Studies on transcatheter arterial embolization in dogs

（犬の肝動脈塞栓術に関する研究）

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CONTENTS

General introduction

Hepatocellular carcinoma (HCC) has become one of the most common types of liver cancer in both humans and dog[1, 2]. To provide context, the liver blood supply is provided by two vessels, the hepatic artery (approximately 20%) and portal vein (approximately 80%)[3]; however, HCC is mainly perfused by the hepatic artery[4]. Therefore, embolization of this tumor-supplying artery would decrease arterial perfusion of the tumor, resulting in selective ischemia and tumor size reduction[1]. Previous studies in humans have demonstrated improvements in survival rates, pain, and local control following arterial embolization for unresectable HCC[1, 5]. In contrast, the intravenous or single-arterial administration of anticancer agents has not been shown to be effective for treating inoperative HCC in humans[6-8].

Currently, transcatheter arterial embolization (TAE), either with additional chemotherapy (TACE) or without, is considered a standard treatment for advanced HCC in humans[1, 6]. TAE or TACE may also be offered to patients with unresectable primary liver tumors or liver metastases in humans[5]. Many articles have discussed the effects of TACE in humans, and the effects of TACE have been found to derive mainly from embolization of arteries that supply the tumor[6].

Although liver resection is considered most effective for dogs with primary or secondary

hepatic tumors[9], such dogs often present with inoperative liver tumors or a poor clinical condition that contraindicates surgical treatment. Therefore, a minimally invasive treatment for inoperative canine liver tumors would be highly desirable. Although TAE or TACE was recently introduced for the veterinary treatment of liver tumors[10], little information is available on the effects of arterial embolization in the canine liver. Previous experimental canine studies[11, 12] have reported fatalities caused by severe liver necrosis after hepatic arterial embolization. We hypothesized that in contrast, selective embolization using currently available advanced devices would reduce the risk of liver damage.

TAE must be practiced by well-trained veterinarians, and knowledge of hepatic vascular anatomy[13-16] is crucial for both a diagnosis of HCC and determination of the approach to the target artery. In particular, an understanding of hepatic arterial anatomy is most important with respect to the target artery approach[17, 18]. However, in dogs, little information regarding hepatic vascular anatomy is available for interventional radiology in the canine liver. Therefore, in chapter 1, our study aimed to define anatomical variations of the hepatic artery, the portal vein, and the hepatic vein based on integrated 3D images in live, normal dogs.

In chapter 2, we investigated the clinical sings, biochemical data, histological findings,

and frequency of artery recanalization in normal canine livers following selective arterial embolization with gelatin sponge particles (GSPs). On the other hand, in chapter 3, microspheres (MSs) were used as permanent embolic agents for selective TAE in normal canine liver.

In chapter 4, we investigated the practical effects of selective embolization of the tumor-suppling arteries using GSPs in four dogs with HCC.

CHAPTER 1

Anatomical evaluation of hepatic vascular system in healthy beagles using X-ray contrast computed tomography

1-1 Background and objectives

Recently, diagnostic precision has been improving in veterinary medicine due to advanced equipment and techniques. One of the major diagnostic modalities is computed tomography (CT). It is noninvasive, fast, and provides much information for the clinician, including information on the hepatic vessels[13, 19]. In recent years, three-dimensional (3D) CT has assisted preoperative surgical planning, and has contributed to evaluation of the morphology of the liver, hepatic vessels, and tumors in hepatic surgery[20].

There are many reports about the hepatic vascular system in humans and its acceptance in clinical medicine is high[21-23]. However, in dogs, little information regarding hepatic vascular anatomy is available for hepatic surgery. Therefore, knowledge of the details of the hepatic vascular system is highly desirable for development of veterinary clinical medicine. The present study aimed to define anatomical variations of the hepatic artery, portal vein, and hepatic vein based on integrated 3D images in live, normal dogs.

1-2 Materials and methods

1-2-1 *Animals*

Thirty-two beagles, ranging from 2 to 12 years old, weighing 10.78±1.50 kg (mean± standard deviation (SD)), genetic relationship unknown, and without clinical signs relating to liver disease were entered into the study. Dogs meeting this criteria with liver parenchymal diseases have previously been evaluated with CT. The liver volume in the present study was 336.3±46.7 mL (31.4±4.4 mL/kg) and there were no anatomical abnormalities in liver parenchyma. The beagles were caged with free access to water, and food was withheld for 12 hours before the dogs were anesthetized.

1-2-2 *Abdominal CT*

All dogs have anesthesia induced by slow intravenous administration of propofol (7 mg/kg, intravenously) to effect. A cuffed endotracheal tube was placed in the trachea and the dogs were connected to a partial rebreathing cycle system delivering isoflurane (1.0-2.0%) and oxygen. Anesthetic depth was monitored using clinical signs, and dogs were ventilated to maintain eucapnia.

Contrast-enhanced multi-detector computed tomography (MDCT) examinations were carried out using an eight-detector-row computed tomography system (ECLOS 8; Hitachi

Medical Corporation, Tokyo, Japan). Scans were obtained with a collimation of 8 × 1.25 mm, table pitch of 0.875, tube voltage of 120 kV, and tube current of 350 mA. All dogs were placed in ventral recumbency. All CT studies were performed during apnea under anesthesia. All images were acquired using a soft tissue reconstruction algorithm.

The CT angiographic study was performed in four steps. In the first step, a survey helical CT scan of the abdomen was performed from most cranial aspect of the diaphragm to the cranial aspect of the pelvis, and then imaging of the liver was performed from the most cranial aspect of the liver to the middle position of the right kidney. In the second step, dynamic CT was performed with MDCT. Dogs received 2 mL/kg of iopamidol with an iodine concentration of 370 mg/mL (Oiparomin 370; Fuji Pharmaceutical Company, Toyama, Japan) as intravenous contrast for vascular imaging. The contrast medium was injected with an automatic power injector (A-60, Nemoto Kyorindo co., Ltd., Tokyo, Japan, at a speed of 1-2 mL/s), and arterial phase imaging was started 8-20 s after the start of the injection of the contrast medium. The scan parameters were identical to the survey helical scan. The start time of the arterial phase CT scanning depended on patient's heart rate, and CT scanning time was common to all phase, ranging from 10 to 15 s. In the third step, portal phase imaging was started 45-60 s after the start of the injection. In the fourth step, equilibrium phase imaging was started 150 s after the start of the injection.

1-2-3 *Vascular analysis*

3D reconstruction of the hepatic vasculature was made using volume measurement software (Ziostation2; Amin, Tokyo, Japan). The resulting 3D images of the hepatic artery, the portal vein, and the hepatic vein were carefully reviewed and compared with the axial source images to ensure that no important structures were inadvertently deleted from the 3D images. The 3D images of the hepatic artery, the portal vein, and the hepatic vein were adjusted to the position to each other by focusing at the hepatic hilum. Axial, sagittal, and coronal images were further integrated into a single image.

1-3 Results

1-3-1 *Liver segments*

The present study visualized small vessels in the livers of 32 dogs using triple phase helical CT images. Contrast medium was used to distinguish the hepatic artery, portal vein, and hepatic vein of the following liver segments: the papillary process of the caudate lobe, left lateral lobe, left medial lobe, quadrate lobe, right medial lobe, right lateral lobe, and the caudate process of the caudate lobe. The paths of the hepatic artery, portal vein, and hepatic vein were visualized in each lobe, and the results are discussed below (Figures 1-4).

1-3-2 *Hepatic vascular system in papillary process (PP) of the caudate lobe*

Hepatic artery: In four of the 32 dogs (13%), one main artery was confirmed and the artery arose proximally from the left hepatic artery.

Portal vein: In all dogs one main portal vein was confirmed. It arose from the portal vein and entered PP at the level of the bifurcation of the right medial portal vein, running parallel and dorsal to the papillary lobar hepatic vein.

Hepatic vein: All dogs had one hepatic vein that originated from the bifurcation of the right medial lobar and quadrate lobar portal vein. The papillary lobar hepatic vein was

dorsal to the portal vein of the PP.

1-3-3 *Hepatic vascular system in left lateral lobe (LLL)*

Hepatic artery: In all dogs the hepatic artery was confirmed. Most dogs had two main arteries supplying the LLL, and each of the arteries branched to the cranial and caudal directions.

Portal vein: All dogs had two main portal vein branches supplying the cranial and caudal LLL. Each portal vein entered the LLL at the bifurcation of the quadrate and left medial lobar portal vein.

Hepatic vein: All dogs had two main hepatic veins that branched from the left hepatic vein to the cranial and caudal LLL as the portal vein. The hepatic vein was ventral and parallel to the portal vein of the LLL in all dogs.

1-3-4 *Hepatic vascular system in left medial lobe (LML)*

Hepatic artery: In all dogs the hepatic artery was confirmed. Twenty nine dogs (91%) had an artery arising from the left hepatic artery. The other dogs (9%) had an artery arising from the right medial lobar artery.

Portal vein: All dogs had one main branch supplying the LML. The main branch

originated from the left portal vein and was parallel to the right medial lobar hepatic vein.

Hepatic vein: All dogs had one main branch originating from the left hepatic vein. The hepatic vein of the LML was a single vessel, while the portal vein of the LML gave off a branch to the quadrate lobe.

1-3-5 *Hepatic vascular system in quadrate lobe (QL)*

Hepatic artery: In 25 of the 32 dogs (78%) the hepatic artery of the QL was confirmed. Fifteen dogs (47%) had a hepatic artery arising from the right medial lobar hepatic artery. Ten dogs (31%) had a hepatic artery arising from the left hepatic artery. The hepatic artery was immediately adjacent and to the right of the portal vein of the QL.

Portal vein: All dogs had one main branch supplying the QL. The main branch had a common bifurcation of the left medial lobar portal vein and originated from the left portal vein.

Hepatic vein: In all dogs the QL and right medial lobe had a common branch. The common branch originated from left hepatic vein in close proximity to vena cava. In the bed of the gallbladder, the quadrate lobar hepatic vein arose from the common branch to right division.

1-3-6 *Hepatic vascular system in right medial lobe (RML)*

Hepatic artery: In all dogs the hepatic artery was confirmed and arose from the proper hepatic artery after the gastroduodenal artery.

Portal vein: All dogs' RML were supplied by one portal vein which was located to the right of the gallbladder and originated from the left portal vein at the bifurcation of the papillary division.

Hepatic vein: In all dogs two hepatic veins were confirmed. One hepatic vein directly branched from the vena cava and was found to the right of the portal vein. Another hepatic vein originated from the common hepatic vein with the QL.

1-3-7 *Hepatic vascular system in right lateral lobe (RLL)*

Hepatic artery: In 30 of the 32 dogs (94%) the right lateral lobar hepatic artery was confirmed. Twenty six dogs (81%) had one artery arising from a common hepatic artery before the gastroduodenal artery. Four of those 26 dogs had another artery arising from the right medial hepatic artery and one of those 26 dogs had another artery arising from gastroduodenal artery. Three dogs (9%) had an artery arising from the right medial hepatic artery. One dog (3%) had an artery arising from the gastroduodenal artery.

Portal vein: In all dogs one portal vein was confirmed. Some dogs had one to three main

branches supplying the RLL. The main branch originated from a main portal vein after the gastroduodenal vein entered a main portal vein, and shared the foundation with the caudate lobe portal vein.

Hepatic vein: In all dogs the hepatic vein was confirmed. The hepatic veins directly branched from caudal vena cava. All branches were dorsal to the lobar portal vein, and located deep within the hepatic parenchyma. In some dogs, an additional smaller vessel of the RLL entered into the caudate lobe.

1-3-8 *Hepatic vascular system in caudate process of the caudate lobe (CP)*

Hepatic artery: In 21 of the 32 dogs (66%) the hepatic artery was confirmed. The hepatic artery branched from the right lateral lobar hepatic artery and was caudal to the lobar portal vein.

Portal vein: In all dogs the portal vein was common with the bifurcation of the right lateral lobar portal vein. The portal vein of CP branched from the right lateral lobe portal vein to the caudal direction.

Hepatic vein: In all dogs the hepatic vein was confirmed and directly branched from the vena cava. Most dogs had one main caudate lobar hepatic vein with one to three accessory branches. All vessels were caudal and slightly dorsal to the portal vein of the CP.

1-4 Discussion

1-4-1 *Running patterns of the hepatic vessels*

In the veterinary field, early detection and diagnosis has become possible due to advanced equipment including CT scanners. However, the available literature on normal blood supply of the hepatic lobes in the dog is scarce, and to some extent is also conflicting[15, 17]. Therefore, the present study tried to define the running patterns of the hepatic vascular system in healthy beagles. Hepatic arteries, portal veins and hepatic veins in live dogs were successfully visualized by using 3D-CT and the hepatic vessels were identified in detail. In the present study, some running patterns of the hepatic arteries and the vascular anatomy of the portal vein and hepatic vein were found to be consistent between normal beagle dogs.

1-4-2 *Characteristic of canine hepatic vascular system*

According to Ursic et al.[17], the branching pattern of the portal vein was found to be similar in all examined dogs. This is in agreement with the present results which found that there were no significant differences between running patterns. For the hepatic artery, they showed that the right lateral, right medial, and left branches were actually major arteries which supply the liver. However, their origin, course, and ramification patterns

differed among individual livers. This was especially true for the right medial branches. In their study[17], there was no description about the running pattern of the hepatic vein. In contrast to their report[17], in the present study, the right medial hepatic artery was not significantly different between the 32 beagles. There were two patterns of origin of the quadrate lobar artery: arising from the left hepatic artery and right medial hepatic artery. The right lateral hepatic artery arose from a common hepatic artery, the right medial hepatic artery, or the gastroduodenal artery. In addition, the hepatic artery usually adjoined the portal vein. Occasionally, a part of the hepatic artery twisted around the portal vein. These findings may prove to be useful for interventional radiologists. The origin, course, and number of the hepatic artery in the present study were different from Ursic's report[17]. According to Hall et al.[24], there was a broadly consistent pattern in the hepatic and portal supply to the individual liver lobes. The branching pattern of the major portal vein and hepatic vein in their study[24] agrees to a certain extent with the present study; however, in their study, there was no description about the hepatic artery.

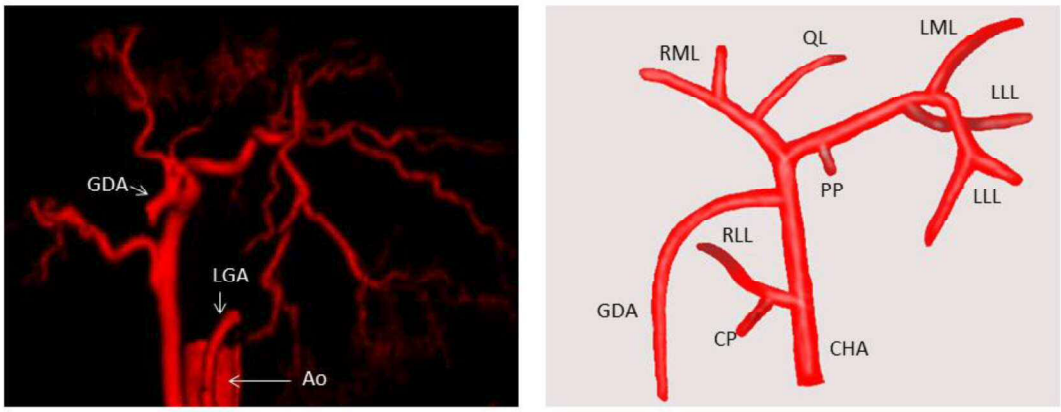
1-4-3 *Evaluation of canine hepatic vasculature using contrast CT*

These two previous reports[17, 24] using corrosion casting technique lacked visualization of the hepatic vein or hepatic artery. However, triple phase CT can visualize the entire

hepatic vascular system at one time. Unfortunately, the present report failed to identify some vascular branches such as the arteries of PP, CP and QL in several dogs. Although previous reports[17, 24] showed no minimum diameter of hepatic vessels observed in corrosion casting, the diameter of the vessels that could not be identified in the present study might be smaller than 1 mm. Recently, CT with thinner slices and multi detector CT have become available, and these modalities may achieve better spatial resolution to visualize small vessels. The present study used CT scanning to investigate hepatic vascular anatomy in live dogs, whereas previous reports[14, 17, 24, 25] used the corrosion casting technique to confirm hepatic vascular anatomy. Corrosion casting can visualize tiny vessels better than CT. However, the diameter and position of hepatic vessels may differ in live dogs. In clinical practice, it is important to obtain biological information while the individual dog is alive. Therefore, the present report tried to define hepatic vessels using CT in normal, living dogs. As a result, it was possible to visualize small vessels better using CT imaging than with the corrosion casting technique[14, 17, 24]. Interestingly, it was found that the hepatic artery differed between each individual dog (Table 1) whereas the portal vein and hepatic veins were consistent. Therefore, the anatomy of the hepatic artery of each individual dog should be confirmed in situations involving interventional radiology.

1-4-4 *Conclusion*

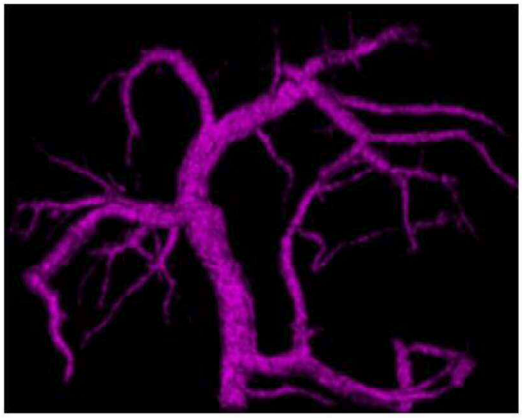
In conclusion, consistencies in vascular anatomy were found in the livers of 32 beagles. This information could be useful for diagnosis of liver failure and in creating a standardized description of the approaches to liver lobectomy in the dog. In addition, the hepatic vascular anatomy described in the present report provides the surgeon with an anatomic guideline to consult when planning and performing liver lobe resection and interventional radiology in the dog.



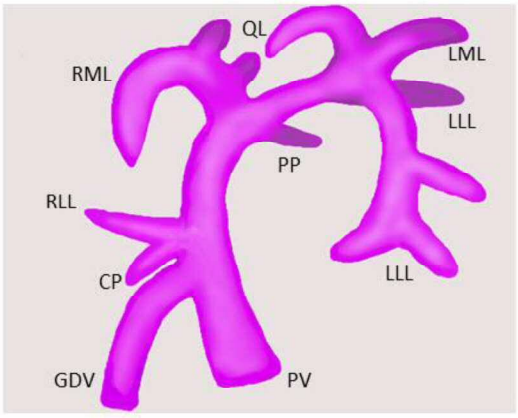
1A 1B

Figure 1. (1A) Ventral-dorsal arterial phase of a normal dog. The abdominal artery, left gastric artery, and gastroduodenal artery were removed from the images for clarity. (1B) Schema of the hepatic artery. Shown is a main branch of the artery to each lobe.

Ao, aorta; LGA, left gastric artery; GDA, gastroduodenal artery; CHA, common hepatic artery; PP, papillary process of the caudate lobe; LLL, left lateral lobe; LML, left medial lobe; QL, quadrate lobe; RML, right medial lobe; RLL, right lateral lobe; CP, caudate process of the caudate lobe



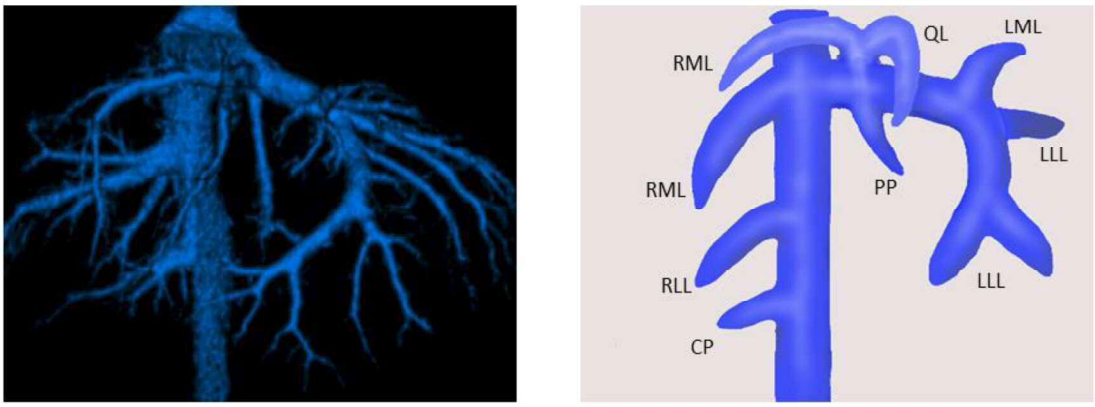
2A



2B

Figure 2. (2A) Ventral-dorsal portal phase of normal dog. (2B) Schema of portal vein. A main branch of the portal vein to each lobe is shown.

PV, portal vein; GDV, gastroduodenal vein; PP, papillary process of the caudate lobe; LLL, left lateral lobe; LML, left medial lobe; QL, quadrate lobe; RML, right medial lobe; RLL, right lateral lobe; CP, caudate process of the caudate lobe



3A 3B

Figure 3. (3A) Ventral-dorsal hepatic vein of a normal dog. (3B) Schema of the hepatic vein, with a main branch of the hepatic vein to each lobe shown.

PP, papillary process of the caudate lobe; LLL, left lateral lobe; LML, left medial lobe; QL, quadrate lobe; RML, right medial lobe; RLL, right lateral lobe; CP, caudate process of the caudate lobe

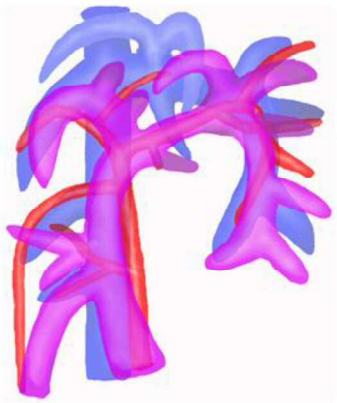


Figure 4. Integrated image of the ventral-dorsal hepatic artery, portal vein, and hepatic vein.

Table 1: Origination of vessels of the hepatic artery for each lobe

Liver lobe	Number of cases	Originated vessels
PP	4/32 (12.5%)	Left hepatic artery
	28/32 (87.5%)	Unconfirmed
LLL	32/32 (100%)	Left hepatic artery
LML	29/32 (90.6%)	Left hepatic artery
	3/32 (9.4%)	RMLa
QL	15/32 (46.9%)	RMLa
	10/32 (31.2%)	Left hepatic artery
	7/32 (21.9%)	Unconfirmed
RML	32/32 (100%)	A main hepatic artery
RLL	26/32 (81.2%)	Only CHA (21), CHA + RMLa(4),CHA + GDA(1)
	3/32 (9.4%)	RMLa
	1/32 (3.1%)	GDA
	2/32 (6.3%)	Unconfirmed
CP	21/32 (65.6%)	RLLa
	11/32 (34.4%)	Unconfirmed

Origination of vessels of the hepatic artery in each lobe. Individual differences of the QL and RLL were especially large. In addition, some dogs had two arteries originating into the RLL.

PP, papillary process of the caudate lobe; LLL, left lateral lobe; LML, left medial lobe; QL, quadrate lobe; RML, right medial lobe; RLL, right lateral lobe; CP, caudate process of the caudate lobe; RMLa, right medial lobar artery; CHA, common hepatic artery; GDA, gastroduodenal artery; RLLa, right lateral lobar artery

CHAPTER 2

Transcatheter arterial embolization in normal canine liver

2-1 Background and objectives

Hepatocellular carcinoma (HCC) is one of the most common types of liver cancer in man and dog. The blood supply of the liver normally relies on the hepatic artery (approximately 20%) and portal vein (approximately 80%)[3]. HCCs are primarily perfused by the hepatic artery[4], providing a rationale to consider embolization of this artery as a strategy for selective ischemia and tumor size reduction[1]. Previous studies in human patients documented improvements in survival rates, pain, and local control after arterial embolization of unresectable HCC[1, 5]. In contrast, intravenous or single-arterial administration of anticancer agents has not been proven effective for intraoperative treatment of HCC in humans[6-8]. Transcatheter arterial embolization (TAE), with or without adjunct chemotherapy (TACE), is a standard treatment for advanced HCC in man, such as those with unresectable tumors or liver metastases[1, 6].

Although liver resection is considered most effective for dogs with primary or secondary hepatic tumors[9]. However, this option is not for dogs with unresectable neoplasms or

medical contraindications to surgery, justifying the development of minimally invasive therapy. Anecdotal reports of TAE are available in the veterinary literature[10], but little the effects of arterial embolization on the canine liver remain poorly documented. Experimental hepatic arterial embolization in dogs[11, 12] has led to fatalities due to severe liver necrosis. In contrast to these reports, we hypothesized that selective embolization would be possible with advanced technology and would reduce the risk of liver damage. Therefore, the objectives of this study were to determine the outcomes of selective arterial embolization with gelatin sponge particles (GSPs), in terms of clinical signs, biochemical data, computed tomographic and histological findings.

2-2 Materials and methods

2-2-1 *Animals*

Five healthy adult Beagles were enrolled in this study. Physical examination, hematology and routine biochemistry were within normal limits in each of these dogs. This study was approved by our institutional ethics committee. Dogs were housed in cages with free access to water and food was withheld for 12 h before anesthesia.

2-2-2 *Transcatheter Arterial Embolization*

Anesthesia was induced via slow intravenous administration of propofol (1% intravenous propofol, 7 mg/kg; Ishimal Pharmaceutical Co., Ltd., Japan) and maintained with isoflurane (Isoflu, 1.4%–2.5%; Dainippon Sumitomo Pharma Co., Ltd., Tokyo, Japan) and oxygen. All dogs were administered antibiotic (cefazolin sodium; 25 mg/kg intravenously) and analgesic (buprenorphine; 20 μg/kg intramuscularly) after induction.

The right femoral artery was punctured with a 20-G needle and cannulated with a 4-French (Fr) introducer sheath (Radifocus introducer sheath; Terumo Co., Ltd., Tokyo, Japan)[26]. A guidewire (Radifocus guidewire M, diameter: 0.89 mm, angled, 80 cm; Terumo Co., Ltd., Tokyo, Japan) and catheter (PA catheter, 4 Fr, 40 cm; Terumo Clinical Supply Co., Ltd., Gifu, Japan) were inserted into the aorta and celiac artery under

fluoroscopic guidance (ARCADIS Varic; Siemens Healthcare Japan, Tokyo, Japan). A microguidewire (Radifocus guidewire, diameter: 0.41 mm, angled, 150 cm; Terumo Clinical Supply Co., Ltd.) was advanced into the common hepatic artery through the catheter. A 2.1-Fr microcatheter (Sniper 2, 110 cm; Terumo Clinical Supply Co., Ltd.) was placed in the left hepatic artery prior to injection of contrast agent (Optiray 350; Covidien Co., Ltd., Tokyo, Japan) under digital subtraction angiography (DSA). Embolization was achieved with 80 mg of porous gelatin particles (Gelpart 1 mm; Nippon Kayaku Co., Ltd., Tokyo, Japan) mixed with 10 mL of contrast medium. In all dogs, GSPs were injected until an overflow of contrast medium was observed with DSA. Post-embolization arteriograms were obtained to confirm complete occlusion of the left hepatic artery (Figure 1). After removing the sheath, the puncture site was manually compressed. 25 mg/kg cefazolin sodium (intravenously) and 20 μg/kg buprenorphine (intramuscularly) were administered after treatment. All dogs were monitored for 12 weeks after TAE.

2-2-3 *Evaluations*

After embolization, a common physical examination was performed once a day for 2 weeks, then twice a week for remaining 10 weeks.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total bilirubin, and lipase levels were measured before and 1 day after TAE. These tests were repeated 1, 2, 4, and 12 weeks after TAE.

Computed tomography (CT; ECLOS 8; Hitachi Medical Co., Ltd., Tokyo, Japan) was performed before and immediately after embolization, and repeated at Day 3, and Week 1, 2, and 8. Each study included 4 phases: abdominal survey, arterial, portal, and equilibrium phases. Iopamidol (Oiparomin 370; Fuzi Pharmaceutical Co., Ltd., Toyama, Japan) was injected intravenously to provide contrast during vascular imaging. Three-dimensional (3D) reconstructions of the hepatic vessels were generated with Ziostation2 software (Ziosoft, Inc., Tokyo, Japan).

Liver biopsies were obtained via laparoscopy immediately after TAE, and at Day 3, and Week 1, 2, 4, and 8. Samples from the left lateral lobe, left medial lobe, and right lobes of the liver were fixed in 4% paraformaldehyde and embedded in paraffin. Tissue sections were stained with hematoxylin–eosin, Gitter, periodic acid Schiff, and Azan stains.

2-2-4 *Data analysis*

Body weights and duration of TAE are reported as means ± standard deviations (SDs).

Pre-embolization and post-embolization values were compared using a paired *t* test. A *P* value of <0.05 was considered statistically significant.

2-3 Results

2-3-1 *Successful TAE*

The mean body weight of dogs in our study was 9.76 ± 0.32 kg. The microcatheter was successfully inserted into the left hepatic artery under DSA guidance, and resulted in successful embolization of the left hepatic artery in all dogs. Approximately 1.0–2.0-mL of GSPs and contrast medium was injected before overflow was observed. Complete occlusion of the left hepatic artery was confirmed on post-embolization hepatic arteriograms in all dogs. The mean duration of TAE was 96 ± 12.08 min.

Clinical examination of dogs remained within normal limits throughout the study. No abnormality was detected on preoperative evaluation of CT studies. The branches of the hepatic artery that supplied the left lobe were clearly identified in the arterial phase of the study. Immediately after embolization, complete arterial occlusion was confirmed. In all dogs, recanalization of the arteries occurred within 2 weeks after treatment (Figures 2 and 3).

2-3-2 *Changes of liver enzymes*

Hepatic enzyme levels increased temporarily after treatment (Figure 4), but gradually returned to normal. ALP levels were elevated compared to preoperative values at 4 weeks

after TAE but remained within normal range. Other parameters were not altered in any of the dogs.

2-3-3 *Histological changes*

No relevant differences were observed between tissues from treated and untreated liver lobes after TAE (Figure 5). No evidence of perivascular hemorrhage or inflammation was found in the vessel wall or surrounding tissues.

2-4 Discussion

2-4-1 *Safety of selective TAE in normal canine liver*

Embolization is defined as a blockade of arterial flow induced by injection of an agent[27, 28]. The main findings of our study are that selective TAE of the left hepatic artery was well-tolerated, provided less than 2 weeks of occlusion, and did not induce long-term ischemic changes in affected liver lobes.

TAE-mediated vascular occlusion may also be used for preoperative infarctions of tumors[29]. In this setting, TAE limits blood loss, facilitates the identification of dissection planes, and a decreases the risk of neoplastic cell dissemination during surgical manipulation[30]. However, acute ischemia of an HCC is often followed by abdominal pain, discomfort, and fever in human patients[1, 31]. These signs have been recognized as "post-embolization syndrome"[29]. No such signs were observed after hepatic arterial embolization in the 5 healthy dogs of our study, and the procedure was overall, well tolerated. Previous reports[32, 33] described that hepatic enzyme levels increased temporary after TAE. Hepatic enzyme levels increased slightly in some dogs, which is consistent with post-treatment liver damage. However, these values remained within normal limits and were not associated with clinical signs of liver dysfunction.

2-4-2 *Selective embolization*

Selective embolization of hepatic arteries is needed to avoid the lethal consequences associated with TAE of the common hepatic artery in dogs[12]. The relative proximity of cystic and gastroduodenal arteries relative to each hepatic further justify selective embolization to avoid gallbladder infarction or other organ damage[18]. TACE has been reported in dogs with hepatocellular adenoma, but the left hepatic artery could not be catheterized with a 5.0-Fr catheter[33]. In the present study, 2.1-Fr microcatheters were successfully inserted into the left hepatic arteries of all dogs.

2-4-3 *Size of embolic agents*

Embolization with smaller particles has been found to increase tumor ischemia but is also more likely to cause complications such as bile duct injury or liver necrosis[1, 34]. According to Sonomura et al[34], GSPs measuring 500 μm or less in diameter increased the incidence of bile duct injury. The particles selected for our study measured 1mm in diameter, which may explain absence of histological abnormalities.

2-4-4 *Characteristic of gelatin sponge particles*

Recanalization post TAE is influenced by the absorption characteristics of the

embolization agent. A gelatin sponge, absorbed within 48–72 h, was found to induce complete arterial occlusion with recanalization in approximately 2 weeks, in man[35]. Our results are similar, as recanalization occurred within 2 weeks after arterial embolization in dogs. Further studies are needed to determine whether alternative agents such as polyvinyl alcohol and microspheres would extend the duration of occlusion in canine livers although the necessary duration of vascular occlusion to induce tumor necrosis is unknown[36, 37].

2-4-5 *Study limitations*

Our investigation was limited to left-side embolization in a small number of beagle dogs. In the clinical setting, dogs with liver tumors may need TAE or TACE of the right lobe. However, individual variations in origins, courses, and ramification patterns of the right hepatic arteries among dogs[17, 18] prompted us to study the left lobes of the liver. In addition, only three biopsy samples were collected from each liver in this study. Although a small amount of tissue was harvested under laparoscopy, we cannot eliminate the potential impact that these samplings may have had on liver enzymes after embolization.

2-4-6 *Conclusions*

A strong knowledge of the vascular anatomy of the liver[13-16] and specific training in interventional radiology are crucial to locate HCC and select the best approach to the target artery[17, 18]. Once these skills are acquired, clinicians should expect selective TAE of the left hepatic artery with GSPs to be relatively safe in healthy dogs, and result in recanalization within 2 weeks. Further studies are needed to focus on safety of TAE in dogs with HCC. We believe that selective TAE to canine hepatocellular carcinoma is applicable to assess anti-cancer effects.

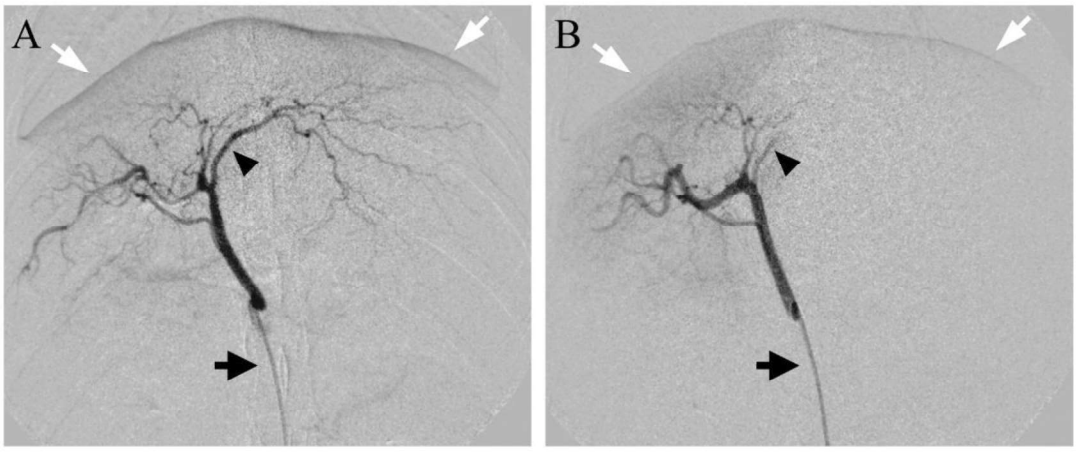


Figure 1. Ventral–dorsal digital subtraction images of a dog. (A) Pre-embolization hepatic arteriogram, demonstrating the injection of contrast medium through a 4-Fr catheter (black arrow) placed in the common hepatic artery. (B) Post-embolization hepatic arteriogram showing complete occlusion of the left hepatic artery. The white arrow indicates the diaphragm line, and the black arrowhead indicates the left hepatic artery.

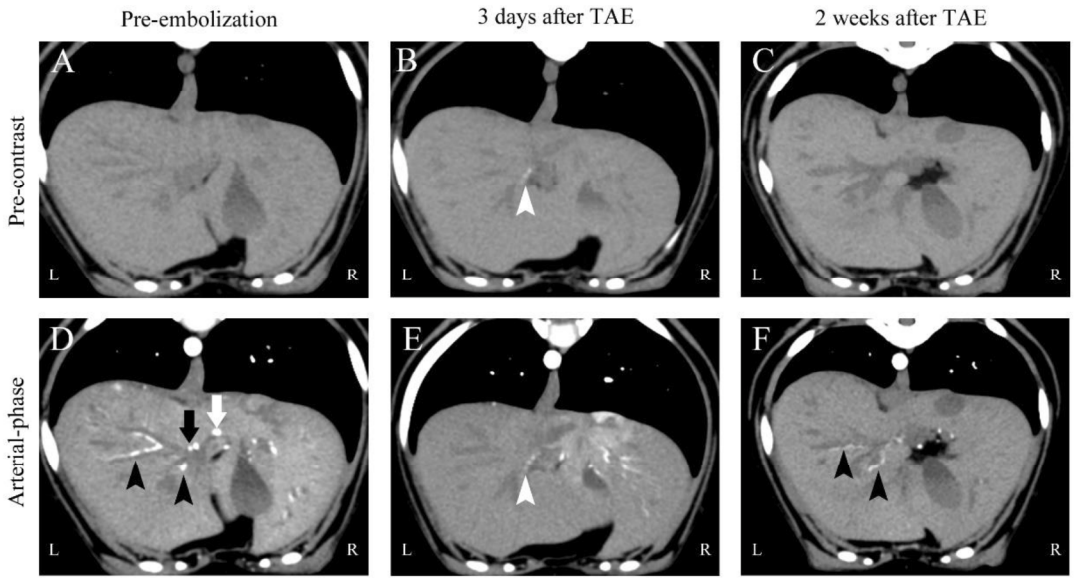


Figure 2. Transverse computed tomography (CT) images. (A) A non-contrast CT image obtained before embolization. (B) A non-contrast CT image obtained 3 days after embolization, demonstrating slight embolic agents with contrast medium uptake (white arrowhead) in the left hepatic artery. (C) A non-contrast CT image obtained 2 weeks after embolization. (D) An arterial-phase CT image obtained before embolization revealed the left hepatic artery (white arrow), the left medial lobar hepatic artery (black arrow), and the left lateral lobar hepatic artery (black arrowhead). (E) An arterial-phase CT image obtained 3 days after embolization, demonstrating no arterial enhancement in the left lobe of the liver and arterial enhancement in the right lobes. (F) An arterial-phase CT image obtained 2 weeks after embolization showing recanalization of the left hepatic artery.

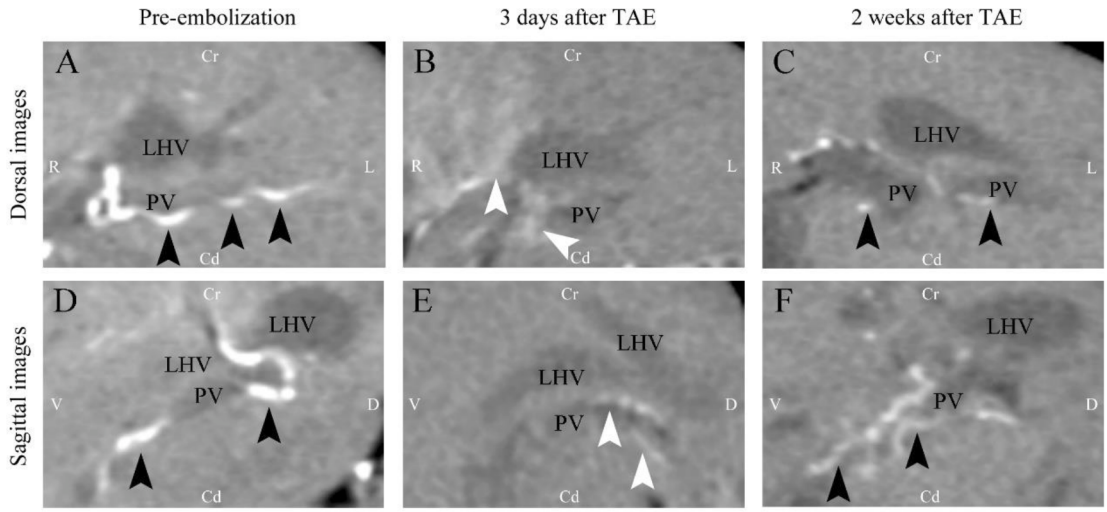


Figure 3. Arterial-phase computed tomography (CT) images. (A) A dorsal CT image obtained before embolization showing the left lateral lobar hepatic artery (black arrowhead) running in a spiral manner around the portal vein (PV) of the left lateral lobe. (B) A dorsal CT image obtained 3 days after embolization, demonstrating no arterial enhancement and slight embolic agents with contrast medium uptake (white arrowhead) in the left lateral lobe. (C) A dorsal CT image obtained 2 weeks after embolization showing recanalization of the left lateral lobar hepatic artery. (D) A sagittal CT image obtained before embolization. (E) A sagittal CT image obtained 3 days after embolization, demonstrating no arterial enhancement in the left lateral lobe. (F) A sagittal CT image obtained 2 weeks after embolization showing recanalization of the left lateral lobar hepatic artery. LHV, left hepatic vein; Cd, caudal; Cr, cranial; D, dorsal; V, ventral.

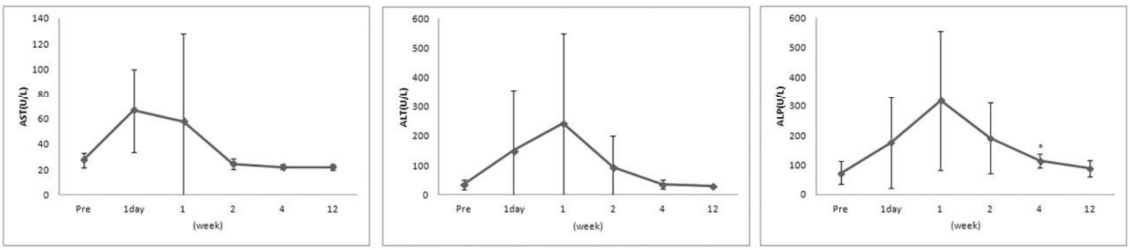


Figure 4. Changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels during the examination period. AST, ALT, and ALP levels increased transiently after TAE but gradually returned to normal (*: $P < 0.05$).

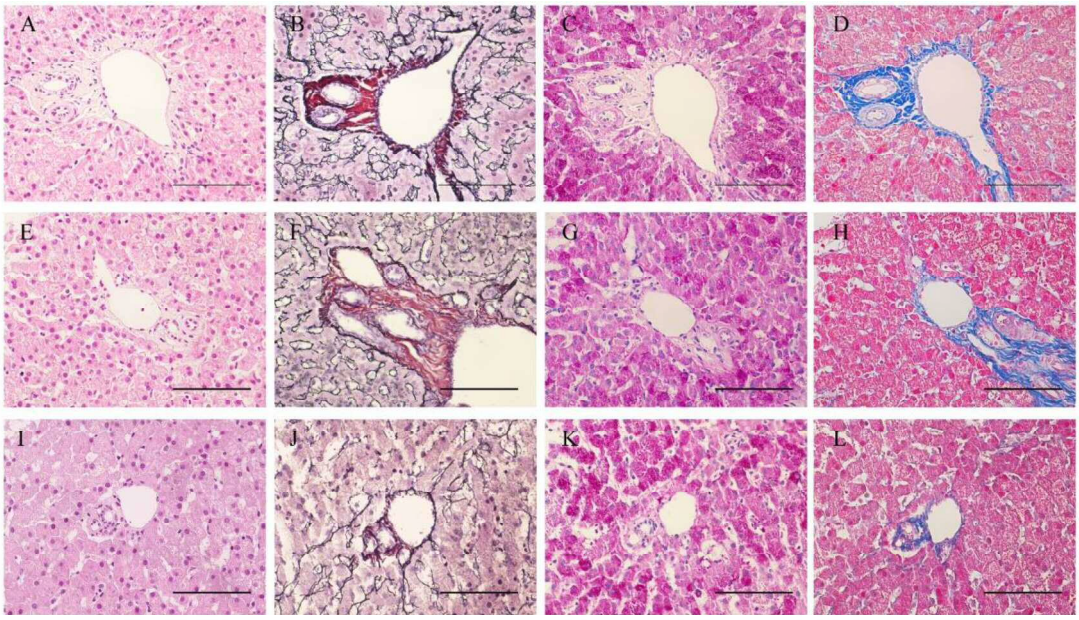


Figure 5. Histological liver findings of the untreated lobe after staining with (A) hematoxylin–eosin, (B) Gitter, (C) periodic acid-Schiff, and (D) Azan. Histological liver findings of the left lobe at 1 week after treatment shown via staining with (E) hematoxylin–eosin, (F) Gitter, (G) periodic acid-Schiff, and (H) Azan; at 2 weeks after treatment, staining with (I) hematoxylin–eosin, (J) Gitter, (K) periodic acid-Schiff, and (L) Azan. No relevant abnormalities were observed in treated liver tissues relative to untreated tissues. Scale bar = 100 μm.

CHAPTER 3

Transcatheter arterial embolization in normal canine liver with MSs

3-1 Background and objectives

Embolic agents differ with respect to absorption characteristics. In chapter 2, GSPs were used as embolic agents for selective TAE in normal canine liver. A gelatin sponge, absorbed within 48–72 h, was found to induce complete arterial occlusion with recanalization in approximately 2 weeks. Recently, microspheres (MSs) are used as permanent embolic agents for human HCC. MSs are not resorbed, and then can be expected to induce long term effects of arterial occlusion compared with GSPs. However, little information is available regarding MSs in veterinary field. Therefore, we investigated clinical symptoms, biochemical analysis, pathological findings, and recanalization of the arteries after selective TAE with MSs in normal canine liver.

3-2 Materials and methods

3-2-1 *Animals*

Four beagles without clinical signs relating to liver disease were entered into the study. All dogs had no abnormal blood test and clinical symptoms. All dogs were caged with free access to water, and food was withheld for 12 hours before the dogs were anesthetized. All dogs treated with microspheres (Embosphere 100–300μm, Nippon Kayaku, Tokyo, Japan).

All dogs were induced by slow intravenous administration of propofol (1% propofol, Ishimal pharmaceutical company, Japan, 7mg/kg, IV) to effect. A cuffed endotracheal tube was placed in the trachea and the dogs were connected to a partial rebreathing cycle system delivering isoflurane (Isoflu, Dainippon sumitomo pharma Co., Ltd., Tokyo, Japan, from 1.4 to 2.5%) and oxygen. Anesthetic depth was monitored using clinical signs, and the dogs were ventilated to maintain eucapnia. All dogs were monitored under anesthesia using a respiratory profile monitor to measure end tidal carbon dioxide, peak inspiratory pressures and oxygen saturation. All dogs were recovered from anesthesia and injected antibiotics, painkiller, and hemostatic.

CT (ECLOS 8; Hitachi Medical Corporation, Tokyo, Japan) scanning was obtained before and after TAE in four phases; survey of the abdomen, arterial phase, portal phase

and equilibrium phase. Dogs received iopamidol with an iodine concentration of 370 mg/mL (Oiparomin 370; Fuzi pharmaceutical company, Toyama, Japan) as intravenous contrast for vascular imaging. 3D reconstruction of the hepatic vessels was made using the software (Ziostation2; Amin Stock Co.).

3-2-2 *Procedure of TAE*

Hepatic arterial embolization was performed under general anesthesia. Access to the right femoral artery was obtained using the Seldinger technique[26]. The right femoral artery was punctured with a 20-G needle (Terumo, Japan) and cannulated with a 4-French introducer Sheath (Radifocus introducer sheath, 4Fr, Terumo Co., Tokyo, Japan). Guide wire (Radifocus Guide wire M, diameter of 0.89 mm, Terumo Co., Tokyo, Japan) and catheter (PA catheter, 4 Fr, 70 cm, J curve, Terumo Clinical Supply Co., Gifu, Japan) was inserted into the celiac artery, and then advanced into common hepatic artery with injecting contrast medium (Optiray 350, Covidien Co., Tokyo, Japan) under digital subtraction angiography (DSA) (ARCADIS Varic, Siemens Healthcare Japan, Tokyo, Japan). Microguidewire (Radifocus Guide wire M, diameter of 0.41 mm, Terumo Clinical Supply Co., Gifu, Japan) was inserted through the catheter. A 1.7 Fr microcatheter (Derniere Plus, 1.7Fr, Create Medic Co., Tokyo, Japan) was used. Left hepatic artery was

visualized using contrast medium through microcatheter on DSA image. MSs were mixed with the contrast medium. Super selective embolization of the left lateral hepatic artery was performed until there was sluggish hepatic arterial flow.

3-2-3 *Evaluations*

Liver biopsies were obtained with laparoscopy or laparotomy from the embolized and non-embolized hepatic lobes more than three times during 8weeks after embolization. Tissue samples were fixed in 4% paraformaldehyde and processed to paraffin tissue blocks. These blocks were cut and stained with hematoxylin and eosin, Gitter, Azan, Periodic Acid-Schiff, and Sirius red.

Biochemical analysis was obtained before, 1 day, once a week during 4 weeks, 8, and 12 weeks after the treatment. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total bilirubin, and lipase were included.

3-2-4 *Data analysis*

The post-embolization values were compared to pre-embolization values using a paired *t* test. A *P* value <0.05 was considered to indicate a statistically significant difference.

3-3 Results

3-3-1 *Successful TAE*

In all dogs, microcatheter could be inserted successfully into the left hepatic artery using DSA guidance. Super selective embolization of the left lateral hepatic artery succeeded in all dogs. Embolic agent was injected until sluggish hepatic arterial flow was observed. Post-embolization hepatic arteriograms demonstrated complete occlusion of the target arteries (Figure 1).

3-3-2 *CT scanning*

CT scanning was performed before and after TAE in all dogs. There was no abnormality relating to liver disease before the treatment. In arterial phase, the branches of the hepatic artery to the left lobe were obviously visualized. Immediately after the treatment, embolic agents were observed in the left hepatic artery with CT scanning. Arterial phase of CT scanning showed complete occlusion of the arteries (Figure 2). The embolic particles were not resorbed during 12 weeks observation period.

3-3-3 *Changes of liver enzymes*

The results of biochemical analysis are below (Figure 3). Hepatic enzymes increased temporarily after the treatment but were almost within normal range. In all dogs, GGT

and total bilirubin were no remarkable.

3-3-4 *Histological changes*

In the histopathological examination, there were no significant differences between the liver with or without the treatment. There was no damage to the vessel wall and no perivascular hemorrhage. No inflammatory changes of the vessel wall or the surrounding tissues were observed.

3-3-5 *Clinical symptoms*

All dogs showed no clinical symptoms like as pain, fever, and anorexia during the examination. Only one dog transiently showed a purple spot in the femoral skin after the treatment.

3-4 Discussion

3-4-1 *Super selective embolization*

The present study demonstrates that super selective embolization is possible in canine hepatic artery. Embolization using smaller particle produces greater ischemic effect in tumors, however, is more likely to produce complication such as bile duct injury or liver necrosis[1, 34]. According to a study by Sonomura et al[34], GSPs not larger than 500 μm cause a high incidence of bile duct injury. In present study, even MSs of 100–300μm caused no pathological abnormality. It suggests that super selective embolization can avoid bile duct injury.

3-4-2 *Characteristic of MSs*

In chapter 2, gelatin sponge caused a temporary vascular occlusion and recanalization occurred in approximately 2 weeks. The present study also demonstrated that early recanalization within 2 weeks after arterial embolization occurs in dogs treated with GSPs. On the other hand, MSs were not resorbed during 12 weeks. Therefore, long term effects of arterial occlusion can be expected by using MSs. However, we should be careful to avoid overflow of embolic agents when MSs is used as embolic agents.

3-4-3 *Conclusions*

In chapter 2, GSPs caused no clinical symptoms in all dogs whereas some dogs showed liver enzymes elevated after embolization. In the present study, none of the dogs showed clinical signs such as pain, fever, and anorexia during the observation period and hepatic enzyme levels increased temporarily after treatment, but gradually returned to normal. These results suggest that MSs is appeared to be safe as same as GSPs and selective embolization could reduce liver damage. In conclusion, TAE is comparatively safe to liver in clinically healthy dogs.

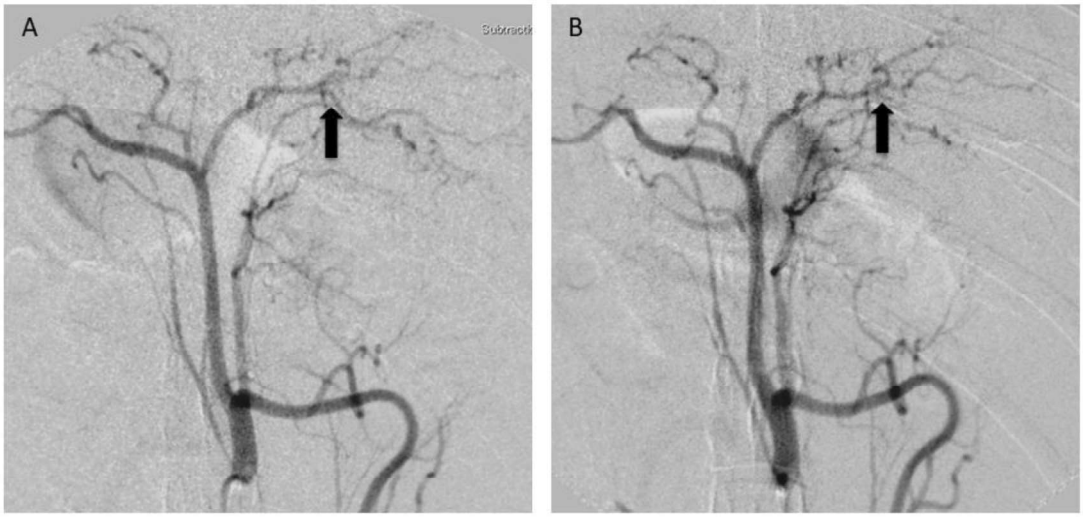


Figure 1. Ventral–dorsal digital subtraction images of a dog. (A) Pre-embolization hepatic arteriogram, demonstrating the injection of contrast medium. Black arrow indicates branches of the left lateral hepatic artery. (B) Post-embolization hepatic arteriogram showing occlusion of the left lateral hepatic artery.

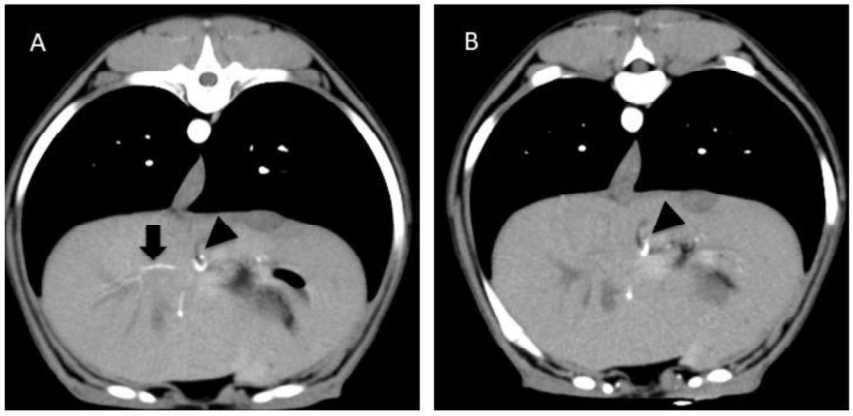


Figure 2. Transverse computed tomography (CT) images. (A) An arterial-phase CT image before embolization revealed the target artery (arrowhead) branch of the left hepatic artery (arrow). (B) An arterial-phase CT image obtained 3 days after embolization with microspheres (MSs), demonstrating no recanalization of the target artery.

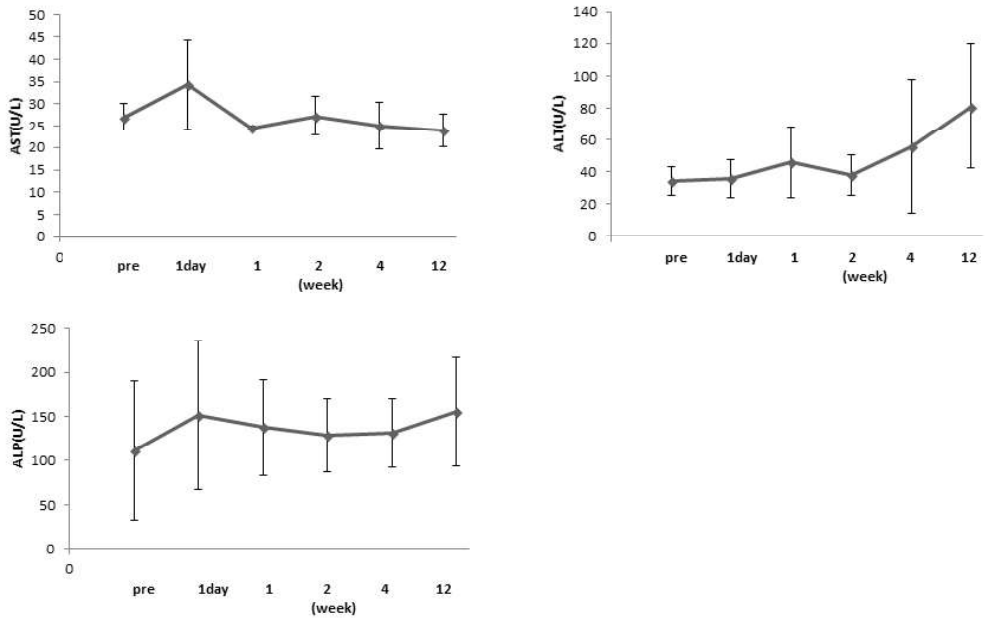


Figure 3. Changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels during the examination period. AST, ALT, and ALP levels increased temporarily after the treatment but were almost within normal range.

CHAPTER 4

Transcatheter arterial embolization in dogs with hepatocellular carcinoma

4-1 Background and objectives

Hepatocellular carcinoma (HCC) is one of the most common types of liver cancer in dogs[2]. It is well known that a liver lobectomy is the preferred method of treatment for dogs with primary hepatic tumors[9]. However, inoperative liver tumors present a difficult challenge for dog owners and veterinarians.

In humans, transcatheter arterial embolization (TAE) is considered a standard treatment for advanced HCC. Notably, TAE improves pain and local control and prolongs survival rates in patients with unresectable HCC[5, 38]. This method has recently been used in veterinary medicine[10, 33, 39]. However, little practical information is available. Here, we report tumor responses, clinical reactions and adverse effects after selective embolization of the tumor-suppling arteries using gelatine sponge particles (GSPs) in four dogs with HCC.

4-2 *Materials and methods*

4-2-1 *Case histories*

Four cases were presented for evaluation of liver tumors. Signalment, case histories, clinical findings, tumor volumes and outcomes are summarised in Table 1. Computed tomography (CT) images (ECLOS 8; Hitachi Medical Co., Ltd., Tokyo, Japan) were obtained before and after treatment under general anaesthesia. Contrast-enhanced CT scan was performed in arterial, portal and equilibrium phase after injection of contrast medium (Oiparomin 370; Fuzi Pharmaceutical Co., Ltd., Toyama, Japan). Scans were obtained with a collimation of 8 × 1.25 mm, table pitch of 0.875, tube voltage of 120 kV, and tube current of 350 mA. Axial, sagittal and dorsal CT images were carefully reviewed to identify vessels that supplied to tumors.

Tumor volume was measured using Ziostation2 software (Ziosoft, Inc., Tokyo, Japan). Tumor contours were outlined using a mouse-driven cursor on each individual CT image. The final tumor volumes were measured by the summation of areas. Tumor biopsies were obtained via laparotomy at the time of another disease (Case 1), laparoscopy (Case 2 and 4) or ultrasound-guided Tru-Cut needle (Case 3), and histopathological findings were consistent with HCC.

4-2-2 *TAE procedures*

TAE was performed under general anaesthesia in all dogs. GSPs were used as embolic agents in present cases because they are widely used in human HCC and available at low cost in Japan. The same TAE procedures and embolic agents were used in all dogs. Equipment used in TAE is summarised in Table 2. Access to the right femoral artery was obtained using the Seldinger technique[26]. The right femoral artery was punctured with a 20-G needle and cannulated with a 4-French (Fr) introducer sheath. A guidewire and catheter were inserted into the coeliac artery using fluoroscopy (ARCADIS Varic; Siemens Healthcare Japan). A microguidewire was inserted into the common hepatic artery through the catheter. A mixture of GSPs and 10 mL of contrast medium was prepared for embolization. The 2.1-Fr microcatheter was placed at the origin of the arterial branches supplying the tumors under digital subtraction angiography. Finally, the mixture was injected into the tumor-suppling arteries until an overflow of contrast medium was observed.

Post-embolization hepatic arteriograms demonstrated occlusion of the target arteries in all dogs. After removing the sheath, the puncture site was manually compressed. All dogs were administered cefazolin sodium (Cefamezin; Astellas Pharma Inc.) (25 mg/kg intravenously q12h) intraoperatively and then cephalexin (Larixin; Taisho Toyama

Pharmaceutical Co., Ltd.) (25 mg/kg per os q12h) for 7 days postoperatively. Buprenorphine (Lepetan; Otsuka Pharmaceutical Co., Ltd.) (20 μg/kg intramuscularly q12h) was administered perioperatively. Therapy with a 7-day tapering course of prednisolone (Prednisolone; Kyoritsu Seiyaku Corp.) was initiated intramuscularly with a starting dose of 1 mg/kg every 24 hours. All dogs recovered from anaesthesia with intensive monitoring.

4-3 Practical cases

4-3-1 *Selective TAE in case 1*

In case 1, the tumor of the right medial lobe was closely positioned to the caudal vena cava, and the dog presented with a poor general condition. The dog owner desired a minimally invasive treatment after understanding the risks and benefits of a surgical approach and TAE approach. Therefore, TAE was selected as a palliative method for treating liver tumor. In this case, the right medial lobar hepatic artery was embolized (Figure 1). After treatment, no side effects, such as abdominal pain, discomfort or fever, were observed. Hepatic enzyme levels increased temporarily after treatment but gradually declined (Table 3). Following discharge, the owner reported that recovery was uneventful, and clinical symptoms were gradually improved. At 224 days after treatment, CT scan revealed that the tumor volume had decreased by 75.3% and compression of the caudal vena cava was improved due to reduction of tumor size (Figure 2). Clinical symptoms were clearly improved but the dog died of renal failure 376 days after treatment.

4-3-2 *Selective TAE in case 2*

In case 2, the tumor of the left medial lobe was close to the left hepatic vein but surgical treatment would be possible. However, dog owner declined abdominal excision.

Therefore, TAE was selected as a palliative method and the left medial lobar hepatic artery was embolized (Figure 3). No side effects were observed after treatment. Hepatic enzyme levels increased temporarily after treatment but then gradually decreased (Table 3). Following discharge, the owner reported that recovery was uneventful, and no clinical symptoms were observed. At 121 days after treatment, CT scan revealed that the tumor volume had decreased by 17.4% (Figure 4). Following a telephone call to the owner, the dog remained asymptomatic for a while but died 1320 days after treatment. The dog owner noticed abdominal distension 1month before death although the cause of death is unknown.

4-3-3 *Selective TAE in case 3*

In case 3, surgical resection was recommended but the dog owner, who is a medical doctor, desired a minimally invasive treatment after understanding the risks and benefits of a surgical approach and TAE approach. Therefore, TAE was offered and the left medial lobar hepatic artery was embolized (Figure 5). No side effects were observed after treatment. Hepatic enzyme levels increased temporarily after treatment but then gradually decreased (Table 3). At 61 days after treatment, CT scan revealed that the tumor volume had decreased by 31.2% (Figure 6). Following a telephone call to the owner, the dog was

asymptomatic but died 562 days after treatment and the cause of death is unknown.

4-3-4 *Selective TAE in case 4*

In case 4, the dog had tumors in two lobes of the liver and surgical resection was recommended. However, the dog owner desired a minimally invasive treatment after understanding the risks and benefits of a surgical approach and TAE approach. Therefore, TAE was offered. The right and left medial lobar hepatic arteries were embolized (Figure 7). After treatment, hepatic enzyme levels increased temporarily but then gradually decreased (Table 3). In this case, lipase level increased 1 to 2 weeks after treatment, and the dog showed vomiting, abdominal pain and diarrhoea due to pancreatitis. Clinical symptoms were gradually improved, and CT scan at 89 days after treatment revealed that the tumor volume of the right medial lobe had decreased by 14.5% and that of the left medial lobe had decreased by 51.7% (Figure 8). However, acute renal failure occurred after the dog accidentally ingested grapes, and the dog was euthanised 145 days after TAE.

4-3-5 *Follow-up*

In all dogs, postoperative tumor volume decreased compared to preoperative volume. Follow-up was conducted by phone after the owners moved away. Median survival time

was 469 days (range, 145 to 1320).

4-4 DISCUSSION

4-4-1 *Tumor reduction after embolization*

In the present cases, postoperative tumor volume decreased in all dogs. A previous study in humans showed that embolization of the tumor-supplying artery results in ischaemia of the tumor and reduction of tumor size[6]. In line with that observation, the present cases demonstrate that temporary occlusion of the tumor-supplying artery without chemotherapy leads to reduction of tumor size in dogs.

4-4-2 *TAE vs TACE*

In humans, TAE with additional chemotherapy (TACE) is also a well-described method of treatment of advanced HCC[1]. It is well known that effects of TACE are mainly derived from occlusions of tumor-suppling arteries. In the veterinary field, adverse effects and treatment response to TACE remain unclear. Although there is still controversy regarding the efficacy of TAE vs. TACE[1], previous research in humans demonstrates that TAE is as effective as TACE while being less expensive and devoid of adverse effects due to the lack of chemotherapy[40].

4-4-3 *Adverse reactions of TAE*

Regarding adverse reactions, cases 1–3 showed no clinical symptoms such as abdominal pain, discomfort or fever (known as 'post-embolization syndrome') after treatment. Postoperative courses were uneventful, and early discharge was possible. Tumor lysis syndrome was suspected based on tumor size, but no indications were shown after embolization in any of the dogs. Conversely, severe pancreatitis was observed after treatment in case 4. Although this finding cannot be adequately explained, it may be attributed to microthrombus by catheterisation or the flow of embolic agents into the pancreatic artery as the gastroduodenal artery is near the targeted artery[18].

4-4-4 *Characteristics of embolic agents*

Permanent or temporary arterial occlusion can be achieved with different agents, including GSPs, polyvinyl alcohol, microspheres and drug-eluting beads. However, there is little in the literature comparing the efficacy of different embolic agents. GSPs used in present cases are well described as embolic materials, and recanalisation of the embolized artery can occur approximately 2 weeks after treatment with GSPs[35]. Although the duration of vascular occlusion required to induce tumor necrosis is unknown, good therapeutic effects were obtained using GSPs in the present cases.

4-4-5 *Conclusions*

The goal of TAE is to extend survival, improve quality of life, and alleviate symptoms. Regarding surgical resection versus TAE, liver lobectomy is considered the gold standard for dogs with HCC when complete resection is feasible[41, 42]. However, the dog owner often desires minimally invasive treatments and such dogs often present a poor clinical condition that contraindicates surgical treatment. Considering canine median survival time of untreated HCC[9], TAE appears to be a feasible method for the treatments of HCC. This is the first case report to evaluate tumor response using volume measurement method after selective embolization with GSPs in canine liver. However, the present cases were limited by short-term follow-up. Further research is needed to evaluate the long-term effects and complications of this procedure.

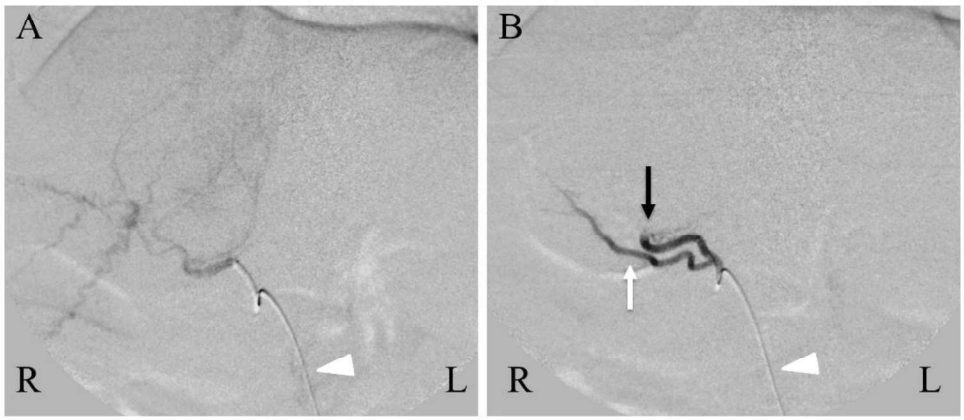


Figure 1. Ventral–dorsal digital subtraction images in case 1. (A) Pre-embolization hepatic arteriogram shows tumor-supplying arteries. (B) Post-embolization hepatic arteriogram demonstrates occlusion of the tumor-supplying arteries at the origin (black arrow). The normal hepatic artery of the right lateral lobe (white arrow) is visualised due to overflow of contrast medium. White arrowheads show microcatheter.

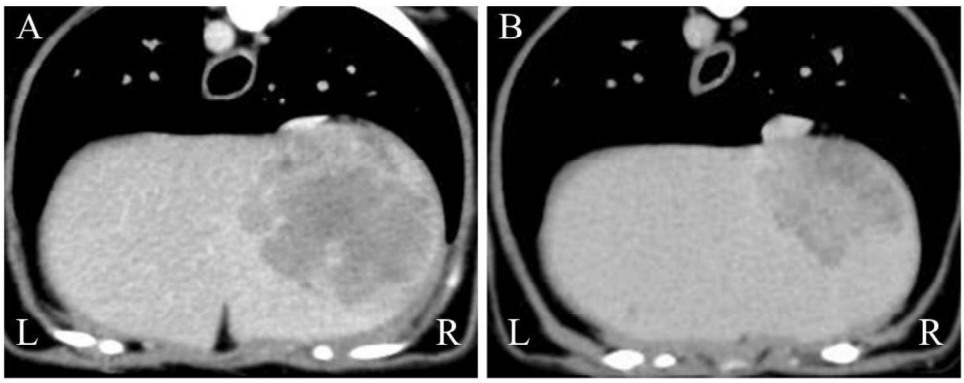


Figure 2. Equilibrium-phase transverse computed tomography (CT) images in case 1. (A) Pre-embolization CT image shows large right-sided liver tumor. (B) A CT image obtained 224 days after embolization, demonstrating tumor shrinkage.

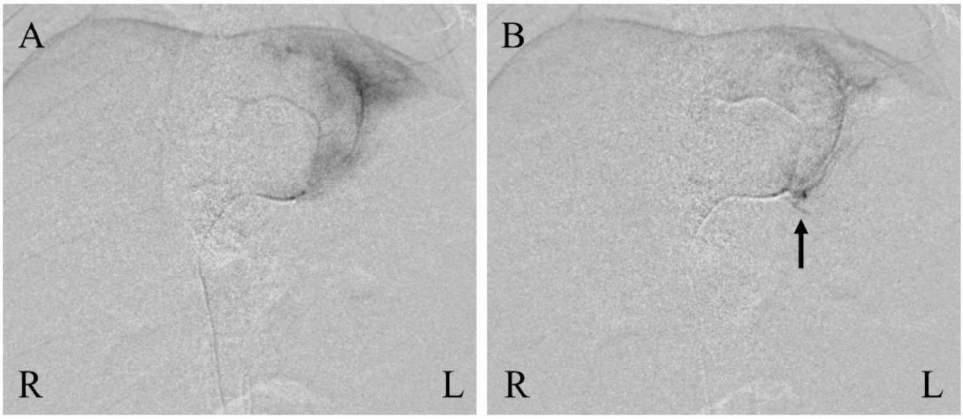


Figure 3. Ventral–dorsal digital subtraction images in case 2. (A) Pre-embolization hepatic arteriogram shows tumor-supplying arteries. (B) Hepatic arteriogram during embolization shows overflow of contrast medium at the tumor-supplying arteries (black arrow).

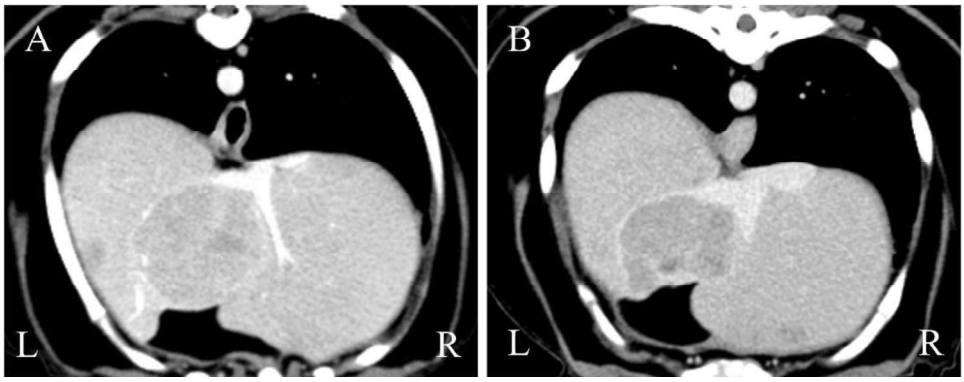


Figure 4. Equilibrium-phase transverse computed tomography (CT) images in case 2. (A) Pre-embolization CT image shows central liver tumor. (B) A CT image obtained 121 days after embolization, demonstrating tumor shrinkage.

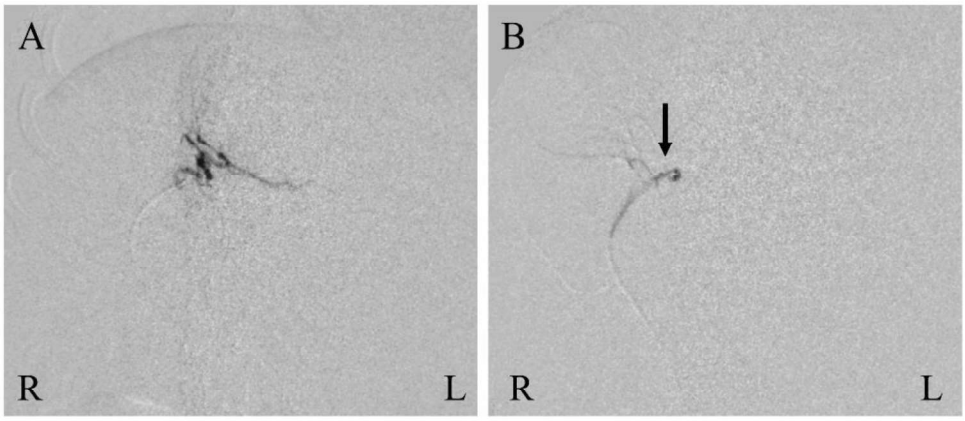


Figure 5. Ventral–dorsal digital subtraction images in case 3. (A) Pre-embolization hepatic arteriogram shows tumor-supplying arteries. (B) Post-embolization hepatic arteriogram demonstrates occlusion of the tumor-supplying arteries at the origin (black arrow).

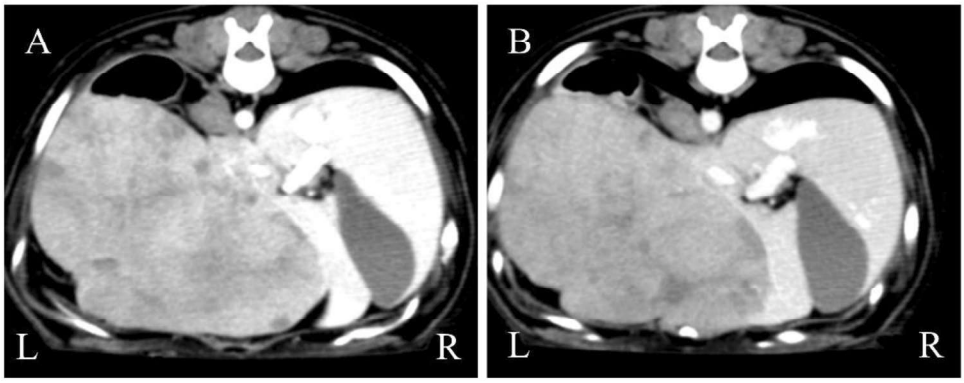


Figure 6. Equilibrium-phase transverse computed tomography (CT) images in case 3. (A) Pre-embolization CT image shows large left-sided liver tumor. (B) A CT image obtained 61 days after embolization.

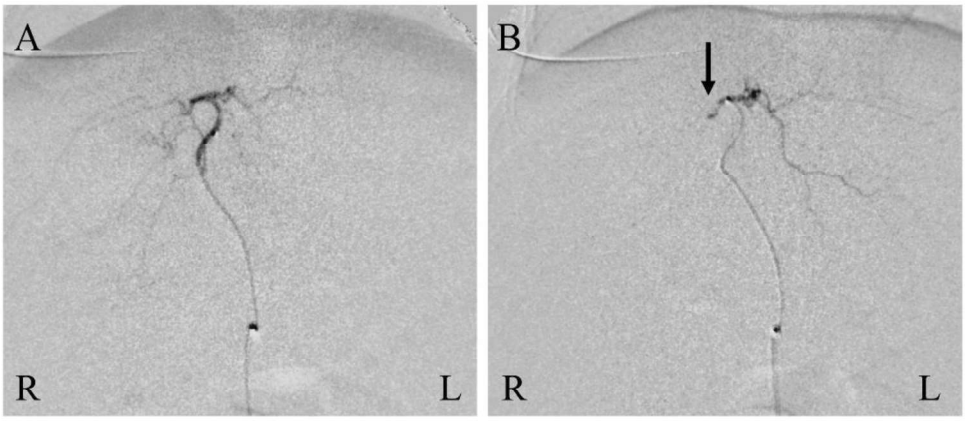


Figure 7. Ventral–dorsal digital subtraction images in case 4. (A) Pre-embolization hepatic arteriogram shows tumor-supplying arteries. (B) Post-embolization hepatic arteriogram demonstrates occlusion of the tumor-supplying arteries at the origin (black arrow).

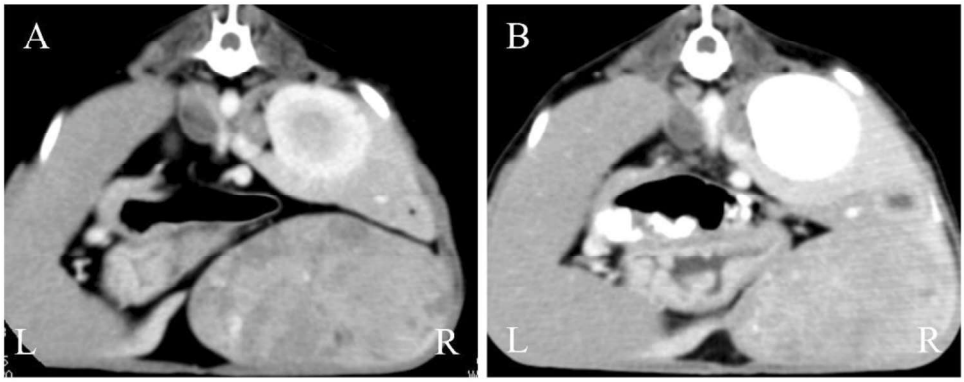


Figure 8. Equilibrium-phase transverse computed tomography (CT) images in case 4. (A) Pre-embolization CT image shows large right-sided liver tumor. (B) A CT image obtained 89 days after embolization, demonstrating tumor shrinkage.

Table 1. Signalments, case histories, clinical findings, tumor volumes and outcomes

Case	Signalment	History and clinical signs	Histologic diagnosis	Side of liver involvement	The main target artery	Pre-embolization tumor volume	Post-embolizaiton tumor volume	Outcome
1	14 YO, beagle dog, ME	1 month history of leg edema and anorexia.	HCC	RML	The right medial lobar hepatic artery	221.8cc	107cc	Clinical symptoms were obviously improved but the dog died of renal failure 376 days after treatment
2	10 YO, American cocker spaniel, ME	Mild generalized seizure (6 years) and abdominal surgery twice for gastrointestinal stromal tumor	HCC	LML	The left medial lobar hepatic artery	44.3cc	36.6cc	The dog remained asymptomatic for a while but died 1320 days after treatment. The dog owner noticed abdominal distension 1month before death although the cause of death is unknown.
3	13 YO, shih tzu, ME	1 month history of anorexia and polydipsia	HCC	LML	The left medial lobar hepatic artery	221.8cc	152.5cc	The dog remained asymptomatic for a while but died 562 days after treatment. The cause of death is unknown
4	13 YO, yorkshire terrier, FS	1 month history of anorexia	HCC	RML and LML	The right medial lobar hepatic artery and left medial lobar hepatic artery	52.9cc (RML), 12.28cc (LML)	45.23cc (RML), 5.93cc (LML)	Severe pancreatitis was observed but grudually improved after treatment. The dog was euthanatized 145 days after treatment because of acute renal failure

YO, year old; ME, male entire; FS, female spayed; HCC, hepatocellular carcinoma; RML, right medial lobe; LML, left medial lobe

Table 2. Equipment used in transcatheter arterial embolization

Equipment	Product name	Characteristic	Manufacturer	Country
Introducer sheath	Radifocus introducer sheath	4 Fr	Terumo Co., Ltd.	Japan
Guidewire	Radifocus guidewire M	Diameter: 0.89 mm, angled, 80 cm	Terumo Co., Ltd.	Japan
Microguidewire	Radifocus guidewire	Diameter: 0.41 mm, angled, 150 cm	Terumo Clinical Supply Co., Ltd.	Japan
Catheter	PA catheter	4 Fr, 40 cm	Terumo Clinical Supply Co., Ltd.	Japan
Microcatheter	Sniper 2	2.1 Fr, 110 cm	Terumo Clinical Supply Co., Ltd.	Japan
Embolic agent	Gelpart 1 mm	Particle diameter of 1 mm	Nippon Kayaku Co., Ltd.	Japan
Contrast medium	Optiray 350	Iodine content: 350 mg/mL	Covidien Co., Ltd.	Japan

Table 3. Changes in AST, ALT, ALP, and GGT levels during the examination period.

	Case 1				Case 2				Case 3			Case 4			
	Pre-TAE	1 day	2 days	5 days	Pre-TAE	1 day	2 days	5 days	Pre-TAE	1 day	60 days	Pre-TAE	1 day	3 days	10 days
AST(U/L)	60	368	315	62	45	1529	459	69	30	826	38	282	15680	538	95
ALT(U/L)	1558	2088	3452	1699	277	3691	2476	953	146	4584	84	118	1809	778	128
ALP(U/L)	10950	10473	9154	8212	1419	2073	2207	1710	1905	2226	1125	4710	5082	5568	2638
GGT(U/L)	18	16	14	14	20	18	17	16	8	8	8	13	12	13	9

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase

General conclusion

The purpose of this thesis is to clarify the effects of TAE in canine liver tumor.

In chapter 1, visualization of hepatic vessels was evaluated in 32 normal beagle dogs by X-ray contrast CT using triple phase images. The following hepatic vessels were clearly visualized: arterial, portal, and hepatic veins. With regards to the running patterns of the portal vein and hepatic vein, there were no significant differences between the dogs. However, the hepatic artery exhibited some differences in each dog. In particular, the hepatic artery of the quadrate lobe and the right lateral lobe had many running patterns. The results of this study could be useful for veterinary diagnosis, surgery, and interventional radiology.

In chapter 2, GSPs were injected through a microcatheter for selective embolization of the left hepatic artery in clinically normal dogs. CT images and histological examination findings were obtained during an 8-week observation period. Biochemical analysis data were obtained during a 12-week observation period after TAE. Embolization was successful in all dogs. Postoperative CT scanning revealed that artery recanalization occurred within 2 weeks after embolization in all dogs. Hepatic enzyme levels increased temporarily after embolization but gradually returned to normal ranges. Histological abnormalities were not observed in any of the dogs in a comparison of treated and untreated liver tissues, and no dog showed clinical symptoms. These results suggest that TAE with GSPs appears to be safe in the livers of clinically normal dogs.

In chapter 3, MSs were injected through a microcatheter for super-selective embolization of the left lateral hepatic artery in normal canine liver. CT images, biochemical analysis data, and histological examination findings were obtained during a 12-week observation period after TAE. Embolization was successful in all dogs. Postoperative CT scanning revealed that no recanalization was observed in dogs during the observation period. The hepatic enzyme levels increased temporarily after embolization but the levels remained almost within normal ranges. Histological abnormalities were not observed in any of the dogs in a comparison of treated and untreated liver tissues, and no dog showed clinical symptoms. TAE appears to be safe in the livers of clinically healthy dogs. Chapter 2 and 3 indicates that GSPs and MSs are minimally invasive agents in the canine liver.

In chapter 4, four dogs with hepatocellular carcinoma were presented for evaluation of transcatheter arterial embolization. In all dogs, the hepatic arteries that supplied the tumor were selectively embolized with GSPs. Postoperative tumor volumes decreased compared to preoperative volume in all dogs. In three dogs, no adverse reactions were observed after treatment although only one dog showed pancreatitis. Taken together, these results suggest that transcatheter arterial embolization can reduce tumor volume and then is an effective treatment for dogs with hepatocellular carcinoma.

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