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Solitary Hepatic VX2 Tumor Model and Its CT Findings in Rabbits

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Abstract : To verify the solitary nodular hepatocellular tumor model induced by intraparenchymal VX2 tumor cell injection and assess the diagnostic imaging character by computed tomography in rabbits. The tumor cell fragment (1~2 mm^3) was loaded in Seldinger needle and directly implanted into the livers of 80 New Zealand white rabbits. The tumor size and patterns of tumor growth were evaluated using spiral computed tomography (CT) at 1, 2, and 3 weeks after tumor implantation. A solitary nodular tumor was successfully created in 82.5% (66/80). The mortality rate was 5% (4/80). The tumor sizes measured were 7.46.3, 14.210.8, 16.212.6 cm at 1, 2, and 3 weeks after tumor implantation on arterial phase CT images. The tumors were round to oval shape with peripheral enhancement and central hypoattenuation on arterial phase, and hypoatteuated wash-out pattern on portal phase. It is considered that inoculation of tumor fragment loaded in Seldinger needle is useful and practical method for creating a solitary hepatic tumor. And CT scanning are valuable to investigate the hepatic tumor and compatible to the observations on macro- and microscopic findings.

Key words **:** Tumor model, liver, rabbit, VX2 carcinoma, computed tomography.

Introduction

Simple and decent tumor models are compulsory to evaluate the efficacy of antitumoral drugs as well as verify the clinical potential of advanced imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI). Since the splanchnic anatomy of rabbit is relatively similar to that of humans (1), rabbit models has been employed for tumor model (10,16). The VX2 tumor, developed in 1937 from a papilloma induced by a Shope virus infection in a domestic rabbit (6,11) is a fast-growing adenocarcinoma with the advantage of a high rate of local invasion and a high level of vascularity.

For the developing tumor model, various methods for inducing a variety of lesions are available. In the previous investigations, a suspension of tumor cells was injected directly into the hepatic parenchyma (8) and tumor fragment or a homogenate was implanted into the liver by using either a surgical procedure (9) or with the aid of a biopsy instrument under sonographic guidance(14). Recently, it was reported that an agarose is a useful material in preventing the leakage in the establishment of VX2 liver tumor models (7). However, the reported incidence of extrahepatic extension of the tumor in those methods was high and a proper size for the tumor relevant study has not been mentioned. Solitary hepatic tumor could be apparently used for the validation of therapeutic agent and imaging modality with ease. Therefore, more practical and convenient method to develop rabbit models of primary hepatic tumor with proper size confined to the liver was attempted and the morphologic characteristics were evaluated with spiral CT in this study.

Materials and Methods

Animals

Eighty New Zealand white rabbits weighing 2.5 to 3.2 kg, 6 month old were used. All animals were fed ad libitum. The animal care and use procedures were in accordance with the Guide for the Care and Use of Laboratory Animals and were reviewed and approved by Animal Care Committee of Chonbuk National University.

Tumor preparation

The VX2 carcinoma was maintained through serial transplantation into the hind limb muscle of the New Zealand white rabbit. Following implantation into the hind legs, the tumor enlarged rapidly. Three weeks after tumor inoculation, the rabbit was euthanized with intravenous administration of 30 mg/kg of sodium pentobarbital, and about 3 cm^3-sized tumor mass was aseptically stripped from the surrounding connective tissues and the inner necrotic portion was removed. The fresh tumor tissue was minced mechanically with scissors until the tumor had a similar consistency, and several pieces of a 1 mm^3-sized solid tumor fragment were selected from the tumor homogenate.

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Direct hepatic inoculation

Eighty rabbits were anesthetized with an intramuscular injection of a mixture consisting of ketamine hydrochloride (Ketalar®; Yuhan Yanghang, Seoul, Korea) and xylazine hydrochloride (Rumpun®; Bayer Korea, Seoul, Korea).

Lateral liver lobe was exposed following a subxiphoid laparotomy and then inoculation was performed into the left lobe. The tumor fragments were implanted by puncturing the left lobe of the liver with a loaded Seldinger needle directly and gentle pressure was placed to avoid leakage for a few minutes.

Computed Tomography

CT scanning was performed with multi-slice spiral CT (Somatom Plus 4, Siemens, Berlin, Germany). After a coronal scout view, a nonenhanced scan was performed through the liver at 120 kVp, 200 mA, with a section thickness of 3 mm, a pitch of 1, and a FOV of 13×13 cm. The intravenous contrast material, 8 ml of iopromide (Ultravist® 370, Schering, Berlin, Germany) was injected at a rate of 0.3 ml/sec using a mechanical power injector. A contrast enhanced CT of the liver was then performed with 1 mm collimation and a pitch of 1 during the hepatic arterial phase (15 seconds after the injection) and the portal venous phase (4 seconds after the end of the arterial phase). Tumor size was measured at arterial phase and enhanced pattern was examined on both phases.

Histopathology

The animals were sacrificed immediately after final CT scanning and an autopsy was conducted on all rabbits. The abdominal cavity and solid organs were inspected for the presence of a tumor. The liver was then fixed in 10% formalin, and subsequently cut into serial 3 mm axial sections in order to maximize lesion detection and match the CT scanning plane. The tumor appeared as distinct white nodules against a background of normal liver parenchyma. Tissue samples of the liver were stained with hematoxylin and eosin for a histopathological analysis.

Statistical analysis

Statistical analysis for tumor size was done with one-way ANOVA test. Significance was assumed for the tests at $p<0.05$.

Results

Tumor yield

Sixty eight out of the 80 rabbits (85%) had single, round to oval hepatic masses. Among them, one extrahepatic nodule on the abdominal wall (size: 7 mm) was observed grossly and one nodule containing no tumor cells only with foreign body granuloma was identified by microscopic examination. The true success rate of tumor growth was 66 of 80 (82.5%). The tumor sizes were 7.46.3, 14.210.8, 16.212.6 cm at 1, 2, and 3 weeks after tumor implantation respectively. The significant tumor growth was observed on 2nd week compared to 1st week, however not between 2nd and 3rd week (Table 1).

Three rabbits were dead. One death occurred immediately after surgery and autopsy revealed no evidence of hemorrhage, extreme stress of laparotomy was considered to be the cause of death. And two rabbits were not recovered from the anesthesia during CT scanning at 3 week after tumor implantation.

Tumor growth pattern

None of the rabbits except for one showed evidence of an extrahepatic tumor extension or visible (> 1 mm) peritoneal seeding. All implanted tumors were well delineated from the surrounding normal liver tissue. The mean tumor size was approximately 1.5×1.5 cm at 3 week after tumor implantation.

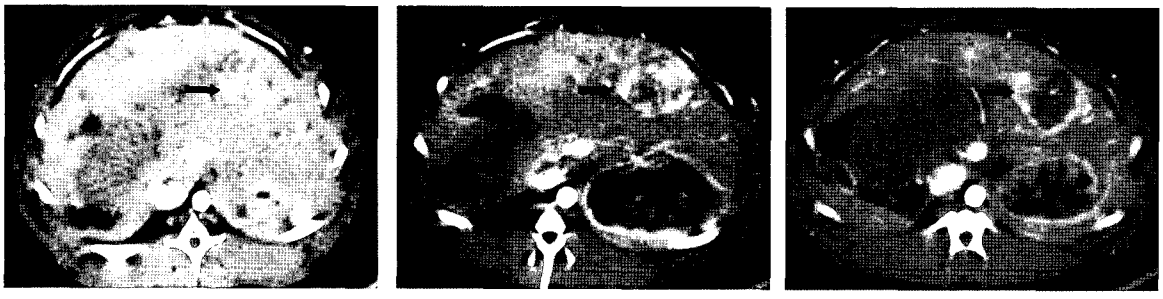
CT findings

The hepatic VX2 tumors were hypodense on the noncontrast images. Peripheral rim-like enhancement with a central necrosis or peripheral nodular enhancement on the arterial phase of contrast enhanced CT (Fig 1). All lesions were hypo-

Table 1. Tumor size measured on enhanced CT images at 1, 2, and 3 week after tumor implantation

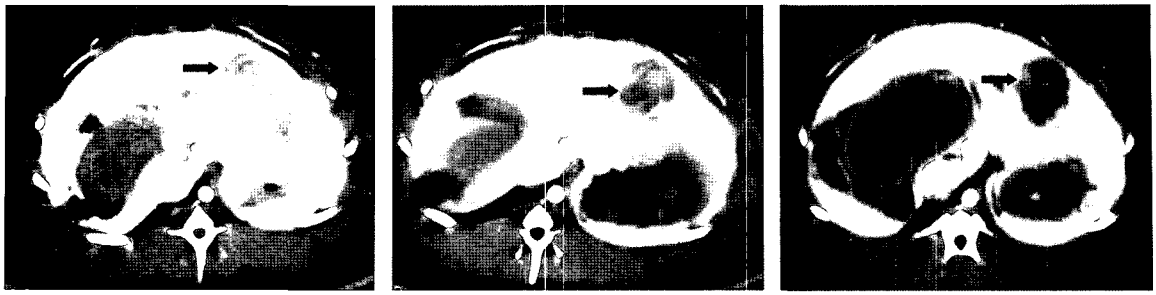
	Arterial phase		
	1st week	2nd week	3rd week
*Size	7.4×6.3	14.2×10.8†	16.2×12.6
SD	2.7×2.6	6.4×4.0	4.6×3.9

* ; expressed as long diameter•TMshort diameter in cm unit.
† : P<0.01



(A) (B) (C)

Fig 1. Arterial phase of enhanced CT images at 1 week (A), 2 weeks (B), and 3 weeks (C) after tumor implantation in a rabbit. Nodular enhancement (A) and peripheral rim-like enhancement with a central necrosis (B,C) are observed (arrows).



(A) (B) (C)

Fig 2. Portal phase of enhanced CT images at 1 week (A), 2 weeks (B), and 3 weeks (C) after tumor implantation in a rabbit. Well-defined, low attenuated mass was noted (arrows).

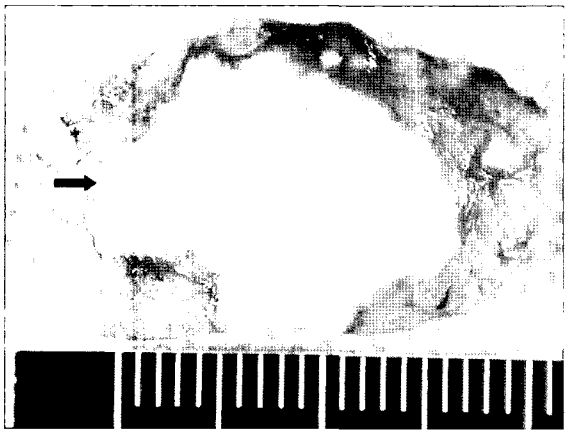


Fig 3. Cut section specimen shows a well-defined and lobulated mass sized 1.5×1.3 cm (arrows) on the surface of the liver.

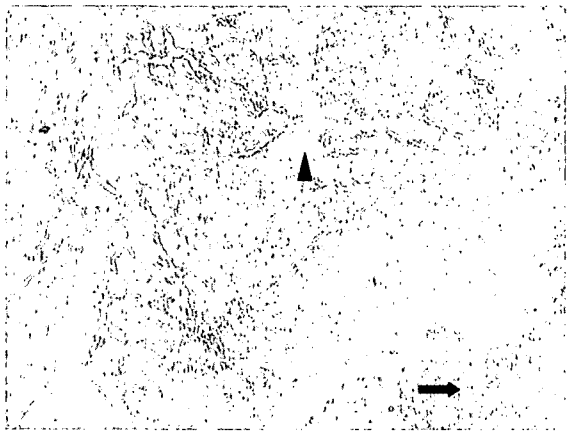


Fig 4. Histopathologic specimen shows tumor surrounding normal liver tissue (arrow). Tumor embedded in coagulation necrosis (arrowhead) show in the center of tumor. (Hematoxylin-eosin stain; original magnification, ×100).

dense on the portal venous phase (Fig 2).

Pathology

The tumor showed light pink to yellowish-white, round to oval shape grossly (Fig 3). The histopathological examination revealed that all lesions except for one were a VX2 carcinoma. The exceptional lesions were identified a granuloma probably foreign body induced lesion without any tumor cells and a severe inflammatory reaction surrounding the lesion. The tumor embedded in coagulation necrosis (arrowhead) was shown in the center of tumor (Fig 4).

Discussion

Various methods of intrahepatic tumor implantation have been devised for hepatic tumor models (3,7,12-14,17). A surgical technique with laparotomy (9) or with an aid of a modified biopsy instrument under US-guidance has been studied (14). Invasive laparatomy technique showed high tumor yield but not in the US-guidance method. Mostly tumor development with a VX2 carcinoma in the liver could be achieved using a cell suspension injected directly into the liver parenchyma for solitary hepatic tumor model (5,7), intraarterial or intraportal injections of tumor cell suspension have also been performed (3), as has injection into the gastrointestinal walls, which resulted in spontaneous tumor metastases to the liver (12). The tumor may spread beyond the target area when a homogenated tumor cell suspension seemed to be injected into the vessels or the liver parenchyma (2,5).

Up to now, those studies with relatively small number of rabbits are not likely to verify the best method to create a soliary hepatic tumor model. Besides, the tumor size has not been manifested. In this study, therefore, a single nodular tumor model used in radiologic research widely was intensively evaluated to verify whether the method could be applied with practical and useful manner for developing high tumor yield and lowering the incidence of extrahepatic extension using great number of rabbits.

A direct hepatic inoculation by a loaded Seldinger needle after laparotomy seems to be simple and practical method for developing single hepatic tumor model in experimental animals. It was reported that tumor yield rate about 86% was produced with the support of 0.2 ml of heated liquid agarose sealing the injection site is more useful method rather than

gentle pressure technique in preventing the leakage in the establishment of VX2 liver tumor models (7). In this study, however, simply gentle pressure by sterilized cotton bud for a few minute to avoid leakage and prevent hemorrhage was quite enough to achieve satisfactory tumor nodule as expected. The implantation of tumor fragment than injection of tumor suspension might contribute to make it easier to control the tumor cell leakage causing seeding. The tumor size was assessed by contrast CT scanning. The mean tumor size about 1.5 × 1.5 cm at 3 week after tumor implantation was relatively smaller than onother study (7) but seems to be appropriate size for the tumor research. The discrepancy was contributed by the using of different tumor cell concentration. Too large tumor containing necrosis or too small to be detected and examined are not adequate for the radiological study. Therefore solitary tumor with about 1 to 2 cm diameter found on 2nd or 3rd week after tumor implantation might be suitable concerning diagnostic imaging and interventional radiology.

Induced hepatic VX2 tumors showing hypodense on the noncontrast CT images revealed peripheral rim-like enhancement with a central necrosis or peripheral nodular enhancement on the arterial phase while all tumors were hypodense on the portal phase of enhanced CT. The CT findings in this study were in full agreement with the previous study (15).

Histopathologically, many malignant tumors have abnormal vessels that are primitive, avascular channels, lacking smooth muscle, and often consisting of only an endothelial layer and connective tissue. In this study, most of the tumors had these vascular structures in their periphery and showed the hyperattenuation on CT, corresponding with previously reported results (2).

The limitations of this study were lack of information of the precise tumor cell concentration contained in the tumor fragment, of various attempting to compare with several methods such as tumor homogenate injection and US-guidance implantation, and of the pathologic information at different tumoral age. And MRI study would be required as the MRI was reported as an useful diagnostic imaging modality in depicting necrosis and characterizing different phases of necrosis in VX2 carcinomas,in further study as well (4). Further study, therefore, should be done for gaining more useful information on the establishment of solitary hepatic tumor model.

A single nodular hepatic tumor model can be achieved from tumor fragment implantation with a loaded Seldinger needle directly and placing a gentle pressure in rabbits. This method could be used for creating an appropriate tumor model in rabbit, which is essential for a radiological investigation and chemotherapeutic agent-relevant study.

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가토에서 VX2 단일간암모델과 CT 소견

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요 약 : 80두의 가토 간에 1~2 mm^3 크기의 VX2종양세포를 직접 주입하여 단일간암모델을 만들고, 종양이식 후1주, 2주, 3주째에CT촬영을 실시하여 생성된 종양의 크기와 조영 양상을 관찰하고 조직병리학적검사로 종양의 진위 여부를 확인하였다. 80두 중 66두(82.5%)에서 단일 종양이 만들어졌다. 나머지 12두 중 1두에서 간외종양의 생성, 그리고 다른 1두에서 육아종성 종괴가 관찰되었으며, 4두는 폐사하였다. 나머지 8두에서는 종양이 생성되지 않았다. 조영 CT에서 관찰된 종양의 크기는 1주, 2주 그리고 3주째에 각각 7.4×6.3, 14.2×10.8, 16.2×12.6 cm로 측정되었다. 모양은 둥글거나 타원형이었으며, 동맥기에서 중앙에 저감쇠와 변연에 조영증강을 보였으며, 문맥기에는 저감쇠를 나타내었다. 종양조직의 이식은 균질한 단일종양모델을 만드는데 매우 유용하고 적합한 방법이며, 조영 CT촬영술은 간종양 진단 및 다양한 치료방법 적용 후 추적검사에 매우 유용하게 활용될 것으로 판단된다.

주요어 : 종양모델, 간, 토끼, VX2 암종, 컴퓨터단층촬영술.