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Considerations for Implantation Site of VX2 Carcinoma into Rabbit Liver

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Abstract

PURPOSE—To assess whether the implantation site of VX2 carcinoma into rabbit liver affects successful vessel selection for transcatheter arterial interventions.

MATERIALS AND METHODS—Twenty-four New Zealand White rabbits were randomly assigned to two groups. All implantations were performed by open laparotomy with minced tumor cells inserted into a 16-gauge Angiocath needle. Group I rabbits (n = 12) had tumor implanted into the left medial lobe of the liver and group II rabbits (n = 12) had tumor implanted into the left lateral lobe. Two weeks after implantation, selective angiography was performed for subsequent chemoembolization, which was part of a different study. Tested variables included maximum tumor diameter, tumor feeding artery size, and tumor vascularity.

RESULTS—Successful tumor growth was achieved in all rabbits. Selective angiography was possible in 33.3% of rabbits in group I and 66.6% of rabbits in group II (P < .05). Tumor size and vascularity were similar between groups. Mean lengths of tumor feeder arteries from the bifurcation of the left hepatic artery were 4.1 mm ± 1.2 in group I (left medial lobe) and 10.8 mm ± 3.0 in group II (left lateral lobe; P < .05). The angulation of the left medial lobar artery (group I) off the left hepatic artery was acute in eight of 12 rabbits (66.6%), but only four of 12 rabbits in group II (33.3%) showed acute angulation of the left lateral lobar artery off the left hepatic artery (P < .05). Mean angiography time was significantly shorter in group II.

CONCLUSIONS—For selective hepatic arterial interventions, the left lateral lobe of the liver may be favorable as an implantation site for VX2 tumors in rabbits.

For the past several decades, rabbits have been used for experimental studies of diagnosis and treatment of malignant liver tumors with use of VX2 carcinoma (1–5). There is no doubt that successful tumor implantation and growth in the liver leads to successful experimental studies and that continuous improvement of all technical aspects of the tumor implantation technique may improve our knowledge regarding the selective use of VX2 carcinoma.

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Interventional radiologists have been using the VX2 liver model for translational interventional oncology studies, especially for transcatheter arterial infusions, in which selective therapeutic drug administration is desirable (2,3). During previous translational experiments (unpublished data), we noticed that the implantation site of tumor may affect the success rate of selective hepatic arterial interventions (2,3,6). We therefore initiated a study to investigate whether the liver VX2 implantation site may have an impact on successfully selecting and/or super-selecting the rabbit's hepatic arteries and tumor feeding arteries. We focused on only the two left lobes (medial and lateral) of the rabbit liver, as we believe the right lobes are not suitable for transcatheter interventions because of an increased number of gastrointestinal complications.

MATERIALS AND METHODS

Our institution's animal care and use committee approved the study, which was part of a study assessing the presence of necrosis after chemo-embolization with magnetic resonance imaging. All animal care and procedures were performed in accordance with institutional guidelines.

Animal and Tumor Model

Adult New Zealand White male rabbits weighing 3.8–4.3 kg (Myrtle's Rabbitry, Thompson Station, Tennessee) were used. Twelve rabbits underwent VX2 tumor implantation to the left medial lobe of liver (group I), and the same number of rabbits received VX2 tumor implantation to the left lateral lobe of the liver (group II).

Anesthesia and Analgesia

As a premedication, a mixture of acepromazine (2.5 mg/kg; Phoenix, St. Joseph, Missouri) and ketamine hydrochloride (44 mg/kg; Phoenix) was injected intramuscularly. Intravenous access was secured via a marginal ear vein, and 0.1–0.2 mL (2.5–5 mg) of sodium pentobarbital (Hospira, Lake Forest, Illinois) was administered intravenously periodically to maintain anesthesia. Endotracheal intubation with a 3.0-mm endotracheal tube (Mallinckrodt Medical, St. Louis, Missouri) was performed to monitor respiration rate and end-tidal CO_2. Postoperatively, analgesic buprenorphine (0.02–0.05 mg/kg) was injected intramuscularly.

Tumor Implantation

To obtain solid tumor for implantation, VX2 tumor cell suspension (approximately 1 × 107 cells, 200 μL) was injected into both thigh muscles of a carrier rabbit. Two weeks later, the bulk of solid tumors was harvested from the carrier rabbit and put into 0.9% sodium chloride. The abdomen of each recipient rabbit was shaved and disinfected with ethanol and povidone iodine. The liver was exposed with a midline subxiphoid incision. In group I, the left medial lobe of the liver was pulled out for implantation. The left lateral lobe was chosen for implantation in group II. The minced tumor cells were packed into a 16-gauge Angiocath (2 inches long), and the thickest portion of the liver was punctured. A 0.035-inch guide wire was then inserted inside the Angiocath to push the minced cells inside the liver. After the Angiocath and guide wire were removed, the pierced liver capsule was then manually compressed. After the absence of bleeding and spillage of tumor cells was confirmed, the liver was then repositioned back to its original intraabdominal space. The abdomen was closed in two layers (peritoneum and muscle layer followed by skin layer) with aseptic techniques. Antibiotic ointment was applied along the suture line.

Hepatic Angiography

Under intravenous anesthesia and intubation as described, selective hepatic angiography was performed for subsequent chemoembolization 2 weeks after the implantation of VX2

carcinoma with a digital subtraction angiographic unit (Toshiba, Tochigi, Japan). Surgical cutdown of the right side of the common femoral artery and insertion of a 4-F sheath (Cook) were done to gain access into the abdominal aorta and select the hepatic artery. A 2-F catheter with a tip in the shape of a hockey stick (JB1 catheter; Cook) was manipulated into the celiac trunk and common hepatic artery. By performing common hepatic arteriography, hepatic arterial anatomy and tumor staining, vascularity, size, and location were verified. Over the 0.014-inch guide wire (Transend; Boston Scientific/Medi-tech, Miami, Florida), a 2-F JB1 catheter was inserted to the tumor feeding artery. All rabbits underwent subsequent chemoembolization of their VX2 tumor.

Tested Variables and Statistics

Successful selective catheterization was defined as catheter engagement of the tumor feeding artery at a distance from its origin, with concurrent catheter advancement and subsequent positioning inside the feeding artery as close to the tumor as possible. With the catheter placed at its final position, successful selective catheterization was confirmed by angiographic demonstration of forward arterial flow and delineation of tumor vascularity with minimal backflow of contrast medium to more proximal arterial branches.

Qualitative variables measured included the presence or absence of successful tumor growth, angiographic presence or absence of hypertrophic tumor feeding artery, angiographic presence or absence of hypervascular tumor stain, and presence or absence of acute tumor feeding vessel angulation at its origin off the left hepatic artery. Quantitative variables included maximum tumor diameter and length of tumor feeding artery. The length of the feeding artery was measured by drawing a straight line parallel to the course of the artery. Short tortuousities were not taken into account. Statistical differences were tested by χ^2 and Student *t* tests ($P < .05$).

RESULTS

The goal of each intervention was to get as close to the tumor as possible. Successful tumor growth in the liver was achieved in all rabbits in both groups. Selective angiography and chemoembolization was possible in four of 12 rabbits (33.3%) in group I and in eight of 12 rabbits in group II (66.6%; $P < .05$; Fig 1, Fig 2). Tumor size (1.7 cm ± 0.3 in group I, 1.8 cm ± 0.5 in group II; $P > .05$), presence of a hypertrophic tumor feeder vessel, and presence of a hypervascular tumor stain were similar between groups (Table 1). However, there were anatomic differences between the tumor feeding arteries of the two groups (Table 2). The mean length of the tumor feeder vessel of the left medial lobar artery (group I) was significantly shorter than that of the left lateral lobar artery (group II) as measured from the bifurcation of the left hepatic artery, at 4.1 mm ± 1.2 versus 10.8 mm ± 3.0, respectively ($P < .05$). The angulation of the left medial lobar artery (group I) at the origin off the left hepatic artery was acute in eight of 12 rabbits (66.6%), but in group II, only four of 12 rabbits (33.3%) showed acute angulation of the left lateral lobar artery ($P < .05$). Mean angiography time in group I was significantly longer than in group II (Table 3). Moreover, the additional use of a vasodilator was necessary in 16% of the group I rabbits.

DISCUSSION

The optimal study execution of rabbit experiments involving hepatic transcatheter arterial manipulations is affected by the operator's ability to successfully select or superselect the tumor feeding vessel. Because of small size and tortuosity of rabbit hepatic arteries, successful selective vessel catheterization is not always possible, even in the presence of a skilled and experienced operator or when a wide range of microcatheters is available.

The rabbit celiac artery gives rise to the splenic artery, the right gastric artery, and the common hepatic artery (Fig 3). The common hepatic artery then passes forward and to the right, giving the rise to the gastroduodenal artery and the caudate lobe artery. The proper hepatic artery then forms the main hepatic artery, which bifurcates to the right and left hepatic arteries. The latter bifurcates to the medial and lateral segmental branches. This branching pattern may be seen in approximately 95% of cases (7). Variations also exist, such as a separate origin of the common hepatic artery off the superior mesenteric artery or a separate origin of the left hepatic artery off the celiac artery. In most cases, the left hepatic artery gives rise to the medial and lateral lobe branches.

In most studies, VX2 carcinoma is implanted into the medial lobe of the liver because it is easy to access during laparotomy and is thick enough to tolerate the tumor implantation manipulations (1–6,8,9). However, many tumors implanted in the left medial lobe have shown angiographically small and tortuous feeding arteries, leading to nonselective and nontargeted intraarterial therapy, with compromised results. This angiographic appearance of the VX2 liver tumor was thought to be an inherent characteristic of the specific tumor vascularity. An initial nonintentional tumor implantation in the left lateral lobe revealed a more convenient angiographic scenario of a hypervascular tumor, with a single and long hypertrophic artery, which smoothly originated off the left hepatic artery. This initial observation led us to further assess whether the site of VX2 tumor implantation affects the way the tumor recruits feeding vessels and the size of these feeding vessels. Interestingly, our initial observations were confirmed, and more importantly, there were statistically significant anatomic differences between the two groups. In the left medial lobar artery group, the tumor feeding artery was small and short, originating at an acute angle off the left hepatic artery. In this group, the tumor feeding artery often showed spasm induced by catheter or guide wire manipulation, leading to longer fluoroscopy times, additional vasodilator administration, eventual thrombosis, and failure of the intervention. In the left lateral lobar artery group, we observed a longer single tumor feeding vessel, which was easier to select without arterial spasm. As a result, the success rate of selective angiography was higher than in cases of left medial lobe tumor implantation. To our knowledge, there is a paucity of literature describing such anatomic differences that may subsequently be exploited for transcatheter arterial interventions. We assume that these differences are related to the rabbit hepatic arterial anatomy (7). The left medial lobar hepatic artery is often short, whereas the left lateral lobar artery is longer. As implanted tumors receive blood from these arteries, it is expected that tumors implanted in the lateral lobe would recruit vessels from the longer lateral lobar artery and that tumors implanted in the medial lobe would recruit vessels from the shorter medial lobar artery.

Our success rate in the left lateral lobe group was not 100%, despite the favorable arterial anatomy. This may partially attributed to the use of JB1 catheters (Cook) instead of microcatheters, which are more flexible and easier to manipulate.

According to these results, we now choose the left lateral lobe of the liver for VX2 tumor implantation in the rabbit. The anatomic advantages of the left lateral lobar artery, combined with fiber braided microcatheters and guide wires, may facilitate selective angiography and chemoembolization and increase the success rate of targeted intraarterial interventions in rabbits.

In conclusion, for selective hepatic arterial interventions in rabbits, the left lateral lobe of the liver may be more favorable than the left medial lobe as a VX2 tumor implantation site.

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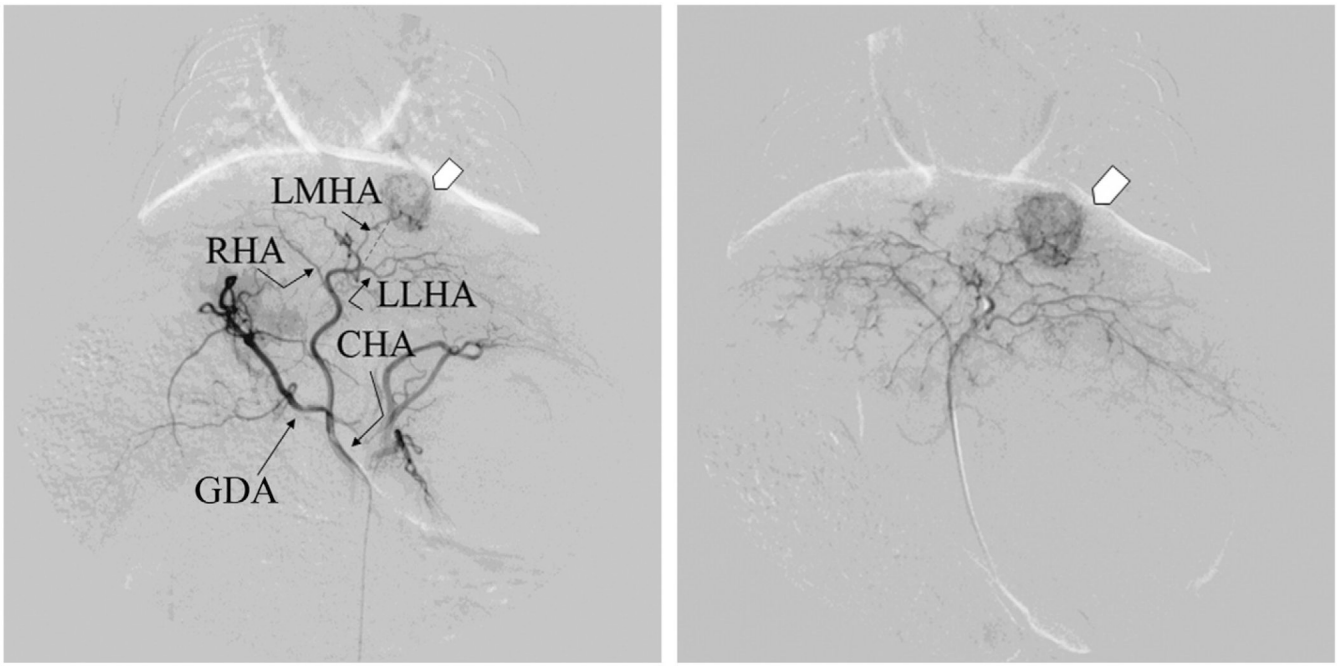
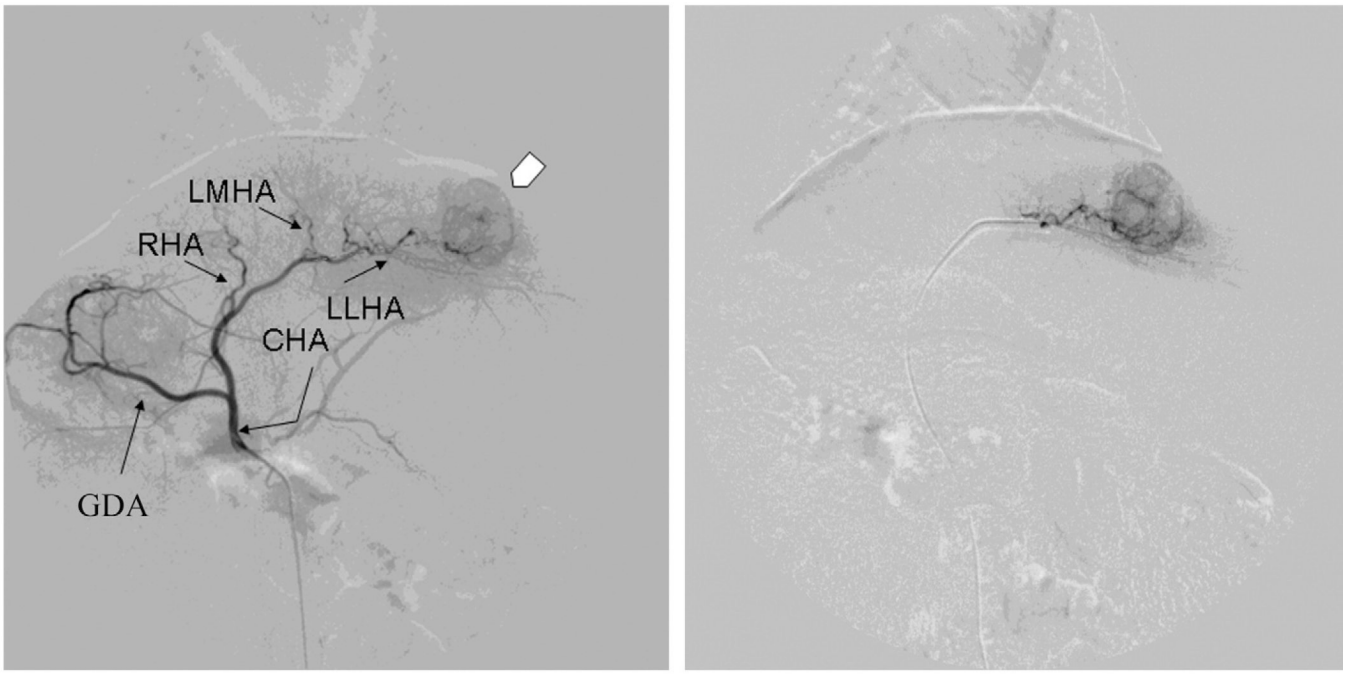


a. b.

Figure 1.

Angiographic appearance of left medial lobe tumor implantation (arrow) at 2 weeks after implantation. Because of the acute angulation and short length of the left medial lobe artery off the left hepatic artery **(a)**, arterial spasm was induced during catheter and guide wire manipulations **(b)**, and subsequent arterial selection failed. Therapeutic drug administration was delivered nonselectively to the right lobe. (LMHA = left medial hepatic artery, LLHA = left lateral hepatic artery, RHA = right hepatic artery, CHA = common hepatic artery, GDA = gastroduodenal artery.)



a. **b.**

Figure 2.
Angiographic appearance of the left lateral lobe tumor implantation (arrow) at 2 weeks after implantation. Because of the obtuse angulation of the left lateral lobar artery off the left hepatic artery **(a)**, the tumor feeding artery could be selected easily without arterial spasm, and sufficient length of the left lateral lobar artery could prevent drug regurgitation **(b)**. (LMHA = left medial hepatic artery, LLHA = left lateral hepatic artery, RHA = right hepatic artery, CHA = common hepatic artery, GDA = gastroduodenal artery.)

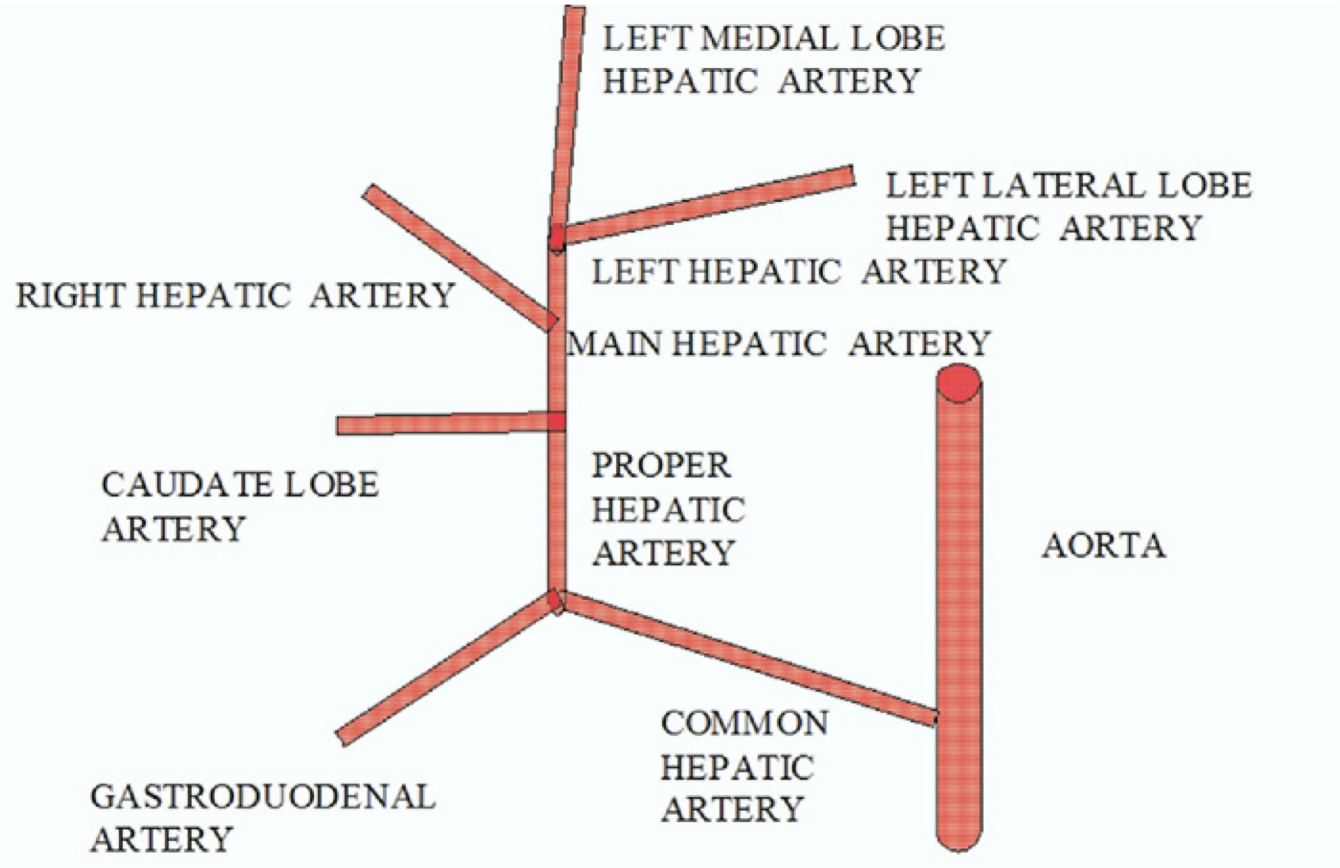


Figure 3.
Illustration of rabbit hepatic arterial anatomy. (Available in color online at www.jvir.org.)

Table 1

Angiographic Variables with Similar Results between Rabbit Groups

Variable	Group I (*n* = 12)	Group II (*n* = 12)
Successful tumor growth (%)	100	100
Maximal tumor diameter (cm)	1.7 ± 0.3	1.8 ± 0.5
Angiographic presence of hypertrophic tumor feeding artery (%)	100	100
Angiographic presence of hypervascular tumor stain (%)	100	100

Table 2

Anatomic Differences in the Tumor-feeding Arteries between Rabbit Groups

Anatomic Characteristic	Group I (LML Artery)	Group II (LLL Artery)
Length of artery (mm)*	4.1 ± 1.2	10.8 ± 3.0
Acute angulation of artery*	8/12 (66.6)	4/12 (33.3)

Note.—Values presented as means ± SD where applicable. Values in parentheses are percentages. LLL = left lateral lobe; LML = left medial lobe.

* $P < .05$ between groups.

Table 3

Procedural Differences between Groups

Procedural Detail	Group I (LML Artery)	Group II (LLL Artery)
Angiography time*	3.5 ± 1.1	2.1 ± 1.1
Additional use of vasodilator	2/12 (16%)	0/12

Note.—Values presented as means ± SD where applicable. LLL = left lateral lobe; LML = left medial lobe.

*$P < .05$ between groups.