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Research Article

Scintigraphic Assessment of Hepatobiliary Functions in Healthy Adult Dogs

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Abstract: Our objective was to investigate hepatobiliary functions in healthy dogs using ^{99m}Tc-mebrofenin hepatobiliary scintigraphy (HBS): 200 µCi/kg ^{99m}Tc-mebrofenin was injected intravenously into 12 healthy adult dogs. One-hour dynamic images and 2, 4 and 24 h static images were obtained. HBS was evaluated for 7 variables. Cardiac washout occurred within 3 min in all dogs. A photophenic area in the region of the gall bladder was seen in 3.03 ± 0.21 min. However, bile ducts were not identified as separate structures in any dogs. Mean Tmax of the liver was 7.95 ± 3.22 min. Initial gall bladder radioactivity was seen 13 ± 1.34 min after radiopharmaceutical injection. Mean hepatic washout time was 44 ± 3.15 min. Small intestinal radioactivity was not detected in the first 60 min, although it was observed within 2-4 h. However, it was not detected in 24 h late static images. In conclusion, ^{99m}Tc-mebrofenin HBS is a sensitive, specific and noninvasive diagnostic method with which to evaluate hepatocellulary functions, intrahepatic bile flow and gall bladder status in dogs. It is thought that the data obtained from this study may guide future research.

Key Words: Scintigraphy, hepatobiliary, dog, ^{99m}Tc-mebrofenin

Sağlıklı Yetişkin Köpeklerde Hepatobilier Fonksiyonların Sintigrafik Olarak İncelenmesi

Özet: Bu çalışmada sağlıklı erişkin köpeklerde ^{99m}Tc-mebrofenin hepatobilier sintigrafi (HBS) ile hepatobilier fonksiyonların değerlendirilmesi amaçlandı. Oniki adet sağlıklı yetişkin köpeğe intravenöz 200 µCi/kg ^{99m}Tc-mebrofenin enjekte edildi. Bir saatlik dinamik görüntüler ve 2, 4 ve 24. saatlerde statik görüntüler elde edildi. HBS 7 değişken yönünden değerlendirildi: Kardiyak arınma, köpeklerde üç dakika içinde şekillendi. Safra kesesi bölgesinde fotopenik alan 3,03 ± 0,21 dakikada görülürken, safra kanalları ayrı yapılar halinde belirlenemedi. Karaciğer için ortalama Tmax 7,95 ± 3,22 dakika olarak gözlemlendi. Safra kesesinde ilk radyoaktivite, radyofarmasötik enjeksiyonundan 13 ± 1,34 dakika sonra görülürken, hepatik arınma ortalama 44 ± 3,15 dakikada izlendi. Tüm olgularda ilk 60 dakika boyunca gözlemlenmeyen ince bağırsak radyoaktivitesi ancak 2-4. saatlerde saptanabildi. Buna karşın 24. saatte alınan geç statik görüntülerde bağırsak aktivitesi gözlemlenmedi. Sonuç olarak, ^{99m}Tc-mebrofenin HBS köpeklerde karaciğer fonksiyonlarının, intrahepatik safra akımının ve safra kesesi durumunun değerlendirilmesinde duyarlı, spesifik ve non-invaziv bir tanı metodudur. Bu çalışmayla ulaşılan verilerin daha sonraki çalışmalara rehber olacağı düşünülmektedir.

Anahtar Sözcükler: Sintigrafi, hepatobilier, köpek, ^{99m}Tc-mebrofenin

Introduction

Nuclear imaging, also known as scintigraphy, is a noninvasive diagnostic procedure (1). In both human and veterinary medicine, scintigraphy is able to visualise and quantitate the distribution of different radiopharmaceuticals in the living organism indicating the normal or abnormal processes of the organ concerned (2).

The radiopharmaceuticals currently used for hepatobiliary scintigraphy (HBS) are ^{99m}Tc-iminodiacetic acid derivatives. The 2 most commonly used are ^{99m}Tc-mebrofenin and ^{99m}Tc-disofenin. After intravenous (i.v) administration, the radiopharmaceutical is removed from the systemic circulation by hepatocytes via the same active transport mechanism used for bilirubin. Once within the hepatocytes, the radiopharmaceutical is secreted unchanged into the bile canaliculi and concentrated in the gall bladder. Progression of the radiopharmaceutical through the body is monitored by the use of a gamma camera (3,4). According to the physiological distribution of the radiopharmaceutical, morphological and functional information such as bile excretion, function of hepatocytes, biliary tract patency, extrahepatic biliary obstructive lesions, and acute or chronic cholecystitis (1,2) can be visualised using HBS.

Scintigraphic studies, which are frequently used in human medicine are rarely used in veterinary medicine in Turkey. We aimed to evaluate hepatobiliary functions in healthy dogs by using ^{99m}Tc-mebrofenin HBS, leading to the diagnosis of hepatobiliary diseases.

Materials and Methods

Animals

Twelve adult dogs of different breeds, ages (30.14 ± 2.19 months old) and weights (26.57 ± 2.65 kg) were used as the study material. Blood cell counts, urine analysis and serum biochemical analysis, including serum alkaline phosphotase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and gamma glutamyl transferase (GGT), total bilirubin and direct bilirubin concentrations, and glucose, urea and albumine measurements were performed to determine the health status of the dogs. Blood samples were measured with a haematology analyser (Abacus Jr Vet, Diatron LTD, Hungary) and the urine samples were analysed using dip sticks (Combur 10, Germany). Routine abdominal radiography and hepatic sonography were also performed. Pratic 1 (Imago Radiology, Italy) roentgen equipment and Concept MCV (Dynamic Imaging, UK) were used for the radiographic and ultrasonographic examinations, respectively. Liver ultrasonography was performed on each dog in dorsal recumbency using a 5 MHz convex probe via contact gel after shaving the relevant area.

Scintigraphic procedure

Scintigraphic procedures were performed in the Nuclear Medicine Department of the Faculty of Medicine, Adnan Menderes University. Dogs were anaesthetised by iv. administration of 1-2 mg/kg of xylazine HCl (Rompun®, Bayer) and 10 mg/kg of ketamine HCl (Ketalar®, Eczacıbaşı). The dogs were positioned in dorsal recumbency, and a dose of 200 µCi/kg of ^{99m}Tc-mebrofenin (bromotriethyl iminodiacetic acid) was injected intravenously. Dynamic acquisition was performed simultaneously using a large field of view gamma camera equipped with a low energy general purpose parallel hole collimator (Siemens e-Soft, Illinois, USA). One-hour dynamic images (60 frames of 1 s. and 59 frames of 1 min) were obtained. Two, four and twenty-four h static images were also acquired (3 h dynamic images were acquired from 2 dogs for evaluating washout of gall bladder activity). Regions of interest were drawn manually for the liver and gall bladder separately, and time-activity curves for these regions were constructed. Images were evaluated visually and quantitatively.

During the scintigraphic procedures, staff used lead aprons and gloves for protection. The radioactive material was injected using a lead shielded injector. Urine catheterisation was performed to avoid the radioactive agents being contaminated by the urine, which was collected in urine sampling bags and disposed of according to the Nuclear Medicine Department's standard techniques. Because of the short half-life of the radiopharmaceutical (less than 6 h), dogs were kept under control in an isolated room for 24 h.

Evaluation of data

HBS was evaluated for 7 variables: 1- time at which the cardiac silhouette could no longer be identified (cardiac washout); 2- identification of a photopenic area in the location of the gall bladder; 3- whether biliary ducts could be identified; 4- time at which liver radioactivity was at maximal intensity (Tmax); 5- time when radioactivity was first seen within the gall bladder; 6- time at which the liver parenchyma could no longer be identified (hepatic washout) and 7- whether radioactivity was seen within the small intestine.

Means ± SD of these values were calculated using SPSS software to derive normal values for HBS in healthy adult dogs.

Results

Means ± SD of the haematological and biochemical parameters are given in the Table. No abnormal findings were observed in haematological or biochemical parameters. Results of urinalysis also revealed normal ranges (density 1018.75 ± 4.83, pH 6.25 ± 0.45, and leucocyte, nitrite, protein, glucose, ketone, urobilinogen, bilirubin, blood and haemoglobin were all negative) in the dogs. Similary, ventro-dorsal and latero-lateral plain abdominal radiographs were normal. In ultrasonographic examinations, liver parenchyma had a uniform echotexture, and large blood vessels and the gall bladder were easily visible. No pathological findings were seen in the parenchymal and vascular structures or gall bladder.

Hepatobiliary scintigraphies were obtained for dogs that were confirmed to be healthy (Figure 1).

Scintigraphy can provide information regarding hepatocellular function, and intrahepatic and extrahepatic biliary patency. Variables for the assessment of hepatocellular function are cardiac washout time and Tmax in the liver.

Table. Biochemical and haematological parameters of the dogs.

Parameters	Mean ± SD	Reference Values	Parameters	Mean ± SD	Reference Values
ALT (IU/l)	41.6 ± 10.9	6-70	RBC (10^6/µl)	6.65 ± 1.2	5.0-8.0
AST (IU/l)	28.7 ± 9.3	10-43	MCV (fl)	66.2 ± 8.2	60-77
GGT (IU/l)	4.3 ± 2.1	1-10	Hgb (g/dl)	14.2 ± 2.2	12-18
ALP (IU/l)	46.9 ± 16.3	8-76	Hct (%)	44.2 ± 6.8	37-55
T. Bilirubin (mg/dl)	0.4 ± 0.2	0.0-0.6	Plt (10^3/µl)	226.8 ± 59.0	200-500
D. Bilirubin (mg/dl)	0.1 ± 0.1	0.0-0.1	WBC (10^3/µl)	10.1 ± 2.5	8.0-17.0
Glucose (mg/dl)	88.5 ± 16.1	60-115	LYM (10^3/µl)	2.52 ± 1.2	1.0-4.80
Urea (mg/dl)	17.0 ± 5.5	10-26	MONO (10^3/µl)	0.74 ± 0.2	0.18-1.35
Albumin (g/dl)	3.6 ± 0.5	3.1-4.5	GRA (10^3/µl)	6.84 ± 2.6	3.0-12.0

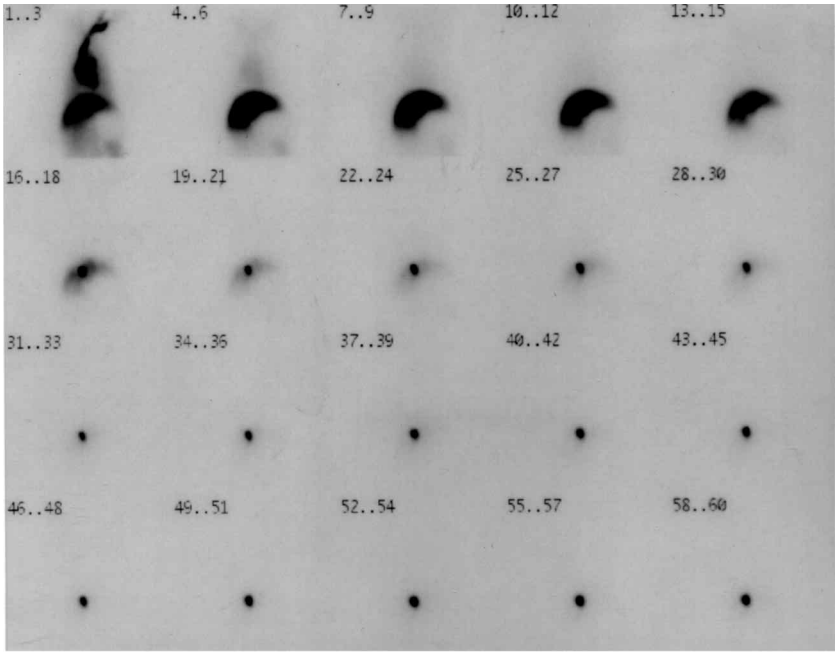

Figure 1. Dynamic pictures (0-60 min) of hepatobiliary scintigraphy in a healthy adult dog.

Cardiac washout occurred within 3 min in all dogs (Figure 2A). Mean Tmax was 7.95 ± 3.22 min.

The status of intrahepatic bile flow and presence of cholestasis were evaluated using 2 variables: 1- time at which the liver parenchyma could no longer be identified (hepatic washout) and 2- time when radioactivity was first seen within the gall bladder. In this study, initial gall bladder radioactivity was seen 13 ± 1.34 min after radiopharmaceutical injection. Mean hepatic washout time was 44 ± 3.15 min.

Several additional variables were identified. A photophenic area in the region of the gall bladder was seen in 2.86 ± 0.21 min (Figure 2B).

Small intestinal radioactivity was not detected in the first 60 min in any dogs, but it was observed within 2-4 h (Figure 2C). However, it was not detected in 24 h late static images.

Bile ducts were not identified as separate structures in any dogs.

For evaluating washout of liver and gall bladder activity, regions of interest were drawn manually for the liver and gall bladder separately and time-activity curves for these regions were constructed (Figure 3).

Discussion

Information that can be obtained from HBS can be classified in 3 categories: hepatocellular function, intrahepatic bile flow, and gall bladder status. The 2 variables that assess hepatocellular function are cardiac washout time and Tmax. Assuming normal cardiac function, the rate of hepatocellular extraction of the radiopharmaceutical from the blood is an indicator of hepatocellular function. Passage of the radiopharmaceutical into the hepatocyte is an active process via the organic anion receptors. Once the radiopharmaceutical is within the hepatocyte, an intact cell membrane is required to prevent diffusion back into the blood. Therefore diseases that slow cellular metabolism (prolong hepatocellular extraction) or damage hepatocyte cell membranes (allow reflux back into the blood pool) are characterised scintigraphically by prolonged cardiac blood pool visualisation of the subsequent delayed Tmax (5). In this study Tmax was 7.95 ± 3.22 min in healthy dogs, slower than that determined in cats (5 min) (5), faster than that determined in humans (10 min) (6), and approximately 1 min faster than that previously reported in dogs (9 min) (7). This may be attributable to the basal metabolic rate or to differences in affinity of the organic anion receptor for the radiopharmaceutical.

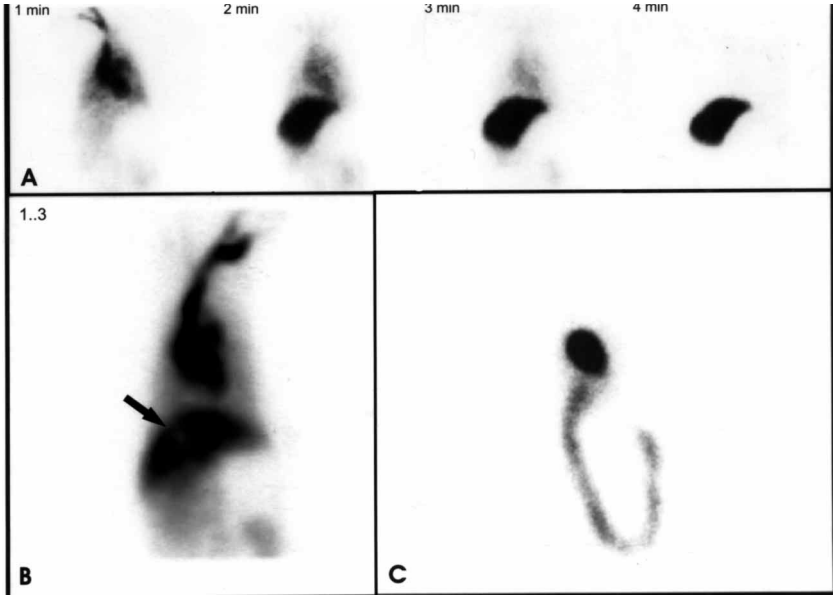

Figure 2. A. Cardiac washout identified within 3 min.
B. Photophenic area in the gall bladder region (arrow).
C. View of radioactivity in the small intestine.

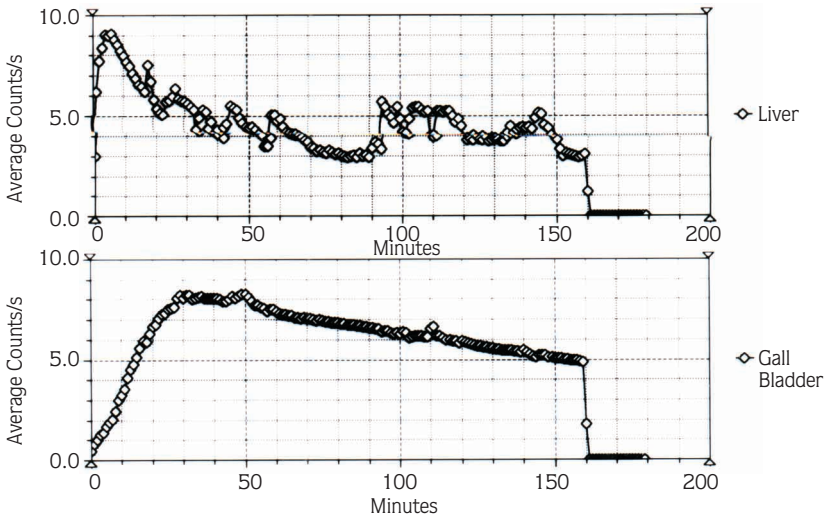

Figure 3. Time-activity curve of the liver and gall bladder.

Cardiac washout time was defined as the time when the cardiac silhouette could no longer be distinguished from the surrounding lung. Prolonged accumulation of the radiopharmaceutical in the cardiac blood pool indicates that the hepatocellular extraction process may be abnormal (5). Considering the results of this study, cardiac washout values >3 min in dogs would be suggestive of hepatocellular diseases.

After extraction by the hepatocytes, the passage of the radiopharmaceutical into the gall bladder depends on several factors, including patency of the bile ducts, tone of the sphincter of Oddi, and rate of bile flow. Inflammation, obstruction or fibrosis of the biliary tree can cause intrahepatic cholestasis with or without concurrent hepatocellular dysfunction. Intrahepatic cholestasis is characterised by a decrease in bile flow rate with variable distension of the biliary system. This is recognised scintigraphically as abnormally prolonged hepatic parencyhmal radioactivity after normal hepatic extraction. HBS is used to evaluate this phase including time of initial gall bladder activity and hepatic washout time (5). In previous studies, initial gall bladder radioactivity was reported as 20 min for humans (6), 15-20 min for dogs (7) and 4.85 min for cats (5). In this study, initial gall bladder radioactivity was seen 13 ± 1.34 min after radiopharmaceutical injection. These results were in agreement with the study in dogs, although mean hepatic washout time (44 ± 3.15 min) was slower than that reported for cats (31.63 ± 5.89 min) (5).

Specific biliary duct anatomic features, including the hepatic ducts, cystic ducts and common bile duct are commonly identified via HBS (7,8). However, no specific bile duct was determined in cats, possibly because of their small size (5). In this study, bile ducts were not visualised in any dogs. Normally, biliary ducts can be visualised in right lateral spot views. However, in this study visualisation of gall ducts could not be performed because of the dogs' dorsal recumbency.

Interestingly, a photopenic area in the gall bladder region has been described in dogs with iatrogenically induced complete bile duct obstruction (9); these investigators speculated that the photopenic area in the image represented a region of the liver with less hepatic parenchyma than other areas owing to displacement by a distended gall bladder. In our clinically normal dogs, a photopenic area in the region of the gall bladder was seen within 3.03 ± 0.21 min. Later, this region was replaced by a hot spot as radioactive bile filled the gall bladder. We assume that this photopenic region represents the normal anatomical localisation of the gall bladder.

The passage of the radiopharmaceutical into the small intestine without cholecystokinin stimulation in healthy dogs within 20-30 min after i.v. administration (1) is common, about 80% of humans undergoing HBS (8), and occurs in 50% of clinically normal, nonfed dogs (7). This was uncommon in our study. In our cases, small intestinal radioactivity was not detected in the first 60

min, although it was observed in 2-4 h. This delayed passage may be explained by spasm of the sphincter of Oddi, resulting from xylazine-ketamine anaesthesia.

Scintigraphy is a rarely used diagnostic method in veterinary medicine in Turkey. Compared with other diagnostic approaches such as radiography, ultrasonography, computerised tomography, magnetic resonance imaging and endoscopy, scintigraphy provides information about the functional status of organs, whereas the others provide information about the morphology of organs. Therefore, ^{99m}Tc-mebrofenin HBS may be considered a sensitive, specific and noninvasive diagnostic method to evaluate hepatocellulary functions, intrahepatic bile flow and gall bladder status in dogs. It is thought that the data obtained from this study may guide future research.

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