Percutaneous US-Guided Implantation of Vx-2 Carcinoma into Rabbit Liver:

A Comparison With Open Surgical Method

Kwang-Hun Lee, M.D. [*,†], **Eleni Liapi, M.D.** [*], **Manon Buijs, M.D.** [*], **Josephina Anna Vossen, M.D.** [*], **Veronica Prieto-Ventura, M.D.** [*], **Labiq H. Syed, M.D.** [*], and **Jean-Francois H. Geschwind, M.D.** [*,1]

[*]Division of Vascular and Interventional Radiology, The Russell H. Morgan Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, Maryland [†]Division of Interventional Radiology, Department of Radiology and Research Institute of Radiological Science, Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea

Abstract

Purpose—To evaluate technical feasibility and experimental usefulness of percutaneous US-guided implantation of Vx-2 carcinoma in rabbit liver.

Materials and Methods—Forty-eight New Zealand White male rabbits were used. Solid tumor mass of Vx-2 carcinoma was harvested from carrier rabbit, and minced tumor cells were implanted. Twenty-four rabbits underwent percutaneous US-guided tumor implantation, and the same number of rabbits underwent open laparotomy tumor implantation. Tested parameters included technical success, procedural time, amount of anesthesia, recovery time, complications, tumor size, and regional tumor seeding.

Results—A new percutaneous US-guided implantation was technically feasible in all rabbits. Evaluation parameters showed that the percutaneous US-guided implantation method is less invasive than the open laparotomy method. Targeting rate for left lateral lobe of implantation site was comparable in both methods (91.7% of percutaneous US-guided; 95.8% in open laparotomy). The success rate of tumor growth in the liver was 100% in both groups. However, in the group with US-guidance, tumor seeding developed more frequently in five of 24 rabbits (20.8%) than in open laparotomy group (2/24, 8.3%). Five rabbits had thoracoabdominal wall needle tract seeding, and two rabbits had tumor seeding at both thoracoabdominal wall and intraperitoneal space.

Conclusions—Percutaneous US-guided implantation of Vx-2 carcinoma in rabbit liver is a less invasive alternative to open laparotomy, achieving equally successful tumor growth in the liver. Although percutaneous US-guidance implantation method may not be considered for long-term survival study design because of the possibility of tumor seeding, it can be considered for nonsurvival study design.

INTRODUCTION

The liver is not only the most common site for metastases from gastrointestinal tumors, malignant melanoma, and primary liver tumors, but also the common organ of primary

[1]To whom correspondence and reprint requests should be addressed at Division of Vascular and Interventional Radiology, Johns Hopkins Hospital, 600 North Wolfe Street, Blalock 545, Baltimore, MD 21287. jfg@jhmi.edu.

hepatocellular carcinoma. So far, the Vx-2 carcinoma implanted into the liver of rabbits has commonly been used for experimental diagnostic and therapeutic research studies. The Vx-2 carcinoma is an animal tumor model that was first introduced by Shope *et al.* [1]. It is a fast growing carcinoma derived from a virus-induced papilloma of rabbits. Although the Vx-2 tumor is of nonhepatic origin, it has been proven to be convenient for the setup of animal liver cancer studies due to its rapid growth and similarities of its blood supply and genotype to that of human hepatocellular carcinoma.

In some early experiments, cell suspension of Vx-2 carcinoma was injected *via* several routes, including the hepatic artery or portal vein [2,3], directly into the liver parenchyma [2], and into the gastrointestinal walls [4] to achieve liver tumor growth. However, this method inevitably had significant problems regarding tumor seeding, with either leakage of cells into the peritoneal cavity during the injection procedure *via* the injection route, or with injection of cancer cells into the blood stream directly *via* the hepatic or portal vein[5,6]. More recent studies have tried to avoid this significant problem by using minced tumor cells harvested from rabbit carrier and implanting them in the liver with an open laparotomy procedure [7-9]. However, open surgery procedures may add significant stress to animals due to longer anesthesia times and postprocedural recovery and possible complications.

In this study, we attempted to investigate the technical feasibility and success rate of percutaneous ultrasound (US-) guided implantation of Vx-2 carcinoma into the liver of rabbit as an alternative method of tumor implantation and compare this technique to the most commonly used open laparotomy.

MATERIALS AND METHODS

Our institution's Animal Care and Use Committee approved the study, and all animal care and procedures were performed under institutional guidelines.

Animal and Tumor Model

Adult New Zealand white male rabbits weighing 3.8 to 4.3 kg (Myrtle's Rabbitry, Thompson Station, TN) were used. A total of 48 rabbits were used in this study. Twenty-four rabbits underwent percutaneous US-guided tumor implantation, and the same number of rabbits underwent standard open laparotomy tumor implantation.

Anesthesia and Analgesia

As a premedication, a mixture of acepromazine (2.5 mg/kg; Phoenix, St. Joseph, MO) and ketamine hydrochloride (44 mg/kg; Phoenix, St. Joseph, MO) was injected intramuscularly. Intravenous access was secured *via* a marginal ear vein, and 0.2 mL (5 mg) of sodium pentobarbital (Hospira, Inc., Lake Forest, IL) was administered intravenously periodically to maintain anesthesia. After the implantation, analgesic buprenorphine (0.02-0.05 mg/kg) was injected intramuscularly for pain relief.

Carrier Procedure

To obtain solid tumor for the implantation, 125 μL of Vx-2 carcinoma cell suspension was injected into each thigh muscle of a carrier rabbit. Two weeks later, distinct solid tumors that had grown in each thigh muscle were harvested from carrier rabbit, and put into 0.9% sodium chloride.

Percutaneous US-Guided Tumor Implantation

Preprocedural preparation was uniform and sterile for all rabbits. The left lateral lobe of the liver was chosen as a target site for all implantations. All rabbits were shaved in the abdominal

area before the procedure. However, for the US-guided procedure, the epicenter of the shaving area was on the left side of the thoracoabdominal wall, and the procedure was performed on a right lateral decubitus position, in order to optimize imaging and facilitate access to the left lateral lobe of the liver. The site of implantation was identified using percutaneous ultrasonography (ALOKA 7.5 MHz probe; Aloka Co. Ltd., Tokyo, Japan) *via* a low intercostal or subcostal sonic window. Both the probe and the ultrasound inspected skin surface were sterile. A small (2-3 mm) skin incision was made with a scalpel at the decided point for percutaneous puncture. A coaxial puncture free-hand method was initially performed, using 14 G Angiocath needles (1-1/4-in. long), which were then exchanged for 16 G Angiocath sheaths (2-in. long) through the initial 14 G Angiocath sheaths, for minced tumor cells delivery (Fig. 1). After confirming the needle tip location of the 14 G Angiocath at the center of the liver thickest portion, the stylet was removed and the 16 G Angiocath sheath containing minced tumor cells was inserted through the 14 G Angiocath sheath coaxially. A 0.035-in. guide wire was then inserted into the Angiocath to further push minced tumor cells inside the liver. After tumor cells implantation, the guide wire was retracted inside the 16 G sheath, which was then retracted inside the 14 G sheath, in order to minimize direct track and peritoneal tumor seeding. All instruments were then removed, and US-guided probe compression of the implantation area was performed for 10 seconds to minimize postprocedural bleeding. A final ultrasonographic image during probe compression was taken in order to verify accurate implantation and absence of bleeding. The implantation site with minced tumor cells was recognized as a round-shaped echogenic area in side the liver parenchyma (Fig. 2). No sutures were required at the puncture site.

Standard Open Laparotomy Tumor Implantation

The abdomen of each recipient rabbit was shaved around the midline epigastric area and prepped in a sterile fashion. Each rabbit was placed on a supine position. The liver was exposed with a midline subxiphoid incision, and the left lateral lobe was gently pulled out with the use of sterile gauze. The minced tumor cells were packed into a 16 G Angiocath sheath (2-in. long), with which the targeted area of the left lateral lobe was punctured. A 0.035-in. guide wire (also 2-in. long) was then inserted inside the Angiocath, in order to push the minced cells inside the liver. After removing the Angiocath and guide wire, the pierced liver capsule was then manually compressed for 10 seconds. After confirming the absence of bleeding and/or spilled minced cells on the surface of the liver, the liver was then repositioned to its original intra-abdominal space. The abdomen was closed in two layers (peritoneum and muscle layer, followed by the skin layer) with aseptic technique. Antibiotic ointment was applied along the suture line.

Postoperative Care

Postoperatively, all animals received a single dose of analgesic buprenorphine (0.02-0.05 mg/ kg) intramuscularly. The animals were covered with cotton blankets in order to maintain their normal body temperature, and were closely inspected for possible signs of distress till full recovery. Thereafter, the animals were inspected daily for postoperative complications and uneventful heeling. The animals were observed daily till their predetermined time point of euthanasia. On day 14 or 21, the animals underwent angiography and were subsequently euthanized.

Imaging Evaluation of Tumor Growth

All animals underwent angiographic evaluation of tumor growth in a dedicated animal angiography suite. A total of 24 rabbits (12 rabbits per group) underwent angiography at 14 days after tumor implantation and the remaining 24 rabbits underwent angiography at 21 days after implantation. Angiographic catheterization was performed under general anesthesia and

via the insertion of a 2Fr sized catheter in the common hepatic artery and if possible, in the tumor feeding artery.

MR imaging was performed at 14 days after implantation in a randomly selected group of rabbits (total of 4 rabbits, 2 per study group). Even though axial imaging, like MRI or CT, may be adequate to monitor tumor growth over time, we chose not to perform such studies in all animals due to their invasiveness (the rabbits should be anesthetized for each scan and they require at least 4 to 5 hours of recovery) and cost.

Definitions of Procedural Measurements

Procedure time for open laparotomy method was measured from the start time point of initially incising each rabbit's abdominal skin surface to the end time point of securing the last subcutaneous suture. Percutaneous US-guided procedure time was measured from the starting time point of initial placement of the probe on the rabbit's skin surface to the ending time point of terminating manual compression. Maintenance of anesthesia during each implantation procedure was measured as the frequency and total doses of 0.2 mL (5 mg) of sodium pentobarbital required. Recovery time was measured from the starting time point of each procedure's ending time point to the time point when each animal would be fully awake. All animals were observed for immediate bleeding and tumor cell spillage, both in the abdominal wall and inside the peritoneal cavity. Wound infection was defined as either an inflammatory reaction or a suppurative infection along the suture line or around the puncture site, requiring daily application of antibiotic ointment. Since our target area was the left lateral lobe of the liver, we also evaluated feasibility of US access to this targeted area. Tumor size was measured as the maximal tumor diameter on all tumor and liver gross specimens at the time of sacrifice. Success rate of tumor growth was defined as the presence of a hypervascular and solid tumor on angiography, which was performed either at 14 or 21 days after tumor implantation, with the presence of a distinct hypertrophic feeding artery that had a lumen diameter large enough to be catheterized selectively with a 2Fr sized angiographic catheter. Tumor seeding was assessed during autopsy, which was performed either at 14 or 21 days after tumor implantation.

Statistics

All groups were compared with Student t-tests or χ^2 tests. Differences were considered statistically significant for $P < 0.05$.

RESULTS

The results of both implantation methods are shown at Table 1. Average procedural time was shorter inpercutaneous US-guided implantation compared with open laparotomy. Maintenance of anesthesia required more frequent injections and larger amount of sodium pentobarbital in open laparotomies than in percutaneous US-guidance procedures. All rabbits that underwent open laparotomy needed additional sodium pentobarbital injections (3-5 times), especially during the initial abdominal cut-down and the final closure. On the other hand, only 4 of 24 rabbits that underwent US-guided implantation needed additional pentobarbital just once (2 mL). The total injected amount of pentobarbital affected the recovery time and rabbits that underwent open laparotomy required more than 3 hours to fully recover, whereas rabbits that underwent US-guided implantation recovered fully 30 minutes after the end of the procedure. No peri-or postprocedural mortality was observed in the US-guided implantation group. In the open laparotomy group, one procedural mortality was observed, possibly to due to anesthesia overdose or hypersensitivity. No bleeding was observed in both groups. No pneumothorax was observed in the rabbits that underwent percutaneous US-guided implantation, despite the fact that some of the puncture sites involved the lower intercostal space. Two of the rabbits that underwent open laparotomy suffered from a wound infection along the incision site, but were

treated with daily application of an antibiotic ointment. Puncture sites were clean in all rabbits that underwent US-guided implantation. Tumor seeding developed in 20.8% of the rabbits (5/24) that underwent US-guided implantation, compared with 8.3% of those that underwent open laparotomy (2/24). Five rabbits had thoracoabdominal wall needle tract tumor seeding, and two rabbit had tumor seeding at both thoracoabdominal wall and intraperitoneal space. Targeted left lateral lobe implantation was achieved in 91.7% of percutaneous US-guided procedures, and in 95.8% of open laparotomies. Failure to implant into the targeted area was confirmed during autopsy. Tumor size was adequate to perform interventional procedures in all rabbits (Figs. 3 and 4), and there was no statistically significant difference in tumor sizes ($P > 0.05$) between two groups (Table 1). The success rate of tumor growth in the liver was 100% in both groups.

DISCUSSION

Issues that arose in the past concerning the technique of Vx-2 carcinoma implantation in the rabbit liver included the route of tumor cell inoculation and the cell preparation method. Burgener and Violante [2] compared three routes of Vx-2 carcinoma inoculation, using intra-arterial, intraportal, and intraparenchymal injections of cell suspensions. At autopsy, in cases of intraarterial and intraportal injection of cell suspensions, the liver was always grossly enlarged and diffusely involved with tumor, whereas in cases of intraparenchymal injections, the Vx-2 carcinoma was always localized and large tumor free areas were found. There-after, researchers chose the direct intraparenchymal route as the standard route of inoculation. Although direct injection of Vx-2 cell suspension into the liver parenchyma is simple and has been widely used in the past, the use of cell suspension has remained problematic. The leakage of tumor cells into the peritoneal cavity *via* the injection route has been frequently reported [5,6]. In a study comparing three direct injection methods (Group 1, Vx-2 tumor cells were injected directly into the liver and no special procedure after removal of the needle; Group 2, the puncture site was gently compressed using an alcoholic cotton gauze for three minutes; Group 3, 0.2 mL of heated liquid agarose was injected to seal the aperture after injection of Vx-2 cells), the leakage rates were 80%, 53.3%, and 6.6% for Group 1, Group 2,and Group 3, respectively [5]. More-over, injection of cell suspensions may lead to direct injection of tumor cells into the blood stream and liver-to-liver and systemic dissemination P [3]. Another study comparing two different cell preparation methods (Group 1, a tumor cell suspension containing 1×10^6 cells in a volume of 0.1 mL injected slowly into the liver parenchyma using a 27-gauge needle during laparotomy; Group 2, a 1 mm^3 fragment of Vx-2 carcinoma inoculated into the subcapsule of the left anterior lobe of the liver) reported that the leakage rates were 50% and 0% for Group 1 and Group 2, respectively [6]. Currently, in terms of cell preparation method, most researchers use minced tumor cells and tumor fragments harvested from carrier rabbit rather than use of cell suspensions [7-9].

In our study, we used minced tumor cells harvested from carrier rabbit for intraparenchymal inoculation, but introduced the less invasive percutaneous US-guided implantation method. According to our results, percutaneous US-guided Vx-2 carcinoma implantations into rabbit liver were technically feasible and had a successful tumor growth at the implanted site in all cases. The reason for testing this less invasive method was that it would significantly reduce animal distress. Thorstensen *et al.* [10] reported a percutaneous US-guided Vx-2 carcinoma implantation method by use of a biopsy instrument in seven rabbits. Although the size of the tumor at 13 to 15 days after the implantation was small (5-8 mm; mean 5.9 mm) because of small loaded tumor volume in the 16 G biopsy device, tumor growth was achieved in all cases at the implanted site. They also reported three cases of peritoneal seeding (3/7, 42.9%).

The advantages of US-guidance are that during each procedure real-time images can be obtained, which facilitate the choice of proper implantation site, show hepatic vessels to be

avoided, the location of needle tip, immediate confirmation of the implantation site as an echogenic area surrounded by liver parenchyma, or demonstrate immediate complications. Moreover, this procedure, by being less invasive, requires a lesser amount of anesthesia and no open abdominal surgical incision. The only disadvantage is the potential iatrogenic tumor cell seeding along the puncture tract route.

CONCLUSIONS

Percutaneous US-guided implantation of Vx-2 carcinoma in rabbit liver is less invasive than open laparotomy implantation method with a confident success rate of tumor growth in the liver. Due to the high iatrogenic tumor track seeding rate, percutaneous US-guidance implantation method should not be considered for long-term survival study design; however, it can be considered for nonsurvival study design.

ACKNOWLEDGMENTS

REFERENCES

1. Moore DH, Stone RS, Shope RE, et al. Ultrastructure and site of formation of rabbit papilloma virus. Proc Soc Exp Biol Med Jul;1959 101:575. [PubMed: 13675323]

2. Burgener FA, Violante MR. Comparison of hepatic VX2-carcinomas after intra-arterial, intraportal and intraparenchymal tumor cell injectionAn angiographic and computed tomographic study in the rabbit. Invest Radiol 1979;14:410. [PubMed: 500305]

3. Izumi B, Tashiro S, Miyauchi Y. Anticancer effects of local administration of mitomycin C via the hepatic artery or portal vein on implantation and growth of VX2 cancer injected into rabbit liver. Cancer Res 1986;46:4167. [PubMed: 3089588]

4. Tabuchi Y, Nakamura T, Saitoh Y. Liver metastases induced by implantation of VX2 cancer into the gastrointestine. J Surg Res 1991;50:216. [PubMed: 1999911]

5. Lin WY, Chen J, Lin Y, Han K. Implantation of VX2 carcinoma into the liver of rabbits: A comparison of three direct-injection methods. J Vet Med Sci 2002;64:649. [PubMed: 12185325]

6. Chen JH, Lin YC, Huang YS, et al. Induction of VX2 carcinoma in rabbit liver: Comparison of two inoculation methods. Lab Anim 2004;38:79. [PubMed: 14979992]

7. Geschwind JF, Artemov D, Abraham S, et al. Chemoembolization of liver tumor in a rabbit model: Assessment of tumor cell death with diffusion-weighted MR imaging and histologic analysis. J Vasc Interv Radiol 2000;11:1245. [PubMed: 11099235]

8. Hong K, Khwaja A, Liapi E, et al. New intra-arterial drug delivery system for the treatment of liver cancer: Preclinical assessment in a rabbit model of liver cancer. Clin Cancer Res 2006;12:2563. [PubMed: 16638866]

9. Hong K, Kobeiter H, Georgiades CS, et al. Effects of the type of embolization particles on carboplatin concentration in liver tumors after transcatheter arterial chemoembolization in a rabbit model of liver cancer. J Vasc Interv Radiol 2005;16:1711. [PubMed: 16371540]

10. Thorstensen O, Isberg B, Svahn U, et al. Experimental tissue transplantation using a biopsy instrument and radiologic methods. Invest Radiol 1994;29:469. [PubMed: 8034455]
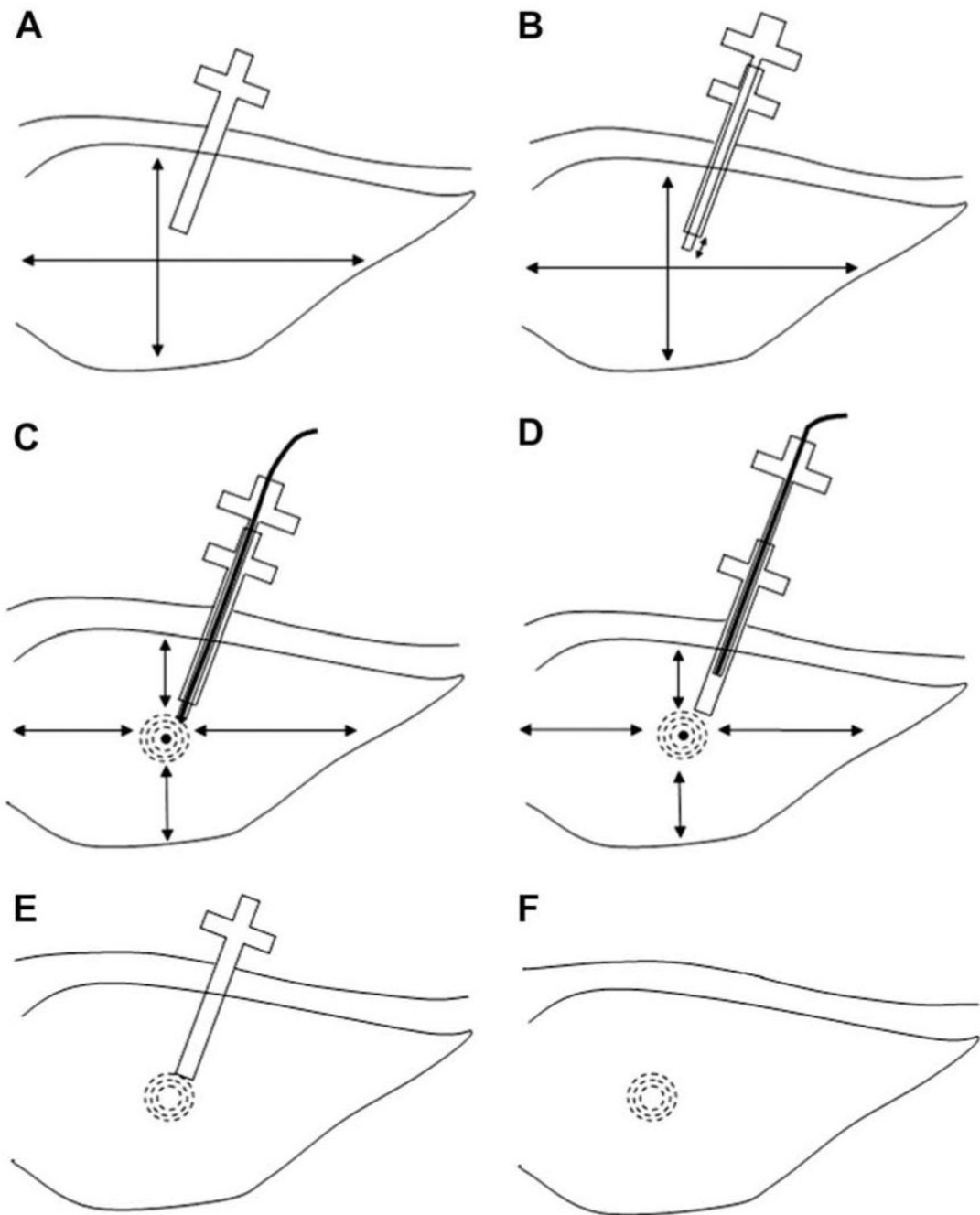
FIG. 1.
A sequential schematic of coaxial percutaneous US-guided Vx-2 tumor implantation. After confirmation of needle tip location of 14 G Angiocath at the center of the liver's thickest portion, the stylet was removed (A), and then 16 G Angiocath containing minced tumor cells was inserted through the 14 G Angiocath coaxially (B). A 0.035-in. guide wire was inserted into the Angiocath to push out minced tumor cells inside the liver (C). Then, conversely, the guide wire was retracted inside the 16 G needle, the two instruments of the guide wire and 16 G needle that were contaminated with minced tumor cells were retracted inside the 14 G, which was thought not to be exposed to minced tumor cells (D), and finally, all instruments was removed (E, F).

FIG. 2.
Ultrasonographic image immediately after percutaneous US-guided Vx-2 carcinoma implantation into left lateral lobe of the liver. Implanted site with minced tumor cells is recognized as round-shaped echogenic area (circle) presented by innumerable tissue interfaces and interposed air bubbles between minced cells.
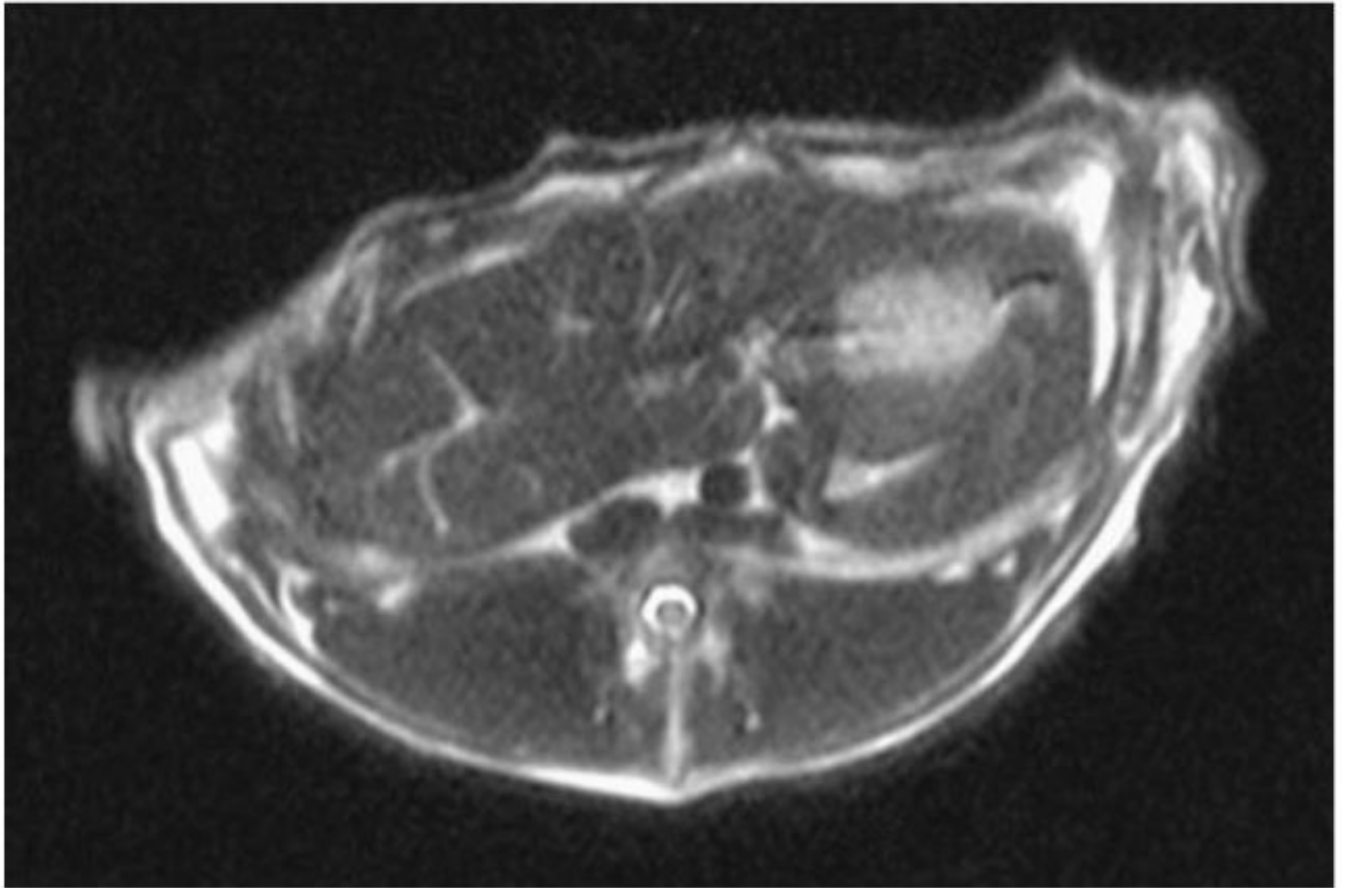
FIG. 3.
Cross-sectional MR image of the case underwent percutaneous US-guided Vx-2 carcinoma implantation. T2-weighted image shows well-located tumor (high signal intensity) at the center of the left lateral lobe of the liver.
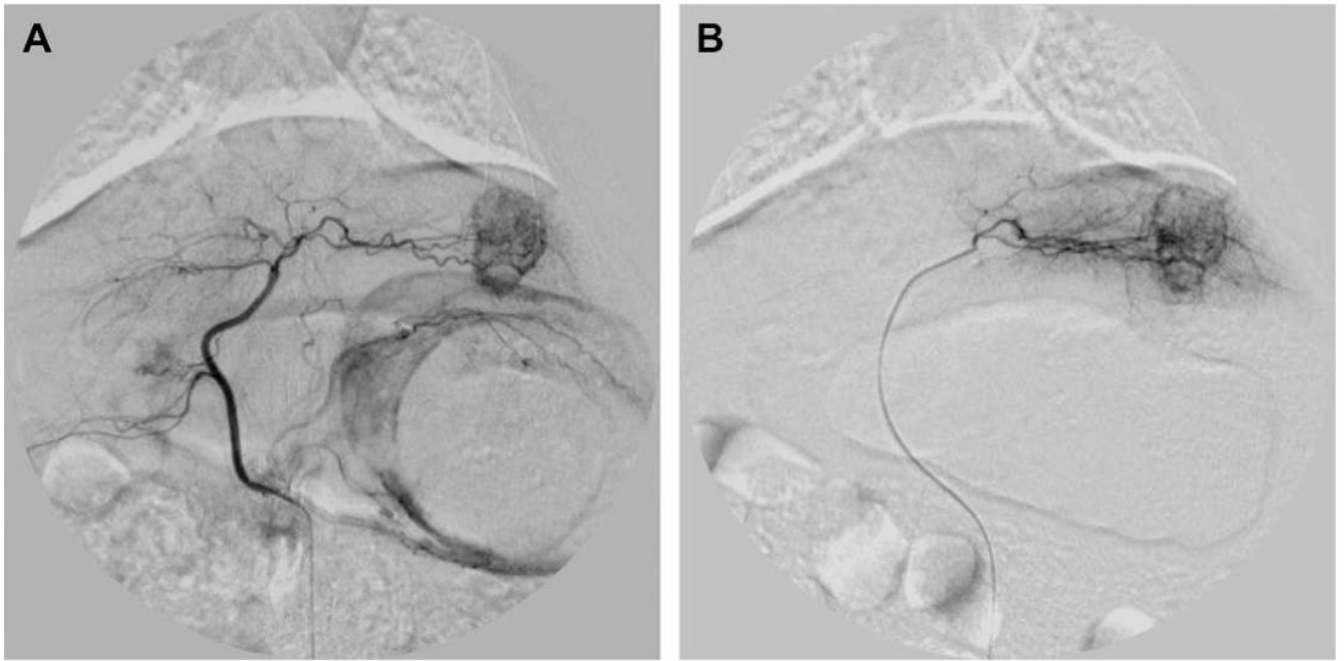
FIG. 4.
Digital subtraction angiography performed 2 weeks after percutaneous US-guided Vx-2 carcinoma implantation. Common hepatic arteriography (A) and selective tumor feeding arteriography (B) with using 2-Fr JB1 catheter show sufficiently large hypervascular tumor, staining with hypertrophic feeding artery allows superselection of feeding artery of left lateral lobe of the liver.

TABLE 1

Results of Vx-2 Carcinoma Implantation by Percutaneous US-Guided and Open Laparotomy

	US-guided laparotomy ($n = 24$)	Open laparotomy ($n = 24$)	P value
Procedure time (average)	7 minutes	15 minutes	<0.0001
Anesthesia (sodium pentobarbital)	<0.2 mL (5 mg)	0.6-1.0 mL (15-25 mg)	<0.0001
Recovery time	<1 hour	>3 hours	<0.0001
Complications			
Death	0/24	1/24 (4%)	>0.9999
Bleeding	0/24	0/24	>0.9999
Pneumothorax	0/24	N/A	N/A
Wound infection	0/24	2/24 (8.3%)	0.48
Tumor seeding	5/24 (20.8%)	2/24 (8.3%)	0.41
Site of implantation (LLL)	22/24 (91.7%)	23/24 (95.8%)	>0.9999
Size of tumor (cm, mean ± SD)			
2 wk	1.7 ± 0.23	1.8 ± 0.24	0.3087
3 wk	3.3 ± 0.34	3.4 ± 0.4	0.5162
Success rate of tumor growth	24/24 (100%)	23/23 (100%)	>0.9999

LLL = left lateral lobe; SD = standard deviation.