The evaluation of rabbits model in bearing VX2 hepatic tumors by multi-slice spiral CT perfusion and real-time contrast enhanced ultrasonography and the correlation research of VEGF

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**Purpose**

1. Primary hepatic carcinoma is one of the most common malignant tumors. The mortality of primary hepatic carcinoma is ranked the second place, just behind lung cancer in China. 2. Following the development of advanced imaging techniques in recent years, computed tomography perfusion has been able to provide functional imaging to evaluate the haemodynamics in tissues and contrast enhanced ultrasound has gradually been generalized in recent years. 3. In this study, we performed multi-slice spiral CT perfusion and contrast enhanced ultrasound in rabbit VX2 hepatic tumors to observe the features of blood perfusion in liver tumors.

**Methods and Materials**

**Materials:** Twenty healthy New Zealand white rabbits (male or female, 2.0~3.0kg) were used to built VX2 hepatic tumor models. The carcinoma cells were subcultured by directly implanted into the root of the rabbit legs. The VX2 hepatic carcinoma mass were implanted into the liver of rabbits in trial group via laparotomic route. Fig. 1 on page 3

**Methods:**

1. **Multi-slice spiral CT perfusion examination**
   - The scan mode called “Toggling - table” was used to performed the CT perfusion from diaphragm to inferior border of liver. The contrast medium (320mgl/ml) was bolus injected by rabbit's auricular vein with the injected speed of 0.7ml/s and injected dose of 1ml/kg. Scan parameter: 100KV, 80mA, slice-thickness was 5mm, 360°rotation time was 0.6s, total scan time was 50s, detectors were configured as 64×0.625mm. Two minutes later, the CT enhancement scan was performed after being injected a contrast medium at the rate of 0.5ml/s and injected dose of 4ml by a high pressure tweein injector, and at the same rate 2ml of normal saline was injected, scan was started when 13s#27s#59s was postponed after injected.
   - The regions of interest were contained the rim of the tumor, non-tumorous regions nearby the tumor and the normal liver. Time density curve, blood flow figures and the CT perfusion parameters: Blood Volume, Blood Flow, Mean Transit Time, Permeability Surface and Hepatic Arterial Fraction were measured automatically. Fig. 2 on page 4

2. **Ultrasound examination**
   - The rabbits were performed by color doppler ultrasonography and CEUS by ALOKA SSD-#10 color doppler diagnostic apparatus. Conventional
detecting the liver by 2-D and recording the location, diameter, shape, color doppler flow imaging inside and outside of the tumor.

- The dynamic features of contrast enhanced ultrasound in the tumor and the normal liver were observed by contrast tuned imaging by bolus injection of SonoVue with injected dose of 0.1ml/kg and normal saline with injected dose of 2ml via peripheral vein. Total detection time was 50s, detector acceptance frequency was 11MHz, and mechanical index was 0.05. Fig. 3 on page 4

3. RT-PCR

- After executed these rabbits, the fresh liver specimen of the rim of the tumor, non-tumorous regions nearby the tumor and the normal liver were obtained respectively.
- The expression of VEGF mRNA in the rim of the tumor, non-tumorous regions nearby the tumor and the normal liver were detected by reverse transcriptase-polymerase chain reaction (RT-PCR). The correlation between the expression of VEGF mRNA and CT perfusion parameters and features of contrast-enhanced ultrasonography were measured.

Images for this section:
**Fig. 1:** The steps of tumor model implantation.

**Fig. 2:** Multi-slice CT enhancement and perfusion were performed in these rabbits at twenty-one day after implantment.

**Fig. 3:** The rabbit was performed by color doppler ultrasonography and real-time contrast enhanced ultrasonography.
Results

1. Pathological features of rabbit VX2 tumor model

- Twenty of 25 white rabbits (83%) were successfully implanted with the tumor, yielding a total of 59 neoplastic nodules with diameters from 0.2 cm to 4.0 cm.
- VX2 hepatic tumors were round-shaped gray nodules with an intact capsule and necrosis in the center of the tumor. The histological analysis showed that VX2 tumors had an arrangement of "nest", with separate fibers and abundant ascendent capillaries. The tumor cells were large and had round or irregular morphology. The nucleus of VX2 cells was hypertrophic and heterogeneous, with different size and shape. The phase of nuclear mitosis was observed in VX2 carcinoma cells. Fig. 4 on page 6

2. CT features of rabbit VX2 tumor model

The CT perfusion results showed increased BF, BV, PS, HAF, HAP but decreased MTT in the rim of the tumor, compared with the surrounding non-tumor areas and the normal liver. Except HAF and HAP, there was no significant difference of other perfusion parameters among tumors, non-tumor areas and normal livers. Fig. 5 on page 7 Fig. 6 on page 8

3. Ultrasound imaging features of rabbit VX2 tumor model

- The lesions were round-shaped iso-echoic tumors with acoustic halo nearby and low level echo of necrosis in the center from 2-D ultrasound. CDFI detected spot-like and strip-like echos of the blood vessels nearby the tumors.
- 3~5 sec after injection of the contrast agent, the tumors gradually showed round or branching hyperechoic enhancement during the arterial phase, and then gradually decreased enhancement with increased enhancement in the liver parenchyma nearby. Fig. 7 on page 9

4. VEGF mRNA expressions in VX2 tumor model

- The expression level of VEGF was examined by RT-PCR. As showed in Fig. 8 on page 10, the normal liver had a low level of VEGF expression. The non-tumor areas adjacent to the tumor expressed a significant higher level of VEGF compared to the normal liver. Furthermore, the rim of the tumor expressed the highest level of VEGF compared to both non-tumor surrounding areas and normal livers. Fig. 9 on page 11
- There were significant correlations between VEGF expression and the data of CT perfusion data in the rim of the tumor, non-tumor regions adjacent to
the tumor and normal livers, but no correlations between VEGF expression and MTT. Fig. 10 on page 12

5. Detecting rate

- There was a significant difference of detection rate in the diagnosis of small hepatic tumor which diameters were less than or equal to 3.0 cm by CT perfusion, 2-D ultrasound and CEUS. CT perfusion and CEUS were better than 2-D ultrasound in detecting small hepatic tumors.
- There was no significant difference of specificity and concordance rate in the diagnosis of small hepatic tumor by CT perfusion, 2-D ultrasound and CEUS. There was a significant difference in detection rate of micro hepatic tumor with diameters less than 1.0 cm by CT perfusion, 2-D ultrasound and CEUS. CEUS was better than CT perfusion and 2-D ultrasound in detecting micro hepatic tumors. Fig. 11 on page 12

Images for this section:
**Fig. 4:** a. The pathological specimen of rabbit VX2 liver tumors. b. VX2 liver tumors with necrosis in the center. c. The HE staining of VX2 rabbit liver tumors (10×). d. The HE staining of VX2 rabbit liver tumors (40×).
**Fig. 5:** a. The tumor showed significantly ring-enhancement on arterial phase. b.-f. The CT perfusion BF,BV,MTT,PS,HAF imaging of the VX2 liver tumor.
Fig. 6: Comparison of different CT perfusion parameters in different regions in rabbits with VX2 liver tumors (n=20)

<table>
<thead>
<tr>
<th>Group</th>
<th>BF (ml/min • 100g)</th>
<th>BV (ml/100g)</th>
<th>MTT (s)</th>
<th>PS (ml/min • 100g)</th>
<th>HAF (%)</th>
<th>HAP (ml/min • 100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The rim of the tumor</td>
<td>311.21 ± 47.57</td>
<td>40.04 ± 4.20</td>
<td>4.91 ± 2.15</td>
<td>37.82 ± 2.01</td>
<td>0.86 ± 0.12</td>
<td>265.44 ± 35.57</td>
</tr>
<tr>
<td>Surrounding non-tumor areas</td>
<td>243.94 ± 35.15</td>
<td>24.60 ± 2.40</td>
<td>14.28 ± 3.07</td>
<td>22.56 ± 1.41</td>
<td>0.47 ± 0.12</td>
<td>115.80 ± 27.10</td>
</tr>
<tr>
<td>The normal liver</td>
<td>231.49 ± 86.48</td>
<td>26.59 ± 2.59</td>
<td>13.62 ± 2.66</td>
<td>23.80 ± 1.77</td>
<td>0.41 ± 0.15</td>
<td>95.42 ± 52.20</td>
</tr>
<tr>
<td>$P_{	ext{ov}}$</td>
<td>0.016</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>$F_{	ext{position}}$</td>
<td>962.437</td>
<td>3794.040</td>
<td>784.352</td>
<td>15968.76</td>
<td>1342.180</td>
<td>800.907</td>
</tr>
<tr>
<td>$P_{	ext{ov}}$</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>$F_{	ext{interaction}}$</td>
<td>8.583</td>
<td>96.877</td>
<td>59.732</td>
<td>485.455</td>
<td>80.301</td>
<td>91.157</td>
</tr>
<tr>
<td>$P_{	ext{ov}}$</td>
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<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* Comparison with the normal liver, $P<0.05$
Fig. 7: a. CDFI showed spot-like or strip-like blood echo in the centre or nearby the tumors. b. 2-D ultrasound showed round-shaped iso-echoic tumors with acoustic halo nearby and low level echo of necrosis in the centre of the tumor. c. CEUS showed round or branches hyperechoic enhancement during arterial phase. d. CEUS showed decreased enhancement with the increased enhancement in liver parenchyma nearby in portal phase.
**Fig. 8:** The expression level of VEGF mRNA at the rim of the tumor, non-tumor regions adjacent to the tumor and normal liver was detected by RT-PCR. M: DNA marker; 1: the normal liver; 2: non-tumor regions adjacent to the tumor; 3: the rim of the tumor.
**Fig. 9:** Comparison of the expression of VEGF mRNA in the rim of the tumor, non-tumorous regions nearby the tumor and the normal liver

<table>
<thead>
<tr>
<th>Diameter (cm)</th>
<th>Number (n)</th>
<th>Detection number by CTP (n)</th>
<th>Detection number by 2-D ultrasound (n)</th>
<th>Detection number by CEUS (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d&lt;1.0</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>1≤d≤3.0</td>
<td>25</td>
<td>28</td>
<td>24</td>
<td>25</td>
</tr>
</tbody>
</table>

**Fig. 11:** Comparison of detection number of the VX2 rabbit liver tumor by CT perfusion, 2-D ultrasound and CEUS.
Fig. 10: Correlation between BV and MTT in the rim of the tumor, non-tumor regions adjacent to the tumor and normal livers and the expression of VEGF mRNA.
Conclusion

1. Multi-slice CT perfusion measured the perfusion parameters by selecting the regions of interest, real-time contrast enhanced ultrasonography diagnosis of solid mass in liver by detecting the blood features in the tumor, which could providing information of perfusion and real-time changes of vascular morphology, and detect the blood supply of hepatic tumors in vivo.

2. There was positive correlation between gene expressions of VEGF mRNA and the display of blood supply feeding vessels by CEUS, the data of CT perfusion. Angiogenesis and haemodynamics could be evaluated by the two imaging technologies.

3. There was high diagnostic value in detecting small hepatic tumors by Multi-slice CT perfusion and CEUS. CEUS was better than CT perfusion and 2-D ultrasound in detecting micro hepatic tumors.

References


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