Establishment of a Rabbit Model of Superior Vena Cava Obstruction

Fa Qin LV, Yun You DUAN, Xi LIU, Tie Sheng CAO, Wen WANG, and Li Jun YUAN

Department of Ultrasound Diagnostics, Tangdu Hospital, Fourth Military Medical University, Xi’an 710038, China

Abstract: Objective: To explore a method of establishing a rabbit model of superior vena cava obstruction (SVCO) by injecting VX2 tumor cell suspension transcutaneously under ultrasound guidance. Methods: A suspension of VX2 tumor cells was prepared under sterile conditions. Fifteen adult healthy New Zealand White rabbits were enrolled in the experiment. Under ultrasound guidance, about 0.1 ml of the living tumor cell suspension was transcutaneously injected in front of the anterior wall of the right superior vena cava (SVC). The lumen, wall, blood flow of SVCs and adjacent tissues were examined with gray-scale and color Doppler ultrasonography, every 3 days starting from the 9th day after injection. Meanwhile, CT scanning and digital subtraction angiography (DSA) were also performed. The rabbits were dissected immediately after death and tissue samples were collected for pathologic examination. Results: Fourteen out of 15 rabbits developed tumors that were located close to SVCs and/or SVCs cavity, which was shown by ultrasonography. The diameters of the tumors were 80.7 ± 4.3 mm. These tumors grew close to SVCs area and resulted in compression and infiltration of SVCs. CT scanning and DSA confirmed the establishment of the SVCO model. The achievement rate of the SVCO model was 93.3%. No rabbit died of complications. Conclusion: A method of establishing a rabbit SVCO model by injecting VX2 tumor cell suspension under ultrasonographic guidance was established successfully, and it proved to be simple, effective and repeatable. The imaging characteristics of this model are in good accordance with those of SVCO in patients. Key words: imaging characteristics, intra-thoracic tumor, ultrasonography, VX2 tumor cells

Introduction

Intraluminal or extraluminal obstruction of the superior vena cava (SVCO) can lead to superior vena cava syndrome (SVCS). Acute or subacute SVCS may be manifested by distinctive clinical features such as respiratory symptoms and venous stasis [16]. With the increase of cancer morbidity, neoplasm-related SVCO has become common in clinical practice. Reports have shown that more than 80% cases of SVCS are caused
by malignant tumors [5]. This syndrome is a very common complication of lung cancer or lymphoma [3, 19]. Because the superior vena cava (SVC) is surrounded by many different types of tissue whose density varies, the diagnosis of SVCS is often based imaging methods other than ultrasonography [9, 14]. Some studies on this disease were performed with transesophageal echocardiography [2], but most of the relevant literature is case reports [15]. The progress of modern diagnostic imaging techniques, especially ultrasonography, has made it possible to locate SVC via a small acoustic window such as the supra-clavicular fossa [12]. Some reports have described normal SVC by transthoracic ultrasonography [11]. At present the reports on transthoracic ultrasonographic study of SVCO are quite few. Furthermore, no detailed information about the establishment of an animal SVCO model can be found in the literature. In this study we experimentally established such an animal model and investigated the relationship between the pathogenesis and the ultrasonographic imaging of SVCO. It may help to evaluate hemodynamic changes of SVCO in humans and may be of use in further studies directed at optimizing the radiotherapeutic dosage for original tumors.

This study was undertaken in accordance with Animal Act, 1986 and its associated Codes of Practice.

Materials and Methods

Experimental animals and anesthesia

Fifteen adult healthy New Zealand White rabbits (8 females and 7 males, weighing 3.5–4.0 kg) were selected randomly from the Experimental Animal Center of the Fourth Military Medical University (FMMU). Ketamine hydrochloride, used as an anesthetic in this experiment, was injected into hind limb muscles of the rabbits at a dose of 30 mg/kg.

Development, passage and preservation of the VX2 strain

A frozen VX2 tumor cell suspension from the Pathologic Department of FMMU was centrifuged for about 5 min after resuscitation, and the supernatant was removed. After washing with phosphate buffered saline (PBS), the cell pellet was resuspended in PBS. Then the cells were stained with Trypan blue. The dead and living cells in this suspension were counted respectively, and the suspension was adjusted to give a VX2 tumor living cell density of \(1 \times 10^7/ml\). To establish a passage rabbit model, about 0.1 ml of cell suspension was injected into the muscles of a rabbit’s hind limb under sterile conditions. Thereafter, when necessary, the growing tumor in the hind limb was removed under sterile condition. After removing the necrotic and connective tissues, the tumor was rinsed twice with saline and homogenated rapidly into 0.1–0.3 mm\(^3\) pieces with a homogenizing apparatus. Then the specimen was suspended in 5 ml saline and drawn into an injector for implanting.

Methods of implanting tumors

The rabbits were fixed on a board in the supine position, shaved of fur on the chest and anesthetized with ketamine injected into the hind limb muscles. The supraclavicular fossa and suprasternal fossae regions were exposed and sterilized, and the surrounding area was isolated with an aseptic towel. The ultrasound probe was covered by a sterilized condom. Gray-scale ultrasonography was performed to show the image of the precordial structure and then to guide the needle to its proper location. Rabbits have two SVCs (also called precaval veins), as well as a transverse jugular vein, and we selected the right SVC as the implant location for the purpose of a good mimesis of human beings. The location was anterior to the medio-superior segment of the right SVC, about 1–2 cm beneath the crotch between the right jugular vein and the right subclavian vein. Sometimes gray-scale ultrasonography did not present the lumen and course of SVC clearly, especially on a thin and small rabbit; in such a circumstance color flow imaging guidance should be considered (Fig. 1). An ultrasound biopsy kit (8-light Diagnostic Criteria Company, Japan) was used. When the structure of the precordial region was obtained clearly on the display by ultrasonography, the injector needle was guided into place. After SVC was identified, 0.1 ml of the VX2 tumor cell suspension was infused slowly under positive pressure beside the SVC. In order to avoid leakage and tumor seeding outside of the peri-venous region, we withdrew the needle rapidly and pressed the wound for a few seconds. The wound was regularly kept sterile and antibiotics were injected into the muscles. After the operation, the behavior, appetite and vital signs,
establishment of rabbit SVCO model

especially rate of respiration, were closely monitored in all the rabbits.

Apparatus and methods of detection
Morphologic and hemodynamic changes of SVCs were observed by using Sequoia 512 (Acuson Corp, America) computed sonography with a 7v3c probe and a frequency of 7.0 MHz. During the experiment, the parameters of the ultrasound device were fixed, at 50 mm echo depth, -20 dB gain, 40 dB color and power gains and 30 cm/s average velocity. As the tumor grew larger, a 3v2c probe was used to show and measure the tumor completely.

The transducer was placed in the right supra-clavicular fossae and long-axis images of SVCs were recorded in all 15 rabbits. Two-dimensional echocardiography was used to observe the tumor size and adjacent structures, caliber, wall morphology and compressibility of SVC. The recorded tumor size is the mean diameter which was calculated as (long diameter + short diameter)/2. Hemodynamic changes of SVCs, including direction, velocity and patterns of blood flow were detected by Doppler echocardiography every 3 days from the 9th day after the infusion. Morbid rabbits were euthanized, with post mortem examinations performed immediately: incided the median sterna, separated soft tissues, exposed SVC and tumors, and investigated the relationship between the tumorous mass and the SVCs.

At the same time as ultrasonography, mediastinum plain scanning was performed on rabbits with tumors by computer tomography (CT) scanning (Philips AVP1), and spiral enhancement scanning with 3-mm layer thickness followed.

Digital subtraction angiography (DSA) (Siemens 1250A) was used to confirm the stenosis extent of SVCs. Following a femoral venipuncture, a micro-duct was inserted into the femoral vein lumen. When the micro-duct tip reached the distal SVCs stenotic segment, an appropriate amount of contrast agent, amidotrizoic acid, was infused. The median and lateral images of SVCs were subsequently recorded.

Statistics analysis
All the data are denoted as mean ± SD. The physiological data were compared by use of the 2-tailed unpaired Student’s t test. The correlation between growth rate and time were analyzed by Excel software. A value of \( P<0.05 \) was taken as indicating statistical significance.

Results
Tumor implanting
Nine out of the 15 rabbits were affected by tumors in front of SVCs on the 9th day after infusion, 3 on the 12th day, 1 on the 15th day and 1 on the 18th day. In one rabbit, no tumor had developed close to SVCs region by 42 days after infusion.

Establishment of SVCO model and detection of ultrasonography
In the normal state, the lumen of SVCs showed echoless and the wall appeared as two hyper-echoic lines by gray-scale ultrasonography. Structures close to SVCs could also be visualized clearly. The blood flow spectrum of SVCs presented laminar flow and normal velocity [3] (Fig. 2). Tumor compression on SVCs was visualized by gray-scale ultrasonography in 8 out of 14 rabbits with tumors on the 15th day after infusion (Fig. 3). Focal mosaic blood flow signals (Fig. 4) and turbulence spectrums (Fig. 5) were detected in SVCs by color Doppler ultrasonography. The early phase of stenosis presented high flow velocity with the maximal velocity approaching 130 cm/s. Stenotic flow of SVCs was detected in 4 rabbits with tumors on the 18th day after infusion, 2 on the 21st and 8 on the 24th day. The success rate of the
SVCO model in rabbits due to VX2 tumor was calculated to be 93.3% (14/15).

Tumors expanded over time in front of SVCs and were observed regularly by ultrasonography. The growth rate was slow in the first two weeks after infusion (Fig. 6). The correlation coefficient of the growth rate with time was 0.9855.

**Autopsy findings**

Autopsy of the rabbits with tumors revealed that the tumors grew in mass without membrane. All tumors were located in front of SVCs and adjacent to its anterior and/or lateral walls, and they all resulted in compression or infiltration of SVCs to different extents. The extent of damage to SVCs depended on tumor size and morphology. During the advanced stage there was liquefaction necrosis in tumors. Obvious lumen dilation was observed both in the distal portion of the stenotic segment of SVCs and in its draining veins (Fig. 7). The tumor diameter was measured as 80.7 ± 4.3 mm by ultrasonography before sacrifice and as 82.2 ± 3.4 mm on autopsy. There was no significant difference between the two methods of measurement (t=0.998, P=0.327405). According to Hematoxylin and Eosin (HE) stained images, the tumor cells presented as an entity nest, densely arranged with few fiber stroma (Fig. 8).

---

**Fig. 2.** Pulsed Doppler image of SVC in a healthy adult rabbit. SVC displays laminar flow spectra, the peak velocity < 50 cm/s.

**Fig. 3.** Gray-scale ultrasonographic image of SVC in a rabbit with SVCO. The arrow indicates that SVC is oppressed and its lumen becomes stenotic. M: mass.

**Fig. 4.** Color Doppler image of SVC in a rabbit with SVCO. The arrow indicates stenotic mosaic flow in SVC. T: tumor.

**Fig. 5.** Pulsed Doppler image of SVC in a rabbit with SVCO. SVC shows turbulent spectra and high velocity in stenotic segment. The peak velocity reached 103 cm/s.
ESTABLISHMENT OF RABBIT SVCO MODEL

**115**

**ESTABLISHMENT OF RABBIT SVCO MODEL**

**DSA and CT findings**

DSA imaging of rabbits with tumors showed that SVCs were compressed, displaced and locally narrowed, and the draining veins were dilated (Fig. 9). When infiltration occurred, anastomosis of tumor veins and the anterior cervical venous plexus was shown. The neoplasm in the superior mediastina appeared deformed in shape and unevenly distributed in density by CT. Compressed SVCs and their diffusely infiltrated walls were visualized at the venous stage by CT contrast scanning (Fig. 10). In some rabbits with tumors, CT showed the SVCs walls as a circular reinforced shadow or filling defect due to intra-cavity low density.

**Discussion**

SVCO can be caused by various factors such as neoplastic compression or infiltration, thrombosis and scar tissue formation, and it commonly leads to clinical SVCS. With neoplasm morbidity increasing, partial or complete obstruction of SVC by tumor compression or infiltration is becoming more common.

In the late 1930’s Kidd utilized Shope virus growing in a rabbit to induce papilloma which subsequently cancerated into squamous carcinoma and after 72 times of passage eventually formed the VX2 strain, which could be implanted in many organs and tissues of the rabbit [10], proving to be quite successful to model rabbit organ tumors [4, 17]. It has been widely believed that the rabbit models developed by the VX2 strain have many virtues including a high success rate, stable biological property et cetera [7, 8, 13, 18]. To our knowledge, we established for the first time the SVCO model in rabbits with the VX2 strain and the success rate was 93.3%.

In our experience, the key points to develop animal models of SVCO are as follows: the tumor survives in the living animal after embedding a tumor mass or infusing a tumor cell suspension accurately close to SVC.
region; and, the growing tumor should lead to partial or complete obstruction of SVC. In this study, the VX2 tumor suspension was infused in front of the SVC area, close to its anterior wall of the medio-superior segment. The injection target was visualized clearly with ultrasonography, which led to no complications and enabled rabbits to survive easily. No rabbit died of complications related to the implantation process during this experiment. Among 15 rabbits, 14 were diagnosed with developing tumors. As the tumors grew, SVCs were displaced, compressed or infiltrated gradually. As a result, the SVC lumen became narrow and the flow velocity increased or even decreased when SVCs were severely stenotic. The draining veins of SVCs dilated. All these results indicated that a typical SVCO model was established.

SVCO was confirmed with ultrasonography, CT and DSA from different aspects. CT scanning indicated the spatial relationship between the tumors and SVCs. DSA displayed abnormal SVC shapes and stenotic lumen, and indicated the changes of SVC hemodynamics to some extent. All these imaging techniques offered different methods complementary to each other. Gray-scale ultrasonography revealed that tumors compressed and/or infiltrated SVC walls, and resulted in lumen stenosis eventually. Color Doppler showed stenotic blood flow images of SVCs, and pulsed Doppler presented abnormal spectral waveforms with high or low flow velocity. In a preliminary clinical study we demonstrated the Doppler flow pattern changes of obstructed SVCs due to neoplasm and its predictive value in patients with SVCS [6]. We found that in all patients before therapy the Doppler spectrum of SVCs presented turbulence, and forward waves manifested irregular turbulent spectrum with high velocity. It seems that the image characteristics of the rabbit SVCO model coincide well with those of patients with SVCO revealed by ultrasonography [1].

In conclusion, a method of establishing a rabbit SVCO model by injecting VX2 tumor cell suspension under ultrasonographic guidance was successfully demonstrated and it proved to be simple, effective and repeatable. The imaging characteristics of this model are in good accordance with those of SVCO in patients. Establishment of the model can offer experimental evidence for the diagnosis, treatment and follow-up study of SVCO.
ESTABLISHMENT OF RABBIT SVCO MODEL

References