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Combining dynamic contrast enhanced magnetic resonance imaging and microvessel density to assess the angiogenesis after PEI in a rabbit VX2 liver tumor model

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ABSTRACT

Objectives: To evaluate the correlation between parameters of dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) and microvessel density (MVD) measurements in rabbit VX2 liver tumor models after percutaneous ethanol injection (PEI) and to observe influence of PEI on angiogenesis in a rabbit VX2 liver tumor model with dynamic contrast enhanced magnetic resonance imaging (DCE-MRI).
Materials and methods: Forty five New Zealand white rabbits were used in this study. VX2 tumor tissue blocks were implanted in the left lobe of liver by percutaneous puncture under CT guidance. 2 weeks later, all rabbits underwent conventional MRI (T1WI, T2WI) to determine the successful models. Then those successful implanted VX2 liver tumor models in the study were randomly divided into the control group and the experimental group, the former did not have processing, and the latter underwent PEI under CT guidance. MRI (T1WI, T2WI and DCE-MRI) was performed 1 week later again, the parameters of DCE-MRI (K^{trans}, K_{ep}, V_e and iAUC60) of viable tumor portions were observed. Then all the liver samples were processed for hematoxylin and eosin (H&E) staining and immunohistochemical staining for CD31 to determine MVD. At last, data (including DCE-MRI perfusion parameters and MVD) were compared between experimental and control groups, correlation of DCE-MRI perfusion parameters and MVD was evaluated.
Results: Twenty six VX2 liver tumor models underwent all examinations (thirteen models for each group) 1 week later after PEI. For the experimental group, the parameters K^{trans} ($r = 0.6382$, $P = 0.0189$) and iAUC60 ($r = 0.6591$, $P = 0.0143$) in viable tumor portions were positively moderately correlated with MVD, whereas the parameters K_{ep} ($r = 0.4656$, $P = 0.1088$) and V_e ($r = 0.2918$, $P = 0.3333$) were not correlated with MVD. For the control group, the parameters K^{trans} ($r = 0.6385$, $P = 0.0188$) and iAUC60 ($r = 0.6391$, $P = 0.0187$) in viable tumor portions were also positively moderately correlated with MVD, while the parameters K_{ep} ($r = 0.5518$, $P = 0.0506$) and V_e ($r = -0.0824$, $P = 0.789$) were not correlated with MVD. K^{trans}, K_{ep}, V_e, iAUC60 and MVD of residual viable tumors in the experimental group 1 week later after PEI were similar to the viable tumors of the control group ($P > 0.05$).
Conclusions: DCE-MRI could be used to evaluate the efficiency of VX2 liver tumor after PEI. The quantitative parameter K^{trans} and semi-quantitative parameter iAUC60 of DCE-MRI are correlated with MVD, which can assess tumor angiogenesis noninvasively of VX2 liver tumor model, and ethanol has no significant impact on angiogenesis of viable tumor 1 week later after PEI.

1. Introduction

Hepatocellular carcinoma (HCC) is the third common cause of death malignancies worldwide [1]. Interventional therapies including percutaneous ethanol injection (PEI), trans-catheter arterial chemoembolization (TACE), radiofrequency ablation (RFA) and so on, have become important adjuvant therapies.

PEI is a well-tolerated, reproducible, simple, and low-cost therapeutic technique [2], it induces coagulation necrosis through cellular dehydration, protein denaturation, and chemical occlusion of small tumor vessels [3,4], and can play an important part in the combination treatment of liver malignancy [5,6]. So it is important to evaluate the effects of PEI.

Angiogenesis significantly associated with tumor proliferation [7], is an important indicator to evaluate the effect after interventional therapy and prognosis of patients. As the "gold standard" measurement to evaluate angiogenesis, microvessel density (MVD) in histological samples is not ideal in clinical practice for the reason that it is invasive and is limited by the histopathologic sampling.

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Noninvasive and repeatable imaging methods such as perfusion imaging to evaluate tumor angiogenesis have gained significant attention in recent years [8].

As a kind of perfusion imaging, DCE-MRI is useful in evaluating early efficacy of antiangiogenesis drugs and vascular disrupting therapies [9–12], and could be applied to assess the efficacy of local tumor ablation such as TACE and RFA [13–15]. However, few studies have investigated the relationship between semi-quantitative and quantitative parameters of DCE-MRI and MVD in the rabbit VX2 animal models, and angiogenesis after PEI has rarely been reported.

As the most commonly used large animal models, the rabbit VX2 liver tumor models can be used to imitate liver tumors of humans, because they have the similar blood supply with advanced human hepatocellular carcinoma [16].

In this experiment, we planned to evaluate the correlation between parameters (K^{trans}, K_{ep}, V_e and iAUC60) of DCE-MRI and MVD and observe the angiogenesis of viable tumors of rabbit VX2 liver tumor models 1 week later after PEI.

2. Materials and methods

2.1. Tumor-bearing rabbits and VX2 liver tumor model

Animal studies were performed according to the guidelines of the Use of Laboratory Animals of the Ministry of Public Health of China and were approved by the ethics committee at our institution. All surgeries were performed under anesthesia, and all efforts were made to minimize suffering.

Forty five New Zealand white rabbits weighed 2.4–2.7 kg were included in the study. After recovery from liquid nitrogen, the VX2 tumor cells were injected into muscles of hind limbs to produce a tumor-bearing rabbit. When tumors grew to a size of about 3 cm in diameter, they were harvested and viable portions of tumors were cut into little tissue fragments about 1–2 mm^3 under sterile conditions. Recipient rabbits (44 rabbits)were anesthetized by intramuscular injection with Lumianning II at a dose of 0.5–0.7 ml and intravenous injection with 1.25% mebumalnatrium at a dose of 1.4–2.0 ml. Then, 2–3 VX2 tissue blocks were implanted in the left hepatic lobe of the recipient rabbits by percutaneous puncture under CT guidance to produce VX2 liver models.

2.2. MRI examination

MRI was performed 2 weeks later after tumor implantation to screen out successful VX2 liver tumor models (a rabbit with a tumor in the left hepatic lobe, the tumor with no necrosis or few necrosis, the max diameter of the tumor is from 1 cm to 2 cm) on a 3.0 T magnet (MAGNETOM Skyra, Siemens, Germany) with an individual coil for rabbits (Shanghai Chenguang Medical Technologies Company Limited, China). Rabbits were anesthetized as described above and maintained in the prone position throughout the examination. Towels were used to wrap the abdomen of rabbits to reduce respiratory motion artifacts. Examination protocols of MRI were shown in Table 1. All sequences were performed with the same geometry. After T1-map and first 5 baseline measurements were finished, 1 ml Gd-DTPA (about 0.2 mmol/kg of body weight) was injected through the left/right auricular vein at a bolus of 2 ml/s using an automated injector(Mallinckrodt, C1213D005X). In total, 100 measurements were acquired to perform DCE-MRI. Identical MRI procedures were performed again 1 week after PEI (Table 1 lists the detailed acquisition parameters).

2.3. Image analysis

DCE-MRI includes a sequential acquisition of magnetic resonance images of tissue before and after the intravenous injection of contrast agent (CA). T2* weighted MRI is based on the first-pass effect of CA. Since the first-pass T2* effect is transient, the interest slice includes only a single. This is of limited value in liver study.

In contrast, T1-weighted DCE-MRI technique is more commonly used in liver cancer research. The T1-weighted DCE-MRI is usually used over a longer time to measure the accumulation of CA in the tissue. The evolution curve as a function of time can be acquired from sequentially sampled T1-weighted magnetic resonance images signal intensity at the tissue.

The acquired DCE-MRI images were sent to Tissue-4D workstation (software based on Toft's model). The following parameters can be derived from the software: (1) K^{trans} (volume transfer constant): determines the transport rate of the contrast agent from the intravascular space to the extravascular extracellular space (EES). (2) K_{ep} (reflux rate constant): represents the volume of the contrast agent from EES to the intravascular space; (3) V_e (volume fraction of EES): an indirect measure representing the cellular density of the tissue; and in the model, $V_e = K^{trans}/k_{ep}$, the model equation is as follows:

$$C_t(t) = K^{trans}\int_0^t C_p(t)e^{-k_{ep}(t-\tau)}d\tau$$

where $C_t(t)$ is the tissue concentration of CA, and $C_p(t)$ is the plasma concentration of CA. (4) iAUC60 (area under curve/initial area under the contrast concentration versus time curve): the most frequently used semi-quantitative parameter determined by both tumor blood supply and endothelial permeability, represents the dose of the contrast agent is taken up by the tumor from injection point to a certain time point, such as the time intensity curve (TIC) to 60 s.

The largest slice or the largest two adjacent slices of the tumor on perfusion imaging was selected to obtain the following parameters K^{trans}, K_{ep}, V_e and iAUC60. "Hot spots" based on the DCE imaging and the perfusion pseudocolor images of the whole tumor (regions of viable tumor portions possess higher perfusion values on the perfusion pseudocolor images of the whole tumor) were chosen as the region of interest (ROI) of the viable tumor portions to obtain the above parameters. The median value offered by Tissue-4D workstation in our study was used as the target parameters.

2.4. PEI

After MRI examination, PEI was carried out on all rabbits of experimental group (the control group was just observed and was not disposed) under CT guidance for the reason that it may be prudent to start treatment at 2 weeks after implantation [17]. After anesthetized well, the rabbit lay on the CT check-bed in the supine position, fur around the xiphoid was shaved off, and a fencelike

Table 1
3.0-T MR imaging sequences and parameters.

	T1WI	T2WI	T1-MAP	DCE-MRI
Sequence type	TSE	TSE	VIBE	VIBE
Flip angle (°)	160°	145°	2°/15°	15°
Field of view (mm)	120	120	120	120
TR/TE (ms)	509/12	4000/98	4.7/1.77	4.7/1.78
Number of averages	4	5	3	1
Thickness (mm)	2.5	2.5	2.5	2.5
Slices	19	19	8	8
Acquisition time (min:s)	3:59	3:26	36 s	6:57

Note: TSE = turbo spin echo, VIBE = volumetric interpolated breath-hold examination, 3D-T1WI-GRE (gradient recalled echo), TR = repetition time, TE = echo time.