine-dependent liver inflammation. J Nucl Med. 2005;46(1):146-52.

21. Martinez-Hernandez A, Delgado FM, Amenta PS. The extracellular matrix in hepatic regeneration. Localization of collagen types I, III, IV, laminin, and fibronectin. Lab Invest. 1991;64(2):157–66.

22. Wang PY, Kaneko T, Tsukada H, Nakano M, Nakajima T, Sato A. Time courses of hepatic injuries induced by chloroform and by carbon tetrachloride: comparison of biochemical and histopathological changes. Arch Toxicol. 1997;71(10):638–45.

23. Sugano M, Cho S, Imaizumi K, Wada M. Hepatotoxicity and lipid metabolism. 3. Changes in phosphatidylcholine and phosphatidylethanolamine during hepatic injury caused by carbon tetrachloride. Biochem Pharmacol. 1970;19(7):2325–33.

24. Coleman JB, Condie LW, Lamb RG. The influence of CCl_4 biotransformation on the activation of rat liver phospholipase C in vitro. Toxicol Appl Pharmacol. 1988;95(2):200–7.

25. Coleman JB, Condie LW, Lamb RG. The role of CCl_4 biotransformation in the activation of hepatocytes phospholipase C in vivo and in vitro. Toxicol Appl Pharmacol. 1988;95(2):208-19.