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PERCUTANEOUS ULTRASOUND-GUIDED CHOLECYSTOCENTESIS IN DOGS

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Percutaneous ultrasound-guided cholecystocentesis was performed on 13 healthy beagle dogs to determine whether percutaneous ultrasound-guided cholecystocentesis in the dog was a feasible and safe procedure. Clinical, laboratory and ultrasonographic examinations were done at 0 and 10 minutes, in the 2nd and 16th hour, and on the 7th day. They included a detailed physical examination of the mucous membranes, cardiorespiratory system and abdominal organs. Laboratory examinations of the blood consisted of a complete blood count, determination of packed cell volume (PCV), haemoglobin (Hb), total plasma protein (TPP), parameters of haemostasis including prothrombin time (PT), activated partial thromboplastin time (APTT), and enzyme activities reflecting hepatobiliary function, i.e. aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT). Ultrasonographic findings of the gallbladder (size, shape, wall, content) and appearance of the biliary tract and the surrounding cranial intraabdominal organs were also evaluated. Percutaneous ultrasound-guided cholecystocentesis was performed easily during the study, and dogs tolerated well the procedure performed without anaesthesia. All laboratory parameters of the blood remained within normal limits throughout the study. However, some follow-up values, i.e. PCV, TPP, APTT and ALT, demonstrated statistically significant differences when compared to baseline measurements, which might reflect the effect of 24-hour fasting before the experiment, as well as day-to-day metabolic fluctuations due to feeding and water supply during the study. There were no visible signs of bleeding from the liver, bile leakage from the gallbladder or accumulation of free peritoneal fluid during repeated ultrasonographic examinations. Percutaneous ultrasound-guided cholecystocentesis seems to be an important diagnostic procedure in canine gallbladder diseases and can be used safely and easily to gain gallbladder bile for diagnosis of bacterial cholecystitis or for investigating hepatobiliary function in the dog.

Key words: Ultrasound-guided cholecystocentesis, dog, gallbladder, biliary tract

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Disorders of the gallbladder and the extrahepatic biliary tract can be studied by biochemical examinations of the blood and urine, as well as by cholecystography, cholangiography and biliary scintigraphy (Fossum and Willard, 1995).

Recently, ultrasonography has been used to image the canine gallbladder providing useful information on both intra- and extrahepatic biliary disorders (Kirpensteijn et al., 1993; Diez-Bru et al., 1997; Rivers et al., 1997; Brömel et al., 1998*a*; Brömel et al., 1998*b*; Vörös et al., 2001). Ultrasonographic gallbladder volume measurements have also been described before and after the application of cholagogue substances to test biliary patency and gallbladder function in the dog (Finn et al., 1991; Sterczer et al., 1996).

In human hepatology, gallbladder puncture and percutaneous cholecystostomy have become increasingly popular means to diagnose and treat various gallbladder and bile duct disorders. In addition to procedures for drainage of acute calculous and acalculous cholecystitis and gallstone dissolution (Nagy and Péter, 1992; vanSonnenberg et al., 1992; Ishikawa et al., 1997; Patel et al., 2000; Puskás and Király, 2000), diagnostic aspiration of bile can be used for bacterial culture in inflammatory gallbladder diseases (Sugiyama et al., 1998).

Changes in the composition of bile and its relationship with the pathogenesis of gallstone formation is an interesting field of clinical research (Wildgrube et al., 1986; Kelly et al., 1993; Tudyka et al., 1994; Washizu et al., 1994; Masamune et al., 1997).

Bile from the gallbladder of human patients can be gained intraoperatively during surgical treatment of biliary disorders (Wildgrube et al., 1986; Armstrong et al., 2000) or, more conveniently, by ultrasound-guided percutaneous cholecystocentesis (Swobodnik et al., 1991; vanSonnenberg et al., 1992; Tudyka et al., 1995). The latter method has been described in the owl monkey (Pekow et al., 1994), in swine (Klapdor et al., 1997; Kessler et al., 2001) as well as in cattle (Braun and Gerber, 1992; Braun et al., 1995). In the dog, bile from the gallbladder was traditionally gained after surgical cannulation of the duodenum (Wildgrube et al., 1986), the common biliary duct (Abedin et al., 1989) or the gallbladder (Kelly et al., 1993), or by cholecystocentesis during laparotomy (Washizu et al., 1990; Masamune et al., 1997).

A brief description of ultrasonographically-guided percutaneous cholecystocentesis was first given by Hogan et al. (1976). This method has recently been applied as a diagnostic tool in canine acalculous cholecystitis and to demonstrate bacterbilia (Rivers et al., 1997). A preliminary study on 6 dogs and 7 pigs was reported by McGahan et al. (1983), who described the technique of sonographically guided cholecystostomy, but did not study the potential risk of this method. Otto et al. (1984) studied the risk of ultrasound-guided fine-needle puncture of various abdominal organs including the gallbladder in dogs. They performed multiple puncture and bile sampling of the gallbladder on anaesthetised dogs and controlled the site of intervention by laparotomy and necropsy

some days later. They results demonstrated in two of six dogs that multiple puncture of the gallbladder might sometimes lead to bile leakage and localised bile peritonitis.

The main goal of the study reported here was to determine whether percutaneous ultrasound-guided cholecystocentesis in the dog was a feasible and safe procedure. Therefore, bile from 13 healthy beagle dogs was gained using this method and followed by clinical, laboratory and ultrasonographic examinations for one week. To our knowledge, no similar investigations have been published previously.

Materials and methods

Thirteen 1-year-old healthy beagle dogs (8 males and 5 females) weighing between 11.6 and 15.5 kg were used in the study. Dogs were kept in kennels and fed on Purina Dog Chow diet and they were fasted for 24 h before commencement of the baseline examinations. Maintenance and care of animals were in accordance with the requirements of the National Institute of Health Guidelines for the Use of Laboratory Animals (USA), and the experiments were performed in compliance with the regulations of the Supervising Committee acting in the Faculty of Veterinary Science, Szent István University, Budapest.

Ultrasonographic examinations were done with a Brüel and Kjaer Panther 2002 ultrasound system, using a 5.0 MHz real-time convex array transducer (Brüel and Kjaer, Naerum, Denmark). Ultrasonographic findings of the gallbladder (size, shape, wall, content) and appearance of the biliary tract were evaluated as described previously (Vörös et al., 2001). Surrounding cranial intraabdominal organs were also examined with the same transducer, and care was taken to detect any free intraabdominal fluid scanning between the diaphragm and ventrally from the urinary bladder when positioning the dogs in dorsal recumbency.

Percutaneous ultrasound-guided cholecystocentesis was performed on dogs in left lateral recumbency using a ventral transabdominal and transhepatic approach of the gallbladder (Swobodnik et al., 1991; Tudyka et al., 1995; Rivers et al., 1997). In the midline of the epigastrium, the skin was prepared aseptically and a 22-gauge (0.7×30 mm) subcutaneous needle with a 5-ml syringe was introduced into the gallbladder under ultrasound guidance, using the disinfected transducer and applying the 'free-hand' technique. Neither sedation nor local anaesthesia was necessary, as the dogs demonstrated only minimal discomfort during physical restraint, similar to an intramuscular injection. The needle was directed through the liver parenchyma towards the gallbladder (Fig. 1) in order to tamponade the puncture site on the gallbladder after withdrawal of the needle. Ultrasound images were recorded on a Panasonic NV-SD3EE VHS video recorder for documentation and further visual analysis.

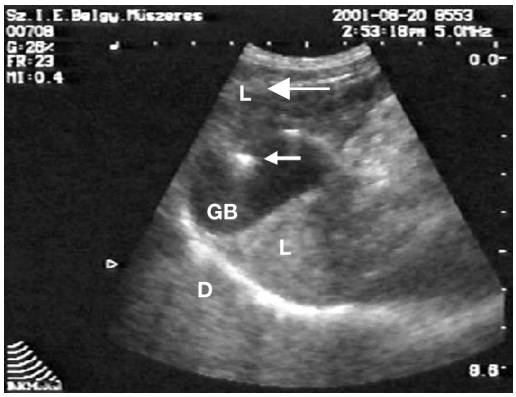

Fig. 1. Sagittal ultrasonographic image of percutaneous transhepatic cholecystocentesis in a healthy dog. D: diaphragm, L: liver, GB: gallbladder. The arrow points to the needle within the gallbladder

Baseline examinations included a detailed physical examination with special regard to the mucous membranes, cardiorespiratory system and abdominal organs. Laboratory examinations of the blood consisted of a complete blood count as well as of determination of the parameters of haemostasis and enzyme activities reflecting hepatobiliary function. Clinical, laboratory and ultrasonographic examinations performed during the study are listed in Table 1. Blood laboratory measurements were evaluated statistically (mean, standard error of the mean, and level of significance compared to basic values) using the MS Excel 97 programme. In addition, serum bile acids and bile acid profile of the bile gained from the gallbladder were also analysed. These latter results will be published elsewhere.

Results

Percutaneous ultrasound-guided cholecystocentesis was performed easily during the study. Dogs tolerated well this procedure and 5.5 to 6.0 ml bile was gained usually during the first attempt (i.e. the used 5.0-ml syringe could be filled with bile up to its maximal volume). A second intervention was necessary only in two dogs, mainly because the length of the applied needle proved to be somewhat short to reach the gallbladder. These two dogs weighed more than 14.0 kg. In one dog, bile was mixed with blood and was unusable for biochemistry. Neither this nor any other dog demonstrated any abnormalities during follow-up clinical examinations, and all showed good general status (behaviour, movement, appetite) during the follow-up period.

Blood count (not listed separately) and blood laboratory parameters shown in Table 2 remained within normal limits throughout the study. However, some follow-up values, such as PCV, TPP, APTT and ALT, demonstrated statistically significant differences ($p < 0.05$) when compared to baseline measurements.

Table 1: List of examinations

Examination	Time of examination				
	0 min	10th min	2nd hour	16th hour	7th day
Clinical examination	P, R, muc. mb., resp., circ., abd.	P, R, muc. mb., resp., circ., abd.	P, R, muc. mb., resp., circ., abd.	P, R, muc. mb., resp., circ., abd.	P, R, muc. mb., resp., circ., abd.
Blood parameters	total blood count, PCV, Hb, AST, ALT, GGT, TPP, APTT, PT	total blood count, PCV, Hb, AST, ALT, GGT, TPP, APTT, PT	total blood count, PCV, Hb, AST, ALT, GGT, TPP, APTT, PT	total blood count, PCV, Hb, AST, ALT, GGT, TPP	total blood count, PCV, Hb, AST, ALT, GGT, TPP
Ultrasound examination	abdomen, liver, gallbladder	abdomen, liver, gallbladder	abdomen, liver, gallbladder	abdomen, liver, gallbladder	ND

P: pulse rate (/min.); R: respiratory rate (/min.); muc. mb.: mucous membranes; resp.: respiratory system; circ.: circulatory system; abd.: abdominal organs; total blood count: quantitative and qualitative examination of the RBC and WBC, number of thrombocytes; PCV: packed cell volume (haematocrit); Hb: haemoglobin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase; TPP: total plasma protein; APTT: activated partial thromboplastin time; PT: prothrombin time; ND: not done

Table 2: Results of laboratory examinations

Parameter	Normal limits	Sampling times				
		0 min	10th min	2nd hour	16th hour	7th day
		Mean ± SD	Mean ± SD (Sign.*)	Mean ± SD (Sign.*)	Mean ± SD (Sign.*)	Mean ± SD (Sign.*)
PCV (litre/litre)	35–55	50 ± 9.8	49 ± 6.6 (0.54)	43 ± 5.6 (0.03*)	42 ± 2.7 (0.001*)	46 ± 3.6 (0.13)
Hb (g/litre)	120–180	154 ± 20.6	154 ± 14.8 (0.77)	149 ± 12.6 (0.43)	158 ± 11.3 (0.97)	158 ± 7.8 (0.30)
TPP (g/litre)	55–75	69.3 ± 7.3	68.2 ± 6.1 (0.63)	65.6 ± 5.6 (0.03*)	65.2 ± 4.4 (0.06)	56.8 ± 3.0 (0.0001*)
APTT (sec)	15–45	18.7 ± 8.3	19.2 ± 6.3 (0.84)	33.0 ± 8.9 (0.001*)	–	–
PT (sec)	< 15	7.3 ± 0.7	7.1 ± 0.5 (0.56)	7.5 ± 0.7 (0.30)	–	–
AST (NE/litre)	< 40	40 ± 17.4	39 ± 16.0 (0.83)	46 ± 12.2 (0.40)	36 ± 15.0 (0.66)	44 ± 7.8 (0.41)
ALT (NE/litre)	< 55	43 ± 18.2	43 ± 21.0 (0.97)	53 ± 21.8 (0.24)	56 ± 21.3 (0.054)	54 ± 16.9 (0.1)
GGT (NE/litre)	< 10	9.3 ± 10.3	9.7 ± 12.1 (0.94)	8.3 ± 7.0 (0.78)	8.1 ± 7.7 (0.46)	4.7 ± 1.9 (0.15)

*Statistically significant differences compared to baseline (0 min) values. Abbreviations are explained in the footnote to Table 1

There were no visible signs of bleeding from the liver, bile leakage from the gallbladder or accumulation of free peritoneal fluid during repeated ultrasonographic examinations.

Discussion

Percutaneous ultrasound-guided cholecystocentesis was easily performed by the described technique. Although no chemical restraint and analgesia were necessary in our dogs, mild sedation and/or local anaesthesia might be necessary in some clinical patients. The advantage of the thin 22-gauge needle used in the present study is the minimal physical damage and elicited pain. In larger dogs, however, longer but thin fine needles might be necessary. Rivers et al. (1997) applied the same technique also using a 22-gauge needle but with a length of 3.5-in (9-cm) long spinal needle. This latter needle might be long enough for larger canine breeds, as well. We did not use any mandrin when penetrating the abdominal wall, liver and gallbladder, but this might be also applied as described earlier (Swobodnik et al., 1991; Pekow et al., 1994). Nevertheless, a relatively long and thin needle can be more flexible, and proper guidance of the needle might be somewhat complicated.

Neither clinical nor laboratory pathological alterations were observed during our experiment within the one-week-long follow-up period, and no clinical abnormalities were reported when the responsible veterinarian was asked one month later. During the first week, all dogs were alert, showed no discomfort, and blood laboratory parameters remained within normal limits during the whole study. Significant differences between some baseline and follow-up data of PCV, total plasma protein, APTT and ALT might reflect the effect of 24-h fasting before the experiment, as well as day-to-day metabolic fluctuations due to feeding and water supply during the study.

These clinico-laboratory investigations demonstrate the feasibility and safety of percutaneous ultrasound-guided cholecystocentesis with the technique reported here, and are supported by the follow-up ultrasound examinations made between 0 and 16 hours with no signs of hepatobiliary complications.

Our results confirm the advantage of the transhepatic approach in comparison to direct puncture of the gallbladder through the abdominal wall in accordance with the opinion of others (Kiss et al., 1987; vanSonnenberg et al., 1992; Pekow et al., 1994; Tudyka et al., 1995). Otto et al. (1984) reported bile leakage and local peritonitis in 2 of their 8 experimental dogs after direct, multiple gallbladder puncture with a 0.68 × 200 mm needle. We conclude that a single transhepatic puncture with a fine 22-gauge needle can be performed in most of the cases without harmful risks. Rivers et al. (1997) reported no complications of their ultrasound-guided cholecystocentesis on 4 dogs with cholecystitis, but they did not aim at any follow-up examinations regarding the technique.

Even ultrasound-guided cholecystostomy for drainage is considered as a useful therapeutic intervention especially in impaired patients with acalculous cholecystitis or as a palliative method preceding surgery in acalculous cholecystitis (Kiss et al., 1987; Sugiyama et al., 1998; Puskás and Király, 2000; Patel et al., 2000).

Potential risks of percutaneous cholecystocentesis and especially those of cholecystostomy are rare but can occur. These rarely include bile leakage with bile peritonitis, significant bleeding from the liver and acute vagal reaction with severe hypotension and bradycardia (vanSonneberg et al., 1992). However, some other authors did not find any significant complications of fine-needle puncture even studying a large number of human patients (Swobodnik et al., 1991; Tudyka et al., 1995; Puskás and Király, 2000).

An interesting and useful application of the technique described in this study can be the investigation of bile metabolism in the dog. Previously applied invasive cannulation methods of the duodenum (Wildgrube et al., 1986), the common biliary duct (Abedin et al., 1989), or the gallbladder (Kelly et al., 1993) are not only complicated but can influence hepatobiliary function as well.

In conclusion, percutaneous ultrasound-guided cholecystocentesis seems to be an important diagnostic procedure in canine gallbladder diseases and can be used safely and easily to gain gallbladder bile for investigating hepatobiliary function in the dog.

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