A study on the clinical application of transarterial embolization (TAE) and transarterial chemoembolization (TACE) with microspheres in normal dogs

(正常犬を用いたマイクロスフィアによる肝動脈塞栓術、肝動脈化学塞栓術の臨床応用への検討)

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GENERAL INTRODUCTION

Hepatocellular carcinoma (HCC) has become one of the most common types of liver cancer in both humans and dog [7, 11]. HCC is categorized into massive type (61%), nodular type (29%), and diffuse type (10%) based on its gross findings [35]. The massive type is a large solitary tumor confined to one lobe of the liver, the nodular type is multifocal and involves several lobes, and the diffuse type is multifocal and involves all lobes or multiple nodules fused together, or diffuse loss of liver parenchyma, and is considered to be the final stage of tumor disease. More than two-thirds of dogs with massive HCC develop in the left lobe of the liver, with a metastatic rate of 0-37%, and liver resection is recommended for treatment [24]. However, liver resection for tumors developing in the left hepatic region has good outcomes and a good prognosis, whereas tumors developing in the right hepatic region involve the posterior vena cava and may have poor outcomes and high surgical risks [5, 35]. In addition, there are few treatment options for HCC such as nodular or diffuse type that cannot be treated with liver resection in veterinary medicine. Therefore, a minimally invasive and safe treatment for unresectable HCC is

demanded. In human medicine, unresectable HCC is treated with local therapies such as radiofrequency ablation (RFA), laser-induced thermotherapy (LITT), transarterial embolization (TAE), and transarterial chemoembolization (TACE) [46].

TAE is a treatment method in which a catheter is advanced percutaneously to the tumor-supplying artery of the HCC under fluoroscopic guidance, and an embolization material administered to it. TACE is almost the same procedure as TAE, but an anticancer drug is administered along with the embolization material. The liver blood supply is provided by two vessels, the hepatic artery (approximately 20%) and portal vein (approximately 80%) [28]; however, HCC is mainly perfused by the hepatic artery [30]. Therefore, embolization of this tumor-supplying artery would decrease arterial perfusion of the tumor, resulting in selective ischemia and tumor size reduction [7]. Previous studies in humans have demonstrated improvements in survival rates, pain, and local control following arterial embolization for unresectable HCC [7, 36]. Currently, TAE and TACE are considered a standard treatment for advanced HCC in humans [7, 15]. TAE or TACE may also be offered to patients with unresectable primary liver tumors or liver metastases in humans [36].

There is a lot of debate in humans about whether TAE or TACE is better, but the answer is still unknown [14, 17]. Moreover, there are also a variety of embolization materials. In Japan, TAE and TACE using gelatin sponges are the most common, but in Europe and the United States, TAE and TACE using non-absorbable spherical embolization material are the most common. It is not clear which embolization material is superior.

Drug-eluting beads (DEB) is a novel drug delivery system that is specifically designed to deliver a drug directly into the tumor tissue at a slow rate [22]. Drug-eluting bead transarterial chemoembolization (DEB-TACE) is expected to function as both a drug delivery system and an embolic agent for feeding artery occlusion.

In a previous study, TAE with gelatin sponge was performed on healthy beagle dogs [33]. This study showed no abnormalities in liver tissue with TAE and concluded that it may be a treatment option for dogs with unresectable HCC. However, the embolus recanalized 2 weeks after TAE. Gelatin sponge is an absorbable material and cannot be permanently embolized. TAE using microspheres is thought to be capable of permanent embolization due to its properties, and it may be expected to have a longer ischemic effect on the tumor. Microspheres have a spherical shape and smooth

surface with homogeneous size distribution, the occlusion level is predictable according to the given particle size [1, 21, 34].

There are little information on TAE and TACE in veterinary medicine and to our knowledge, there have been no reports of DEB-TACE performed on dogs. The purpose of our study is to examine and compare the clinical applications and safety of bead-based TAE and DEB-TACE. Therefore, in chapter 1, we conducted a study on the effects of bead-based TAE on healthy beagle dogs.

In Chapter 2, we performed TACE using drug-eluting beads and examined the effects and usefulness of the beads. Based on these results, we compared and discussed whether TAE and TACE using beads are clinically applicable.

Chapter 1: The Effect of Transcatheter Arterial Embolization (TAE) in Normal Canine Liver using trisacryl gelatin microspheres (Embosphere)

Abstract

In this study, the effect of selective transcatheter arterial embolization (TAE) using trisacryl gelatine microspheres (TGMs) in the normal canine liver was investigated. Selective embolization was achieved by injecting TGMs into the left hepatic artery through a microcatheter in four healthy dogs. After embolization, computed tomography (CT), biochemical analysis and histological examination were performed during a 12-week observation period. Embolization was successful in all four dogs. Postoperative CT revealed consistent embolization of the artery within the experimental period in three dogs. Hepatic enzyme levels slightly increased after embolization but tapered to normal ranges. Histological examinations revealed no abnormal changes. Thus, selective TAE with TGMs was well tolerated in normal dogs and may be applicable to canine hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is one of the most common types of liver cancer in human and dog, and its progression has been linked to neoangiogenesis [7, 11, 30]. The blood supply of the liver normally relies on hepatic artery (~20%) and portal vein (~80%)[28], but HCCs depend on hepatic artery almost that. Transcatheter arterial embolization (TAE) is expected that tumor regression without degrading a liver function and damaging normal liver tissues.

Generally, the most common treatment of HCC in dogs is liver resection [25], but this option isn't available for unresectable tumors, such as extremely progression, liver metastases and impatient of invasive surgery. The treatment of these lower invasive and more safety has been demanded in veterinary medicine.

In human, TAE and transcatheter arterial chemoembolization (TACE) is standard treatment for advanced HCC, such as unresectable tumors or liver metastases [37]. TAE improves survival rates, pain, and local control after arterial embolization of unresectable HCC in previous studies. Few studies of TAE for dogs are reported [10, 45], but effects of arterial embolization for the canine liver were poorly documented.

Gelatin sponge particles (GSPs) have been mainly used for TAE in Japan. However, as gelatin is a resorbable material, GSPs are considered temporary embolic agents. In addition, because of their irregular shape and rough surface, GSPs tend to aggeregate proximally in the vessel. For these reasons, the occlusion level is difficult to predict [29, 34].

On the other hand, calibrated spherical embolic agents or microspheres have already been used worldwide for TAE of hypervascular tumors such as HCC. As they have a spherical shape and smooth surface with homogeneous size distribution, the occlusion level is predictable according to the given particle size [1, 21, 34].

Our previous report appeared that TAE for normal dogs using GSPs is relatively safe but, recanalization within 2 weeks [33]. So we hypothesized that using trisacryl gelatin microspheres (TGMs) for TAE is more selective and precise embolization than using GSPs. Therefore, the objectives of this study were to determine the outcomes of selective arterial embolization with TGMs, in terms of clinical signs, biochemical data, computed tomographic and histological findings.

Materials and Methods

Animals

Four 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 hours before anesthesia.

Transcatheter arterial embolization

Anesthesia was induced via slow intravenous administration of propofol (1% intravenous propofol, 7 mg/kg; Maruishi Pharmaceutical Co., Ltd., Japan) and maintained with isofulrane (Isoful, 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 dog was held in the ventral-dorsal position and shaved and disinfected (Figure 1). The right femoral artery was punctured with a 20-G needle and cannulated with a 4-French (Fr) introducer sheath (Vaivt A; Medikit Co., Ltd., Tokyo, Japan) [38] (Figure 2). 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) (Figure 3). 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 2.0-Fr microcatheter (Sniper 2, 110 cm; Terumo Clinical Supply Co., Ltd.) was placed in the left hepatic artery toward the left lateral lobe prior to injection of contrast agent (Optiray 350; Covidien Co., Ltd., Tokyo, Japan) under digital subtraction angiography (DSA) (Figure 4). Embolization was achieved with TGMs (Embosphere, 100-300 μm; Nipponkayaku Co., Ltd., Tokyo, Japan) diluted twice with contrast medium. In all dogs, TGMs were injected until an overflow of contrast medium was observed with DSA. After embolization arteriograms were obtained to confirm complete occlusion of the left hepatic artery

(Figure 5). After angiography, removed sheath and compressed hemostasis. All dogs were monitored for 12weeks after TAE.

Evaluations

After embolization, physical examination was performed once a day for 2- weeks, then twice a week for remaining 10weeks. Aspartate- aminotransferase (AST), alanine aminotransferase (ALT), alkaline- phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total bilirubin, lipase and C-reactive protein (CRP) levels were measured before and 1, 3 days 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 weeks 1, 2, 4, 12. 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 reconstructions of the hepatic vessels were generated with Ziostation2 software (Ziosoft, Inc., Tokyo, Japan).

Liver biopsies were obtained via laparoscopy after TAE at day 3 and weeks 1, 2, and 4. All samples took from left lateral lobe of the liver were fixed in 4% paraformaldehyde and embedded in paraffin. Tissue sections were stained with hematoxylin-eosin (HE), Gitter, periodic acid Schiff (PAS), and Azan stains (Figure 6).

Data analysis

Body weights and duration of TAE are reported as means ± standard deviations. Pre-embolization and postembolization values were compared using a paired t-test. The differences with probability values (P) of 0.05 or less were regarded as significant.

Results

The mean body weight of dogs in our study was 9.96 ± 2.04 kg. We could insert microcatheter into the left hepatic artery toward the left lateral lobe under DSA guidance in all dogs and inject TGMs before overflow was observed. Complete occlusion of the left hepatic artery toward the left lateral lobe was confirmed postembolization hepatic arteriograms all dogs, but immediately CT after procedure, one was confirmed left hepatic artery and the others were confirmed occlusion in arterial phase. The mean duration of TAE was 80 ± 47.52 minutes.

Clinical examination of dogs remained within normal limits throughout the study.

The embolization of left hepatic artery was confirmed 12 weeks after treatment by post CT (Figure 7).

Hepatic enzyme levels increased, but almost normal during the period (Figure 8). The other parameters were not altered in any of the dogs.

There were no obvious abnormalities in tissues from left lateral lobes after TAE. No evidence of perivascular hemorrhage or inflammation was found in the vessel wall or surrounding tissues.

Discussion

In this study, we could cannulate selectively left hepatic artery toward the left lateral lobe in all dogs. Our previous study [33], embolization of left hepatic artery was successful, but superselective embolization such as left hepatic artery toward the medial or lateral lobe weren't successful. We believe that superselective embolization reduce the damage of parenchyma of liver. Compared our past study [33], hepatic enzyme levels increased mild in this study. Previous study reported nonselective embolization of common hepatic artery was caused centrilobular necrosis [10]. On the other hand, Another study reported, TAE with small particles less than 500 μm and peripheral embolization cause a high incidence of bile duct injury [39]. However, bile duct injury wasn't observed any dogs in this study. According to Stampfl et al [40], 40-120 μm TGMs did not penetrate the peribiliary plexus and hepatic sinusoids in a pig liver model. This report suggested that superselective embolization was safer than selective embolization.

Generally, embolization of artery is confirmed angiography that lack of vascular shadow. This study, one dog was confirmed occlusion by angiography after

embolization, but post CT was appeared left hepatic artery toward the left lateral lobe. Previous report described vessel spasm cause a recanalization [13]. We might misjudge embolization under angiography by the temporary vasoconstriction.

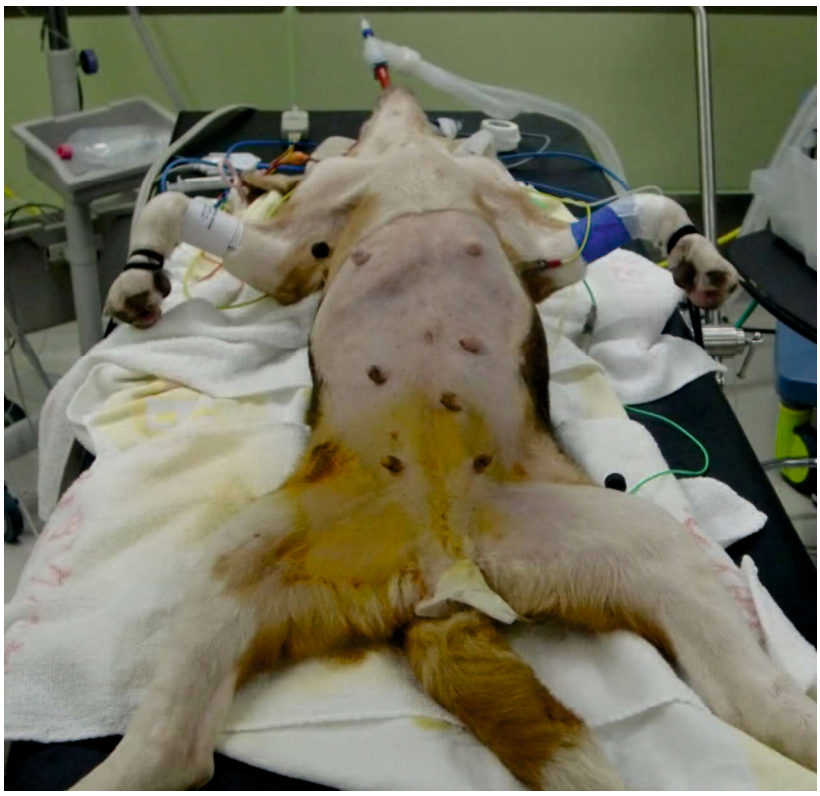
Postembolization syndrome occurred after TAE in human, such as abdominal pain, fever, discomfort etc [7, 19]. All dogs didn't confirmed such signs after procedure in this study. Although these dogs didn't have tumor, TAE seemed to be well-tolerated dogs.

Embolization with TGMs were maintained 12 weeks after TAE in this study. We reported embolization with GSPs were maintained 2weeks [33]. TGMs are nonresorbable particles, so embolization with TGMs expect permanent occlusion of arteries. However it is not clear which is better, the permanent occlusion or temporary occlusion of TAE in human. It is because there is little evidence in the literature comparing the efficiency between different embolizing agents especially for TAE [37]. According to Maluccio et al [26], terminal vessel occlusion should maximize ischemic tumor necrosis. On the other hand, Koçyiğit et al [18], occlusion of the feeding artery for a shorter period of time can be sufficient to achieve satisfactory ischemia. Generally, the most of the antitumoral effects of TAE is local ischemia, so

we believe that long term occlusion enhance the antitumoral effects. Further studies are needed to confirm the usefulness and safety of TAE in dogs with HCC. Our study was limited, but we believe that TAE with TGMs can be a treatment of canine HCC.

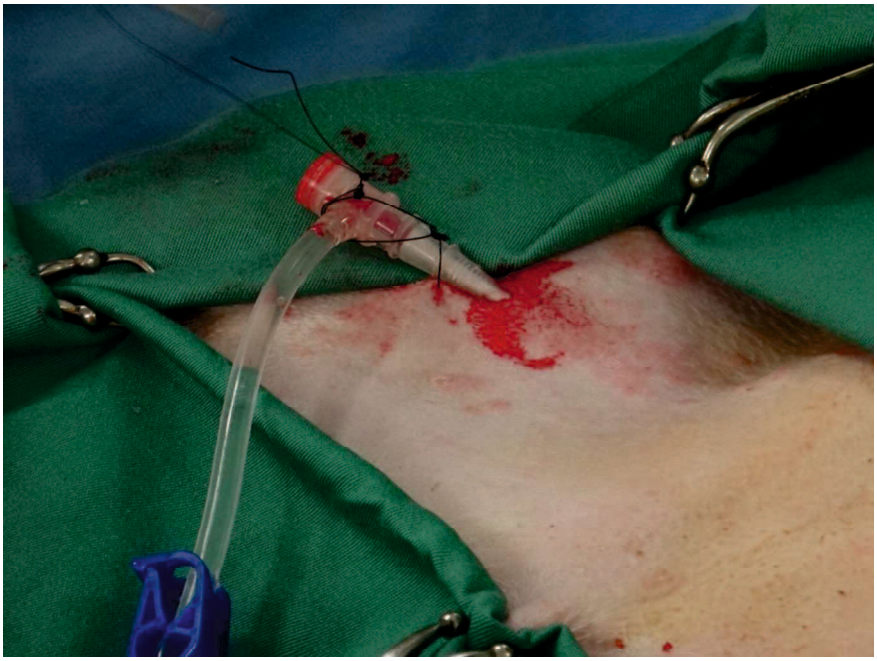
Figures

Figure 1



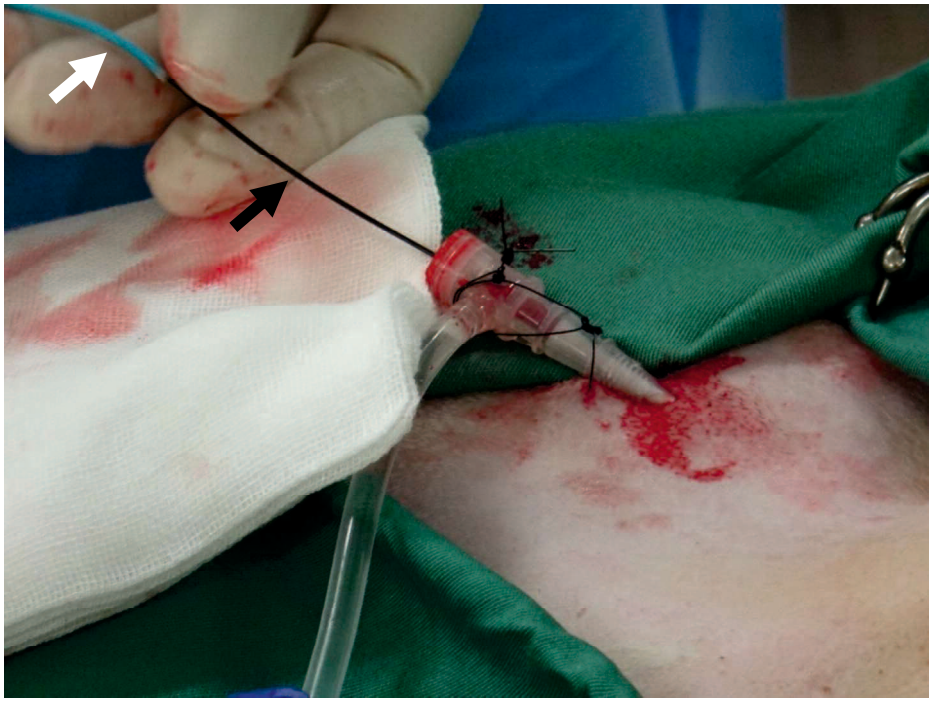
The dog was held in the ventral-dorsal position and shaved and disinfected.

Figure 2



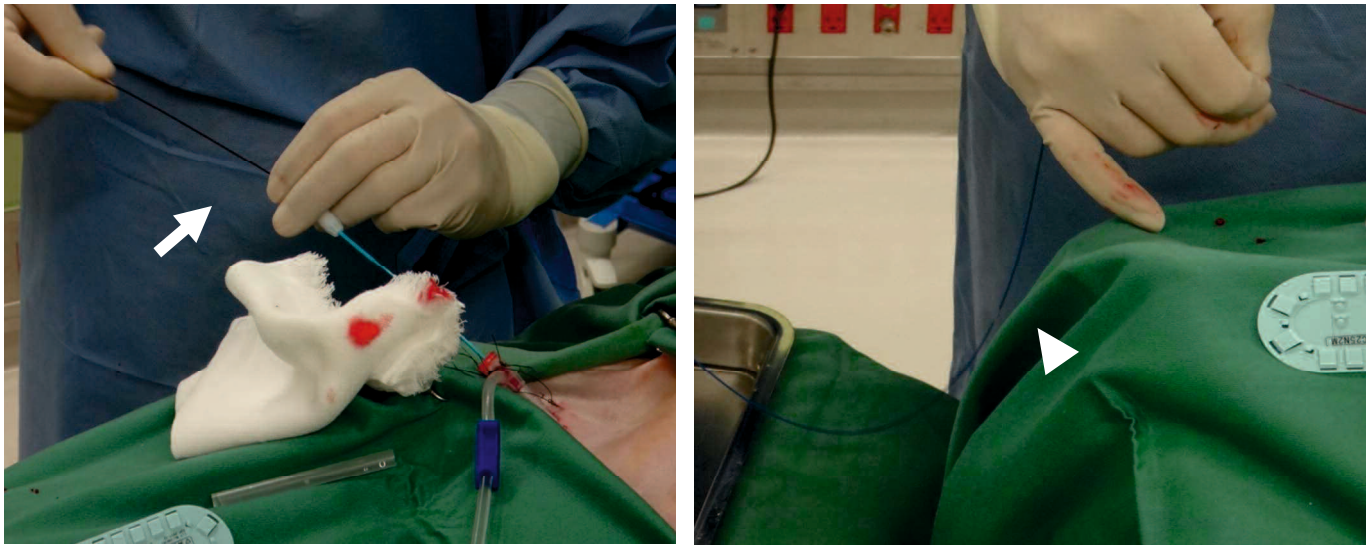
The right femoral artery was punctured with a 20-G needle and cannulated with a 4-French (Fr) introducer sheath.

Figure 3



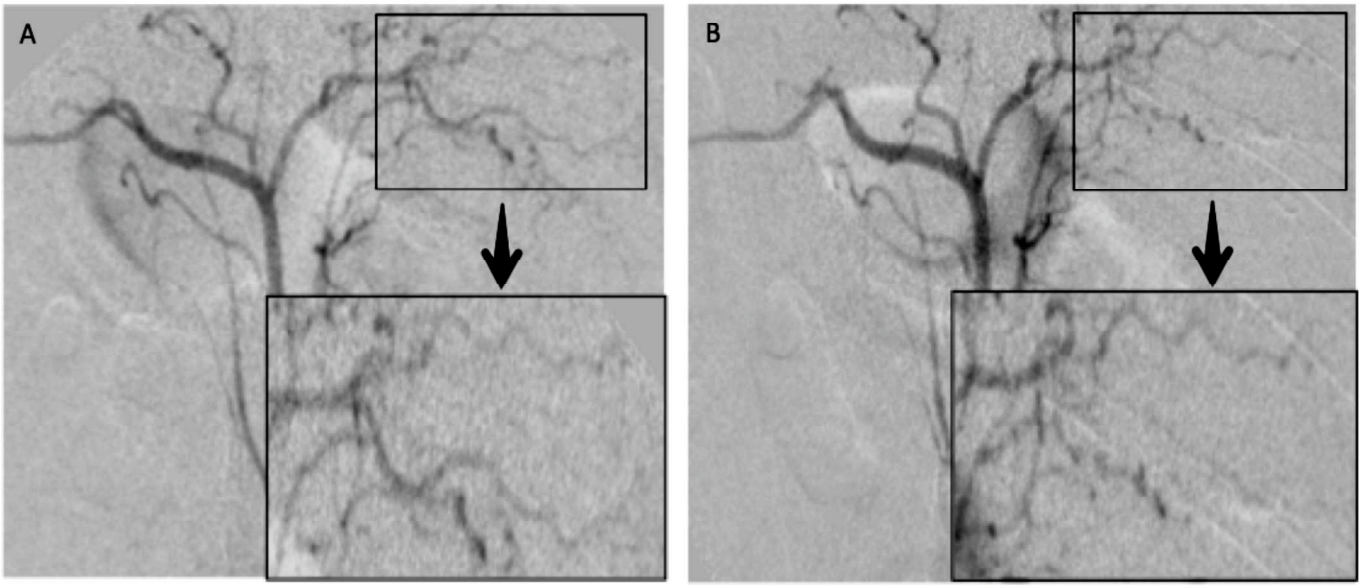
A guidewire (black arrow) and catheter (white arrow) were inserted into the aorta and celiac artery under fluoroscopic guidance.

Figure 4



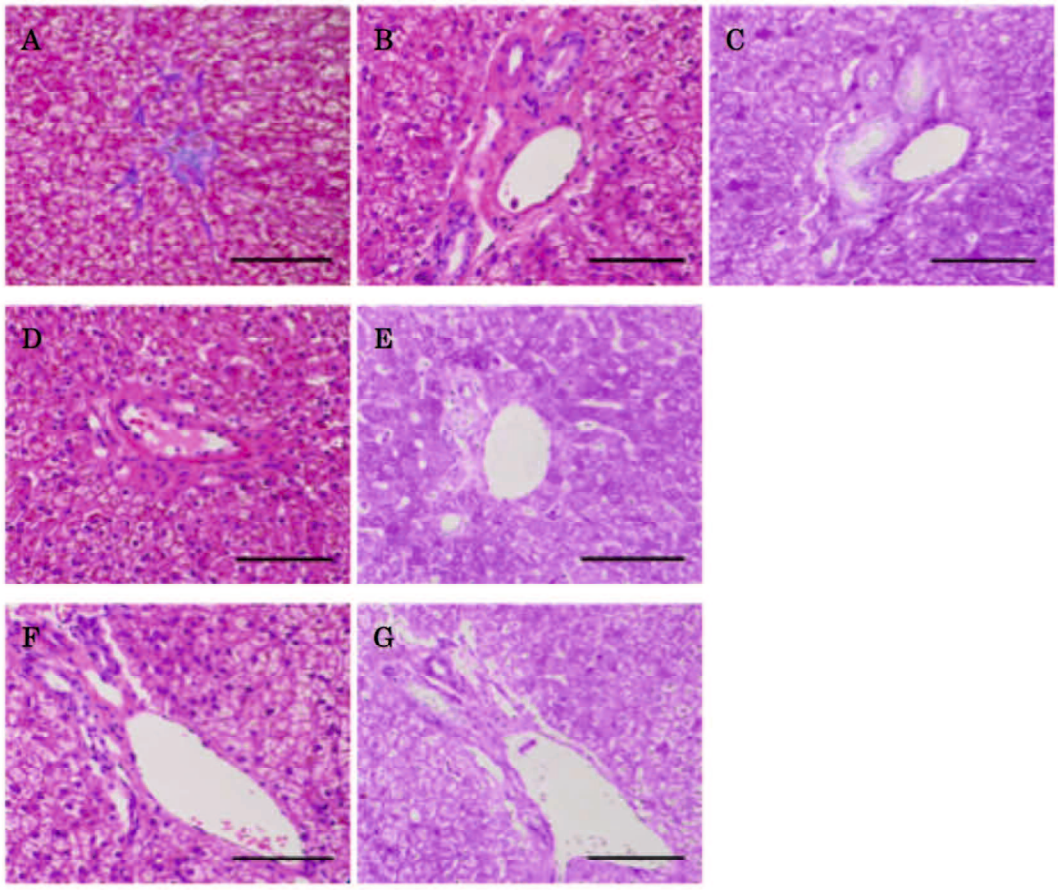
A microguidewire (white arrow) was advanced into the common hepatic artery through the catheter and a 2.0-Fr microcatheter (white arrowhead) was placed in the left hepatic artery toward the left lateral lobe prior to injection of contrast agent under digital subtraction angiography (DSA).

Figure 5



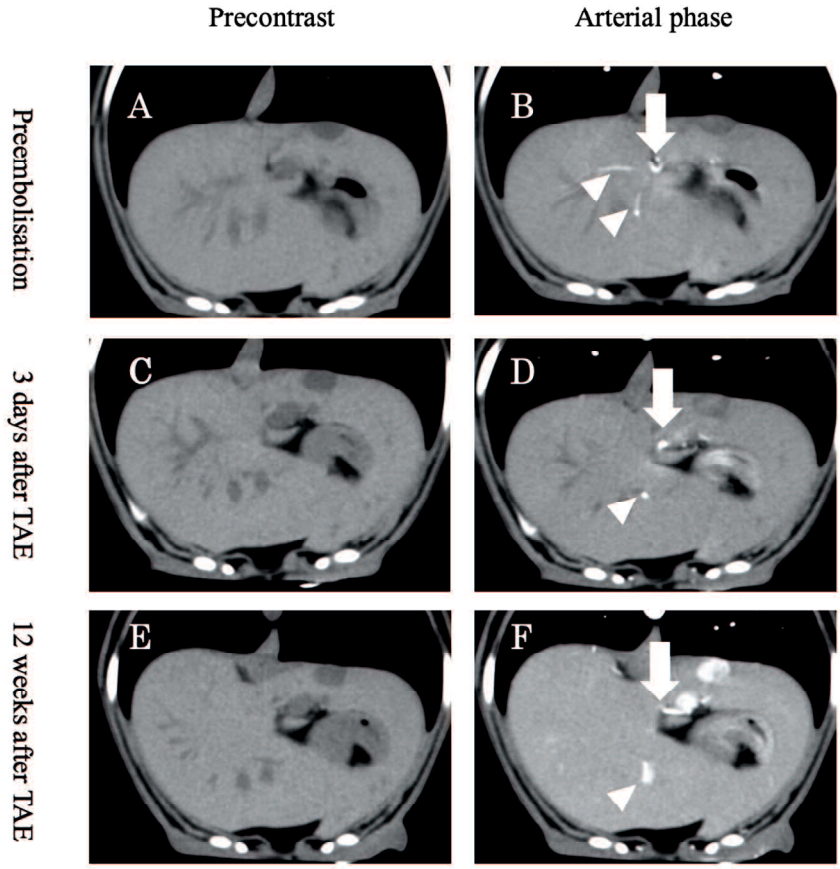
Ventral-dorsal digital subtraction images of a dog. A, Preembolization hepatic arteriogram. B, Postembolization hepatic arteriogram showing complete occlusion of the left hepatic artery toward the left lateral lobe.

Figure 6



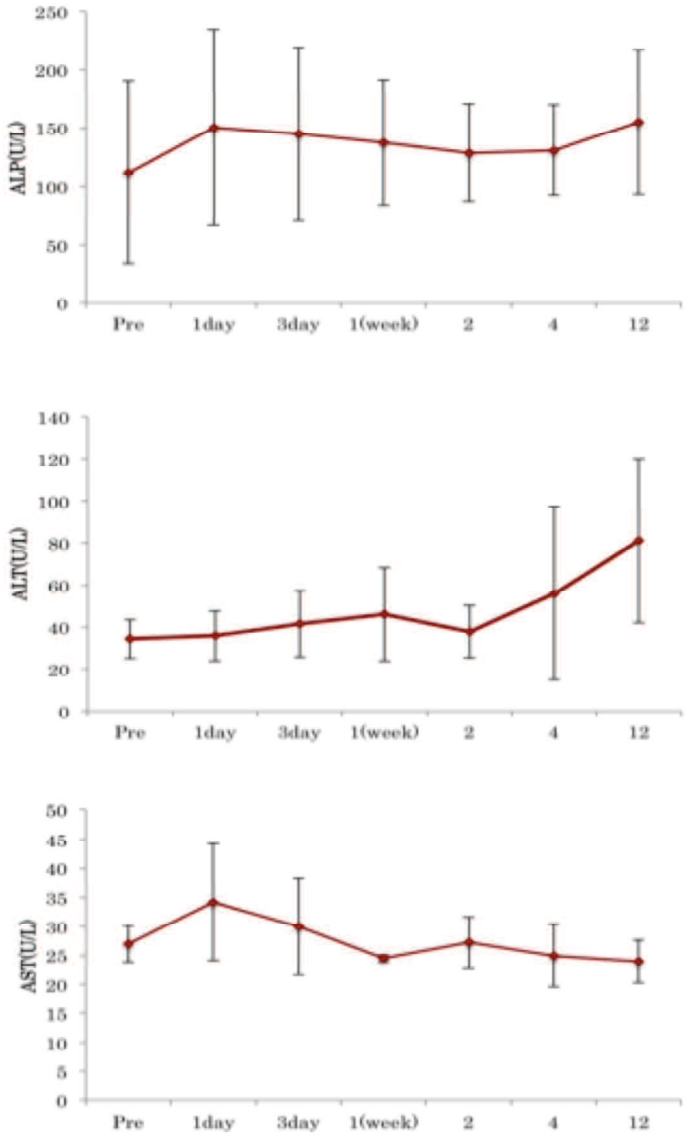
Histological appearance of the left lateral liver lobes 3 days after TAE after staining with (A) Azan, (B) hematoxylin-eosin (HE), (C) periodic acid-Schiff (PAS). Histological appearance of the left lateral liver lobes 1 week after TAE after staining with (D) HE, (E) PAS. Histological appearance of the left lateral liver lobes 2 weeks after TAE after staining with (F) HE, (G) PAS. There was no obvious abnormality in liver tissues. Scale bar = 100μm.

Figure 7



Transverse computed tomography (CT) images. A, preembolization. B, An arterial-phase of preembolizaton: note the left hepatic artery (white arrow), the left hepatic artery toward the left lateral lobe (white arrowhead). C, 3 days after TAE. D, An arterial-phase of 3 days after TAE: the upper left hepatic artery toward the left lateral lobe was embolization. E, 12 weeks after TAE. F, An arterial-phase of 12 weeks after TAE: the upper left hepatic artery toward the left lateral lobe remained an emobolization.

Figure 8



Changes in alkaline -phosphatase (ALP), aspartate -aminotransferase (AST), and alanine -aminotransferase (ALT) levels during the clinical period. These levels were almost normal (*P <. 05)

Chapter 2: Effect of drug-eluting bead transarterial chemoembolization loaded with cisplatin on normal dogs

Abstract

Transcatheter arterial embolization (TAE) and transcatheter arterial chemoembolization (TACE) are standard treatments for advanced hepatocellular carcinoma (HCC) and particularly for unresectable tumors or liver metastases in humans. However, reports on TACE used in veterinary medicine are few. This study aimed to evaluate the feasibility and safety of drug-eluting bead transarterial chemoembolization (DEB-TACE). We performed DEB-TACE in four clinically normal dogs and pharmacokinetically compared the results against hepatic arterial infusion (HAI) of cisplatin in two dogs. Drug-eluting beads (DEB) loaded with cisplatin were injected through a microcatheter for selective embolization of the left hepatic artery. After embolization, computed tomography (CT) images and histological examination findings were obtained during a 4-week observation period. Serum platinum concentrations were measured to evaluate cisplatin after each procedure. Biochemical analysis was performed during a 12-week observation period. Embolization was successful in all dogs, and there were no clinically apparent abnormalities. Embolization was confirmed up to 4 weeks after DEB-TACE in two of

the four dogs and up to 1 week in the other two dogs using postoperative CT images. Cisplatin was not detected in peripheral veins in all dogs after DEB-TACE, but it was detected in trace amounts after HAI. DEB-TACE using cisplatin was safe and well tolerated by normal dogs. DEB-TACE may be useful in terms of determining systemic toxicity and drug concentration within tumors.

Introduction

Unresectable advanced hepatocellular carcinoma (HCC) has a poor prognosis with limited treatment options in veterinary medicine. Transcatheter arterial embolization (TAE) and transcatheter arterial chemoembolization (TACE) are widely used standard treatments for unresectable advanced HCC and liver metastases in humans [7, 15, 36, 37]. Improved survival rates, reduced pain, and local control have been reported after arterial embolization of unresectable HCC. The general concept of TACE is to combine the local infusion of chemotherapeutic agents with selective embolization of the feeding arteries of the tumor. No consensus exists on the most effective embolizing agent. Drug-eluting beads (DEB) is a novel drug delivery system that is specifically designed to deliver a drug directly into the tumor tissue at a slow rate [22]. Drug-eluting bead transarterial chemoembolization (DEB-TACE) is expected to function both as a drug delivery system and an embolic agent for feeding artery occlusion. Bland TAE in healthy beagles was reported to be safe [31, 33], and possibly effective in veterinary practice [32]. Reports of TACE performed in dogs are rare [3, 45], and to the best of our knowledge, no reports have been published on

DEB-TACE performed in dogs. This study sought to determine the feasibility and safety of DEB-TACE in dogs in terms of clinical signs, biochemical data, computed tomography (CT) findings, histological findings, and pharmacokinetics.

Materials and Methods

Animals

Eight 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 hrs before anesthesia.

Transarterial chemoembolization

We performed DEB-TACE on four dogs. Anesthesia was induced via slow intravenous administration of propofol (1% intravenous propofol, 7 mg/kg; Maruishi Pharmaceutical Co., Ltd., Japan) and maintained with isoflurane (Isoful, 1.4%–2.5%; Dainippon Sumitomo Pharma Co., Ltd., Tokyo, Japan) and oxygen. All dogs were administered an 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 (Vaivt A; Medikit Co., Ltd., Tokyo, Japan)[38]. 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 1.7-Fr microcatheter (Derniere, 105 cm; Create Medic Co., Ltd., Kanagawa, Japan) was placed in the left hepatic artery toward the left lateral lobe prior to the injection of a contrast agent (Optiray 350; Covidien Co., Ltd., Tokyo, Japan) under digital subtraction angiography (DSA) (Figure 1). Chemoembolization was achieved with DEB (Hepasphere, 50–100 μm; Nippon Kayaku Co., Ltd., Tokyo, Japan). DEB swelled with contrast medium and cisplatin (IA-call; Nippon Kayaku Co., Ltd.) following the manufacturer's instructions (Figure 2). In all dogs, DEB before overflow was observed (approximately 0.3–0.4 ml of DEB was injected, and cisplatin was contained in 0.21–0.28 mg). After embolization, arteriograms were

obtained to confirm complete occlusion of the left hepatic artery. After removing the sheath, the puncture site was manually compressed. All dogs were monitored for 12 weeks after DEB-TACE.

Hepatic arterial infusion (HAI) and intravenous cisplatin

Anesthesia administration and catheter insertion were performed similar to those of DEB-TACE. Cisplatin was injected into the left hepatic artery toward the left lateral lobe in the same dose as that for DEB-TACE for two dogs. To compare pharmacokinetics, we injected cisplatin (50mg/m^2) into the cephalic vein for two dogs.

Evaluations

After chemoembolization, physical examination was performed once a day for 2 weeks and then twice a week for the remaining 10 weeks. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), total bilirubin (T.Bil), lipase (LIP), C-reactive protein (CRP), blood urea nitrogen (BUN), and creatinine (CRE) levels were measured before the procedures were performed and at 1 and 3 days after DEB-TACE. These tests were

repeated at 1, 2, 4, 8, and 12 weeks after DEB-TACE. To evaluate serum platinum (Pt) concentration, blood sampling was immediately performed after each procedure and was repeated at 5, 15, 30, and 60 min and at 2, 6, 12, 24, 48, and 72 hrs after the procedures. Serum Pt was measured by atomic absorption spectrometry (Z5700; Hitachi High-Tech Co., Ltd., Tokyo, Japan).

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

Liver biopsies were obtained via laparoscopy after DEB-TACE at 1 and 2 weeks. All samples were taken from the left lateral lobe of the liver, fixed in 4% paraformaldehyde, and embedded in paraffin. Tissue sections were stained with hematoxylin–eosin (HE), periodic acid-Schiff, and Azan stains.

Data analysis

Body weight and duration of DEB-TACE were reported as mean ± standard deviation.

Results

The mean body weight of the dogs in our study was 9.57 ± 1.32 kg. Postembolization hepatic arteriograms (Figure 3) and CTs of all dogs confirmed complete occlusion of the left hepatic artery toward the left lateral lobe. The mean duration of DEB-TACE was 39.25 ± 7.36 min and that of HAI was 21.5 ± 0.5 min. Postoperative CT confirmed recanalization at week 1 in two of the four dogs (dogs 3 and 4); however, the remaining two dogs successfully maintained embolization during the 4-week observation period (Figure 4).

LIP was increased in one dog, CRP in four dogs, and ALP in two dogs after DEB-TACE, but these were generally within the normal range throughout the study period (Figure 5). ALP was also increased in all dogs after HAI, but the increase was quite less (around 300 IU/L).

The serum concentrations of Pt were below detection limit in all dogs after DEB-TACE and were found in trace amounts in dogs after HAI (only 6 hrs after administration). In dogs intravenously administered cisplatin, Pt was reduced biphasically as previously reported (Figure 6) [13].

There were no obvious abnormalities in the left lateral lobes further distal to the embolization site and no perivascular hemorrhage or inflammation in the vessel wall or surrounding tissues after DEB-TACE (Figure 7). Moreover, clinical readings remained within normal limits throughout the study.

Discussion

In healthy beagle dogs, DEB-TACE with cisplatin may reduce the side effects of cisplatin because the drug's sustained release keeps the blood concentration of the anticancer drug lower than that with intravenous or intrahepatic arterial administration. In this study, Pt was found in HAI and DEB-TACE below or close to the detection limit, suggesting that the side effects could be greatly reduced compared with intravenous cisplatin. However, only ~42% of cisplatin (approximately 0.12 mg) was contained within DEB, and the remaining 58% was outside DEB. There was more cisplatin outside DEB, and the pharmacokinetics may not be much different between HAI and DEB-TACE. The tumor intravascular volume may be larger in clinical cases with HCC, resulting in a higher dose, which could indicate a significant difference between HAI and DEB-TACE.

The DEB (Hepasphere) gradually released cisplatin into the liver and embolized the hepatic artery so that the time of exposure of the liver to cisplatin was increased and cisplatin accumulated in the liver with a low discharge into the systemic circulation[6, 44]. In humans, cisplatin was administered through the hepatic artery

and through an intravenous line, and serum Pt concentration was lower in the intra-arterial group than that in the intravenous group [41]. Furthermore, in previous reports [2, 8, 41], the anticancer drug concentration in the tumor was higher with HAI than with systemic administration. In addition, in a study on intrahepatic administration of cisplatin in rabbits [9], the cirrhotic group tended to have a higher anticancer drug concentration in the liver, and the serum Pt concentration was initially lower in the cirrhotic group than that in the normal liver group. Pharmacokinetics may be altered in patients with hepatic tissue damage and impaired liver function. For these reasons, DEB-TACE can possibly keep serum concentrations low through the intra-arterial administration of anticancer drugs, prolonging the accumulation of anticancer drugs in the liver by blocking blood flow.

In our study, embolization of the hepatic artery persisted for 4 weeks in two of four dogs, but the remaining two dogs were recanalized 1 week later. Hepasphere is considered a permanent embolic agent, but the beads move distally by redistribution [4, 42]. The permanent occlusion of the hepatic artery imaged by DSA is not achieved with beads as small as 700 μm [43]. A previous report [21] described that beads redistributed to deeper regions could be phagocytosed or ejected out of the vessel. We

used Hepasphere with a size of 50–100 μm before expanding and a diameter of about 540 μm after expanding, so the beads may have moved to more peripheral vessels because of redistribution and recanalization. Furthermore, Hepasphere released the drug gradually, which may have reduced its size and caused recanalization. According to Kocyigit *et al.* [18], occlusion of the feeding artery for a short period of time was found to be sufficient for achieving satisfactory ischemia.In addition, TACE was performed multiple times and was scheduled to be repeated every 2–6 months [20] or on demand [12], during which TACE was added as the tumor remained or relapsed. For these reasons, permanent embolization of the vessel in a single treatment may not be necessary.

In this study, no obvious abnormalities in the liver tissue distal to the embolization site were observed. The liver blood supply is provided by two vessels, the hepatic artery (approximately 20%) and portal vein (approximately 80%) [28]; however, HCC is mainly perfused by the hepatic artery [25, 30]. Therefore, TACE is believed to be able to embolize the tumor's feeding artery while sparing the surrounding liver parenchyma [23, 47, 48]. Although this study was conducted in normal dogs, selective

embolization of the tumor's feeding artery is expected to have little effect on the normal liver tissue in HCC.

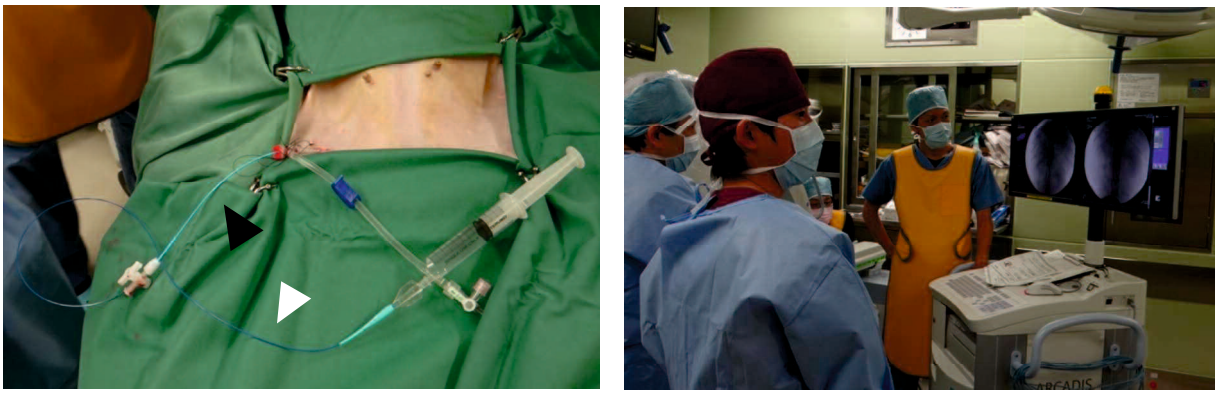
Embolization with smaller particles has been found to increase tumor ischemia, but it is also more likely to cause complications such as bile duct injury or liver necrosis [7, 33, 39]. We used a small-sized particle for DEB-TACE, but no such pathological abnormalities were observed in this study. The use of small particles for DEB-TACE may not be associated with those complications, although a smaller volume of embolization was carried out in this study than that used in a case with tumor.

In veterinary medicine, liver lobectomy is considered the gold standard for dogs with HCC even with incomplete resection [16, 27], and interventional radiology (IVR) is still not widely used. The less number of dogs in each group, lack of a control group and use of DEB doses different from those used in clinical practice were the limitations of this study. These may have affected the accuracy of the investigation. Despite these limitations, DEB-TACE has been demonstrated to be safe in normal dogs. It was noted that DEB-TACE embolized the hepatic artery while reducing systemic exposure to the anticancer drug through sustained release in normal dogs.

Further studies are needed to assess whether the application of DEB-TACE is more clinically relevant in dogs with unresectable hepatocellular carcinoma.

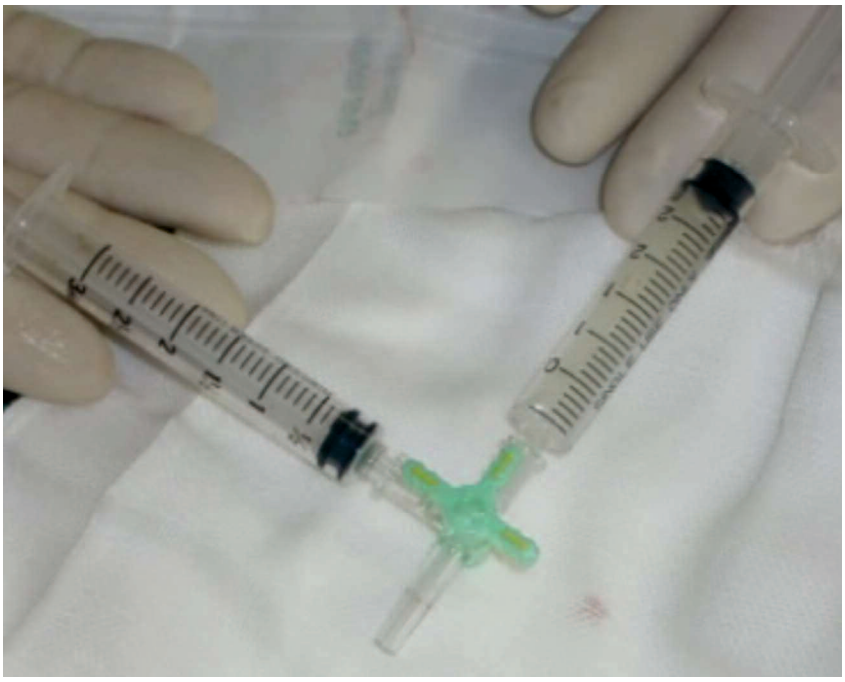
Figures

Figure 1



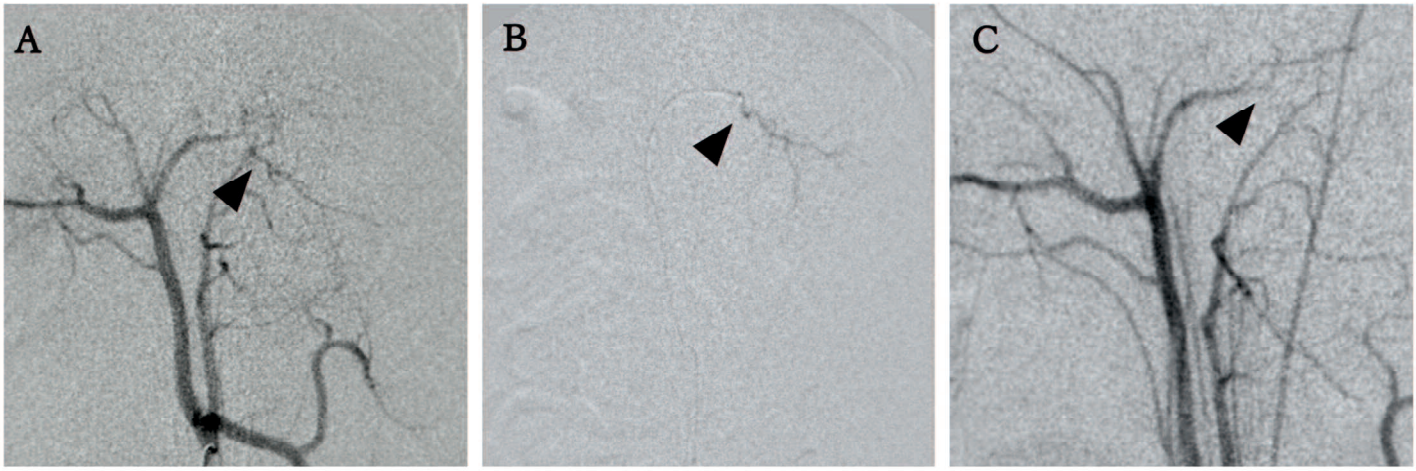
A catheter (black arrowhead) and 1.7-Fr microcatheter (white arrowhead) were placed in the left hepatic artery toward the left lateral lobe prior to the injection of a contrast agent under digital subtraction angiography (DSA)

Figure 2



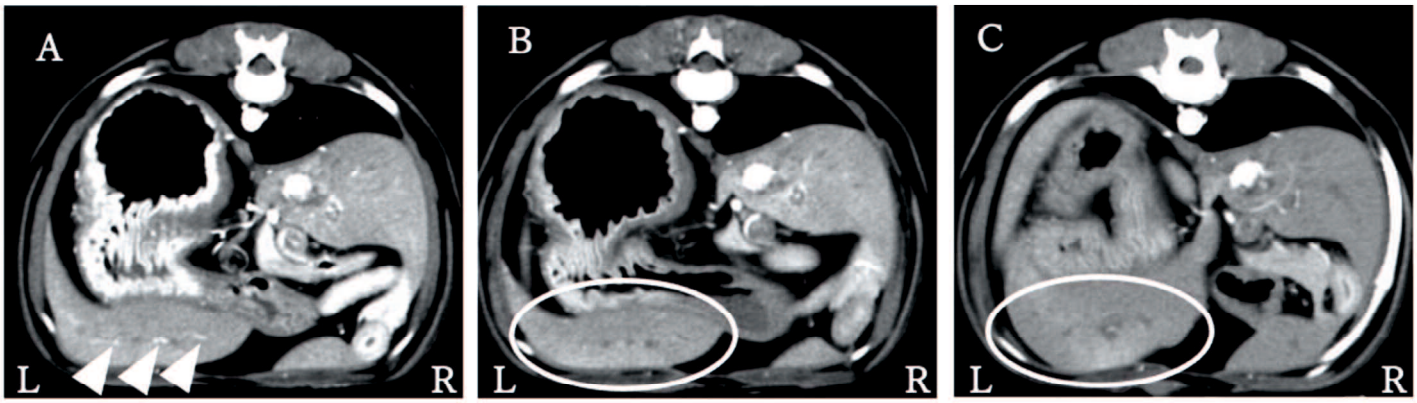
DEB containing anticancer drug is mixed with contrast medium using a 3-way stopcock before DEB was injected.

Figure 3



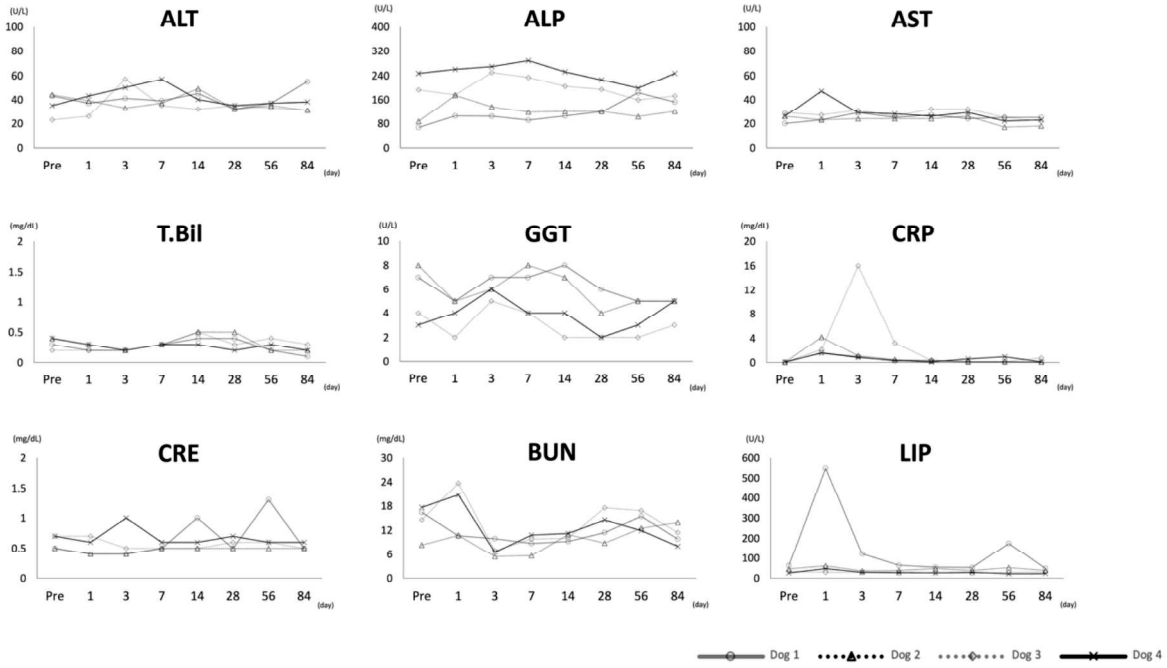
Ventral–dorsal digital subtraction images of a dog. A. Pre-embolization hepatic arteriogram. B. During embolization, injecting Hepasphere into the left hepatic artery toward the left lateral lobe. C. Postembolization hepatic arteriogram showing complete occlusion of the left hepatic artery toward the left lateral lobe. The black arrowhead indicates the left hepatic artery toward the left lateral lobe.

Figure 4



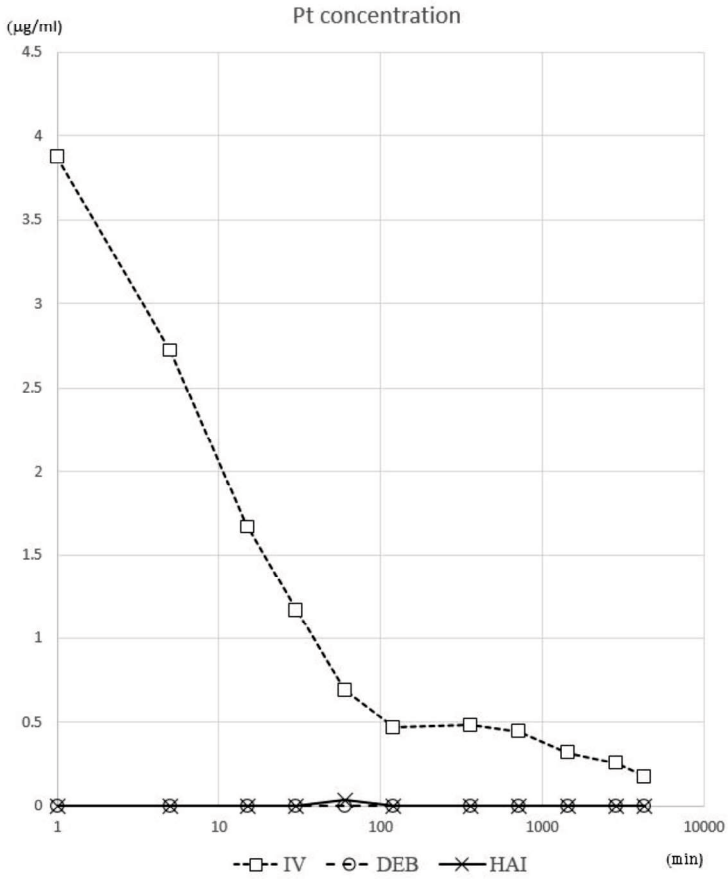
Transverse computed tomography (CT) images. A. An arterial phase CT of pre-embolization (white arrowheads indicate the left hepatic artery toward the left lateral lobe). B. An arterial phase CT 1 week after DEB-TACE. The left hepatic artery toward the left lateral lobe (surrounded by a white circle) was embolized. C. An arterial phase CT 4 weeks after DEB-TACE. The left hepatic artery toward the left lateral lobe (surrounded by a white circle) remained embolized.

Figure 5



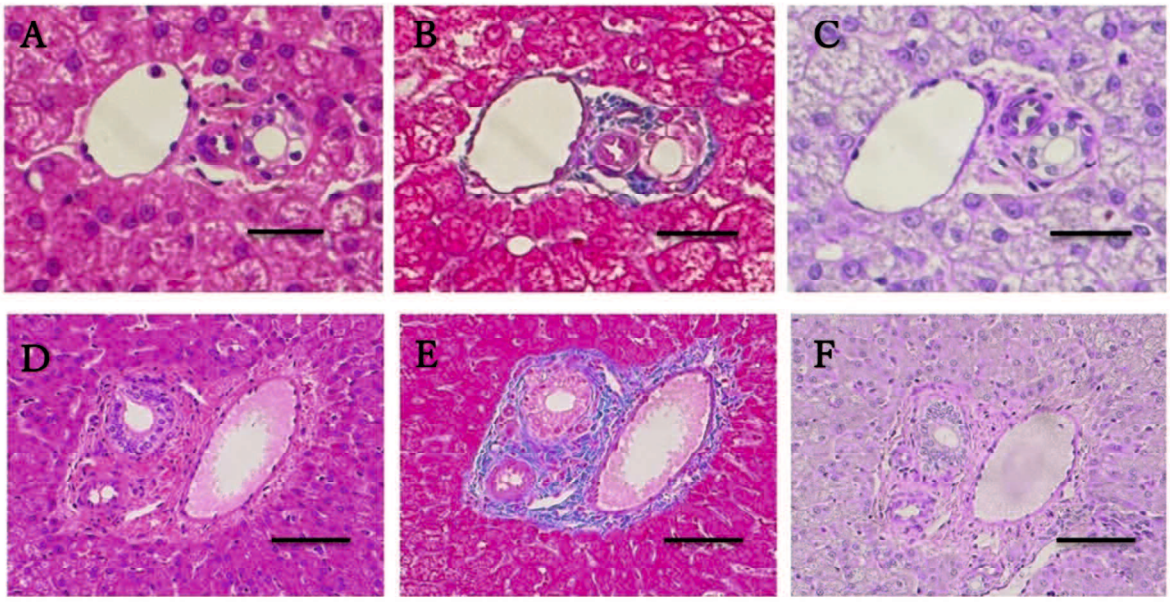
Changes in alanine -aminotransferase (ALT), alkaline -phosphatase (ALP), aspartate -aminotransferase (AST), γ -glutamyl-transpeptidase (GGT), total-bilirubin (T.Bil), lipase (LIP), C-reactive protein (CRP), blood-urea-nitrogen (BUN) and creatinine (CRE) levels during the clinical period. These levels were almost normal.

Figure 6



Blood levels of serum platinum (Pt) in dogs after treatments with hepatic arterial infusion (HAI), drug-eluting bead transarterial chemoembolization (DEB-TACE), or intravenous infusion (IV) of the cephalic vein. A trace amount of Pt was detected in two dogs only 6 hr after administration, although it was close to the detection limit.

Figure 7



Histological appearance of the left lateral liver lobes 1 week after treatment. Staining with A. hematoxylin–eosin, B. Azan, and C. periodic acid-Schiff. At 2 weeks after treatment, after staining with D. hematoxylin–eosin, E. Azan, and F. periodic acid-Schiff. No obvious abnormalities were observed in these liver tissues. Scale bar = 100 μm.

OVERALL DISCUSSION AND CONCLUSION

The present study suggested that TAE with microsphere and DEB-TACE may be a relatively safe and useful treatment for canine HCC. There was little effect on the normal liver in both treatments, but there was a difference in the embolization period. As described above, the particle size of DEB-TACE is smaller than that of microspheres used in TAE due to the sustained release of the drug, and therefore, it was thought to be more likely to cause recanalization. In terms of embolization, TAE may be superior to DEB-TACE, because TAE causes little change in size. However, DEB-TACE is very attractive to increase the drug concentration in the tumor and reduce the systemic side effects by sustained release of anticancer drugs with embolization. Considering the tumor necrosis effect of both embolization and anticancer drugs by DEB-TACE, it cannot be concluded that TAE is superior just because of the longer embolization period.

There is no significant difference between the TAE and DEB-TACE in terms of safety in normal dogs. Furthermore, considering that the goal of TAE and DEB-TACE therapy is to extend survival and improve quality of life by reducing tumor size, it is

difficult to judge which is superior from the results of this study alone. This study is limited, and it is necessary to perform TAE and DEB-TACE in canine HCC to determine which is superior for comparison. There is no answer, but we think the treatment strategy is slightly different between TAE and DEB-TACE. Because of its long embolization period, TAE with microspheres is expected to be used in situations where long-term embolization is required in a single procedure, such as in the central liver region where surgery is considered difficult and tumors are not very large. On the other hand, DEB-TACE can be used for unresectable large tumors with active angiogenesis because it can release anticancer drugs and has a short embolization period, allowing for multiple treatments. It is important to consider which treatment to select based on the condition of the patient, the size of the tumor, and the location of the tumor.

Further study is needed, we believe that TAE with microsphere and DEB-TACE can be new treatments for unresectable hepatocellular carcinoma.

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