Comparison of 64-slice CT Perfusion Imaging with Contrast-enhanced CT for Evaluating the Target Volume for Three-dimensional Conformal Radiotherapy in the Rabbit VX2 Brain Tumor Model

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Brain tumor/Contrast-enhanced CT/CT perfusion.

CT perfusion imaging is a promising technique for delineating the target volume for three-dimensional conformal radiotherapy, but it is difficult in humans to obtain gross pathological samples at the same level of the brain tumor to evaluate this technique. The aim of this study was to use the BV map of CT perfusion imaging to assess the target volume in the rabbit VX2 brain tumor model, which has similar characteristics to human brain tumor, and compare the results to those of CECT. New Zealand white rabbits were used for the animal model. After tumor cell implantation 21 rabbits underwent 64-slice CT scanning. The target slice was selected and the maximum major axis length and minimum minor axis length of the tumor in the target slice on BV maps and contrast-enhanced CT images were measured. Pathological specimens were obtained from the rabbit brains which were removed intact. The GTV and CTV of the imaging methods were compared. Scanning was successful in 20 rabbits. The CECT images showed the target area for the VX2 tumor in 16 rabbits. The BV maps showed the target area for the tumor in 20 rabbits. The probability was 95% that the GTV determined by pathology can be covered completely when BV maps are used. CT perfusion imaging appears to be a promising technique for delineating the GTV of brain tumors in clinical practice.

INTRODUCTION

Radiation therapy is one of the main treatment methods for brain tumors. It has been reported that survival time can be significantly improved by postoperative radiotherapy.1–3 Delineating the target volume is critical in radiation therapy. According to the International Commission on Radiation Units and Measurements published Report 50, GTV is the tumor extent that can be palpated by clinical examination or is visible on imaging, and CTV includes the tumor revealed by clinical examination or imaging examination, i.e., GTV, plus the extent of subclinical lesions (tumor invasion and/or metastasis). Currently, the delineation of the target area for brain tumors is generally carried out by using contrast-enhanced CT or MR images as the basic images. However, in the head, contrast-enhanced scanning only shows the damaged area of the blood-brain barrier; it cannot show the undamaged area of the blood-brain barrier. Therefore, the results cannot represent the full extent of tumor invasion.4,5 Because of the limitations of these conventional imaging techniques, treatment often results in failure of local control.3,6 Research efforts to provide more effective radiation therapy have resulted in the recently developed technique of three-dimensional conformal intensity-modulated radiation therapy. The critical step in this technique is to determine the target volume which includes visible lesions, microscopic lesions, and necessary margin.2

In recent years, a variety of functional imaging methods have been evaluated for delineating the target volume for
brain tumors, however, these studies have had serious design flaws. For example, to control for results at the same level, stereotactic biopsy was used rather than gross pathology, the gold standard.7–11 One promising area of research has been perfusion imaging.12,13 Magnetic resonance perfusion has been used, however, this technique can only provide the relative values of the perfusion parameters. On the other hand, the latest CT perfusion imaging software can provide the absolute values of perfusion parameters, and therefore, theoretically, using functional CT to delineate the target volume for brain tumors should be superior to using MR perfusion.14,15

One major problem encountered in the evaluation of perfusion imaging is that it is difficult to obtain gross pathological samples at the same level of the brain tumor in humans. However, it has been reported that the molecular biological characteristics of the rabbit VX2 brain tumor model are similar to those of human brain tumors.16 Therefore, this is an ideal model for studying perfusion imaging of brain tumors.5,17 The aim of this study was to use the BV map of CT perfusion imaging to assess the target volume in the rabbit model of VX2 brain tumor and to compare the results with those of contrast-enhanced CT and the gross pathology findings. We hoped that our study would determine the significance of CT perfusion imaging BV maps in the delineation of the target volume for brain tumor, and thereby might provide a basis for the clinical treatment of such tumors.

MATERIALS AND METHODS

Experimental animals

Purebred male and female New Zealand white rabbits (n = 21), weighing 2–3 kg were provided by the Animal Center of Sichuan Academy of Medical Sciences (license number SCXK (Shanghai) 2003–0003 Shanghai Slack Experimental Animal Co., Ltd.). VX2 tumor cells were obtained through intramuscular injection in the hind leg of rabbits with tumor in vivo. Rabbits with tumor were provided by the Institute of Ultrasound, Chongqing Medical University. All animals received care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 85–23, revised 1996). All experimental procedures were approved by the Care of Experimental Animals Committee of Laboratory Animal Center, Sichuan Academy of Medical Science.

Model preparation

The rabbits were anesthetized with intramuscular injection of 0.4% pentobarbital. Solid tumors in the groin area of the rabbits with tumor were stripped off under anesthesia and sterile surgical conditions. The samples were rinsed with normal saline and the capsule was removed. Tissue with rapid growth was collected from the edge of the tumor and was cut into 2-mm blocks using a scalpel. The samples was placed in Hank’s solution and centrifuged at 1000 rpm in a centrifugal scrubber for 5–10 minutes. Then the tissue blocks were placed in a 200–300 mesh sieve homogenizer and homogenized with appropriate amount of 0.9% normal saline. Suspended cells were collected and centrifuged in a centrifugal scrubber at 1000 r/min for 5 minutes until the supernatant was clear. Serum-free RPMI-1640 medium was added to make a cell suspension with a diluted concentration of 1 × 10^7 cells/ml. The number of viable cells were counted using the trypan blue exclusion test; the percentage of viable cells was > 95%.

For tumor cell implantation, experimental rabbits were anesthetized and fixed in the prone position. A 3–4 cm incision was made along the mid-sagittal line of the top of the head. The peristium was cut open, and a metal drill with adjustable drill hole depth was used to drill holes on the right side of the sagittal suture and 0.5 mm away from the sagittal suture, and 5 mm posterior to the coronal suture. The drill holes had a diameter of 1.2 mm and a depth of 1–1.5 mm. Attention was paid to keeping the inner plate of the skull intact. A 1.0-ml syringe was used to aspirate a 0.1-ml VX2 cell suspension (1 × 10^7 cells/ml), and the needle was fixed with vessel forceps so that the puncture depth was 3–5 mm below the surface of the skull plate. The needle of the syringe was inserted into the inoculation site, and the cell suspension was injected slowly. The scalp was sutured immediately after the needle was withdrawn. If no neurological symptoms were present after the animals recovered from anesthesia, the surgery was considered successful. After surgery, 40 to 60 million units of penicillin were given routinely and gentamicin 40,000 units were injected intramuscularly for 3 days. An enhanced MR examination was performed 10 days after implantation. A GE Signa 1.5 T superconducting whole body MR instrument was used to perform the scan. Initially, contrast-enhanced T1-weighted spin-echo sequence T1W1 (TR 340 ms, TE 9 ms) was performed. This was followed by fast spin-echo T2W1 (TR4 200 ms, TE 98 ms). Slice thickness was 10 mm, matrix 256 × 192, field of vision (FOV) 22 cm × 22 cm. After a scan showed a suspected nodule at the site where the tumor was transplanted in the rabbit brain, a high-pressure syringe was used to give a bolus injection of double-dose gadolinium-DTPA (0.2 mmol/kg) via the rabbit ear vein to obtain cross-sectional spin-echo T1W1 (TR 340 ms, TE 9 ms) images of the rabbit brain, and the injection flow rate was 1 ml/s. CT perfusion imaging was performed when there was confirmation of VX2 tumor growth.

General information

The experimental animals underwent CT scanning 12–14 days after inoculation. Among the 21 New Zealand rabbits, one moved during scanning due to poor anesthetic effect and the scan was unsuccessful. The remaining 20 animals that were scanned successfully were included in the final data.
analysis.

**Instruments and methods**

A Philips Brilliance 64-slice CT scanner (Philips medical system Cleveland, OH, USA) was used. A Medrad Stellant CT-pressure automatic syringe was used to inject non-ionic contrast agent (iohexol, 300 mg/ml, GE Pharmaceutical Company, Shanghai, China) at a flow rate of 5 ml/s via the lateral thigh vein. The total volume of injection was 1.5–2 ml/kg. Normal saline was then injected immediately.

The conventional CT scan parameters were tube voltage 80 kv, current 120 mA, matrix 512 × 512, field of view (FOV) 250 mm and slice thickness 2.5 mm. A plain coronal scan was performed.

For perfusion CT, in the plain coronal CT scan images, the slice with the maximum tumor area was selected as the central slice. The perfusion procedure was selected, and dynamic scanning of the same slice was carried out. The continuous scan sequence was set. The parameters were slice thickness 2.5 mm, KV120, 84 mAs, collimation 0.625 mm, and scan time 1 s/360°. Image acquisition was once every 2 seconds, and the duration of continuous scan was 70 seconds. The delay time was 0 seconds. A total of 16 image sequences were obtained and each sequence contained 45 images.

The images were input into a Philips EBW CT image processing workstation and brain perfusion software was used for processing. The BV maps of the 16 slices were processed continuously. The basilar artery was selected as the afferent artery and the superior sagittal sinus was selected as the efferent vein. The target slice was selected, and the maximum major axis and the minimum minor axis of the tumor in the target slice in the contrast-enhanced CT images and BV maps were measured and recorded, respectively.

To confirm the pathological specimen was within the

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same slice, the CT bed position for the target slice, i.e., the slice with the maximum diameter, was recorded. The same tip system was used to move the bed to the target slice. The laser light of the detector was turned on, and the skin surface of the rabbit’s head and the skull surface were marked along the CT laser positioning line. The skull was drilled at the marked site and special labeling material with a thickness of 1 mm and width of 3–5 mm (waste X-ray film cut into 2 × 7 cm pieces) was inserted into the brain along the laser positioning line. Scanning was repeated to confirm the positioning of that slice and that the gap between that slice and the target slice was no more than 2 mm (i.e., the gross pathological specimen was taken from the same position of the target slice in the imaging examination).

Pathological specimens

After scanning, examination of rabbit brain anatomy was carried out. The animals were anesthetized. The intact brain of the rabbit was removed and the target slice was cut according to the position of the label. Marker dye was used to mark the visible tumor margin, and the maximum major axis length and minimum minor axis length of the tumor in the target slice visible to the naked eye were measured. GTV\textsubscript{CECT} was defined as the maximum major axis length and minimum minor axis length of the enhancing lesion in the target slice visible to the naked eye and the maximum distance of microscopic invasion beyond the margin visible to the naked eye were recorded. The two pathologists reached an agreement after discussion if they had differing opinions.

Statistical analysis

The data were presented as the mean and standard deviation. Bland-Altman assessment for agreement was used to compare the GTV and CTV methods. A range of agreement was defined as mean bias ± 2 SD. All statistical analyses were performed using the SPSS 15.0 software package (SPSS Inc., Chicago, IL, USA).

RESULTS

Relationship between tumor GTV and pathological CTV

The measurements of the major axis length and minor axis length are presented in Table 1. The pathological CTV
major axis length ranged from 5.1 to 20.9 mm in the 20 rabbit brains with VX2 tumor and the mean was 12.9 ± 3.7 mm. The minor axis ranged from 4.4–12.6 mm and the mean was 7.7 ± 2.2 mm.

In GTV$_{BV}$ images the major axis length ranged from 7–18.3 mm and the mean was 12 ± 3.3 mm. The minor axis length ranged from 3–10.6 mm and the mean was of 7 ± 1.8 mm. The major axis length and minor axis length were

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**Fig. 2.** A Gross pathological specimen shows GTV of the tumor visible to the naked eye (within the marker dye). B Gross pathological section shows pathological GTV of the tumor (within the marker dye). C The maximum major axis length and the minimum minor axis length of the brain tumor on the BV map were less than GTV$_{pathology}$ of the tumor at the same level. D The maximum major axis length and the minimum minor axis length of the brain tumor in CT images were greater than GTV$_{pathology}$ of the tumor and those lengths on the BV map at the same level. E Microscopic pathology showed that tumor invasion exceeded the GTV visible to the naked eye and the tumor extent showed on the BV map (short arrow indicates the area of GTV$_{pathology}$, and the curved arrow indicates the area of CTV$_{pathology}$).
The proportion of expansion needed for the CTV in rabbit model of VX2 brain tumor when contrast-enhanced CT and BV maps were used

In 16 cases, the contrast-enhanced CT images showed the target area for the VX2 tumor. When contrast-enhanced CT was used to estimate CTV_{pathology}, the proportion of expansion in the directions of major axis and minor axis was 81.8 ± 10.1% and 62.1–101.6, respectively (Table 2). In all 20 cases, BV maps showed the target area for the VX2 tumor. When BV maps were used to estimate pathological CTV, the proportion of expansion in the directions of major axis and minor axis was 8.0 ± 4.0% and 12.5 ± 6.2%, respectively.

Examples of a gross pathological specimen, gross pathological section, a CT perfusion BV map, contrast-enhanced CT scan, and a section stained with HE are shown in Fig. 2.

### DISCUSSION

Previous studies have shown that functional CT and MR blood volume maps can show the range of brain tumor invasion better than traditional contrast-enhanced CT and MR. However, in all these studies stereotactic biopsy was used to validate the results. These studies had the following shortcomings: (1) The association between the perfusion parameter images and anatomical pathology GTV/CTV was unknown, and there was a lack of gross pathology control; (2) The biopsy position was not studied systemically as a target area for radiotherapy. Possible reasons for these shortcomings are that these studies were often focused on diagnostic tests, so that their purpose was not to study the target volume for brain tumor radiotherapy, and it is difficult to obtain a gross pathological specimen at the same level of the target slice in humans in vivo. To overcome these problems we decided to use the rabbit model of VX2 tumor. This model was chosen because there is a similarity between the growth characteristics of VX2 tumor, which include growth along blood vessels and perivascular spaces, micro-invasion, and breaking down the blood-brain barrier in tissue next to the tumor as well in the tumor itself, and the growth characteristics of malignant gliomas in humans. Also, a gross pathological specimen at the same level as the target slice can be obtained in animals in vivo. We believe that our study methods ensured consistency and accuracy of sampling, and that the methodology of our study was reliable, thereby providing a methodological foundation for using CT perfusion to accurately evaluate the anatomical target area of a brain tumor model.

For convenience, we recorded the tumor extent on the BV map as GTV_{BV}. Gross tumor volume in the pathological specimen visible to the naked eye was recorded as GTV_{pathology} and the tumor extent in the gross specimen visible to the naked eye plus the microscopic invasion extent was recorded as CTV_{pathology}. The results showed that the major axis length and minor axis length determined from the BV map was smaller than GTV_{pathology} in only one of 20 tumors as the major axis length and minor axis length shown on the BV maps were not significantly different from GTV_{pathology}, and therefore theoretically, the probability is 95% (1/20) that GTV_{pathology} of VX2 brain tumor can be covered completely when a BV map is used. This indicates the feasibility of using a BV map to delineate GTV for brain tumors. Our results support those of previous reports on the invasion range of human brain tumors. The reason why a BV map can reflect the tumor GTV_{pathology} more accurately than traditional contrast-enhanced CT is that a BV map can accurately assess tumor angiogenesis, and tumor angiogenesis is a good indicator of the boundary of solid tumors.

In our study, on BV maps the major axis length was less than CTV_{pathology} in 16 tumors and the minor axis length was less than CTV_{pathology} in 18 tumors. A possible reason is that the growth of solid tumors can be divided into prevascular stage and vascular stage. In the prevascular stage, tumors obtain nutrients through diffusion. In this stage, the tumor is less than 0.4 mm³, and the cell number does not exceed 10⁷. When the tumor size is larger than 0.4 mm³, the diffusion method cannot meet its growth needs, and the tumor will enter the vascular stage. At that time, the tumor begins to form its own vascular system and the growth is accelerated. A BV map can only show the tumor part with angiogenesis; it cannot show the tumor part at the tumor edge which is still at the prevascular stage. Our data showed that on BV maps, GTV_{BV} was greater than CTV_{pathology} in the
direction of the major axis in 4 cases and \( \text{GTV}_{\text{BV}} \) was greater than \( \text{CTV}_{\text{pathology}} \) in the direction of the minor axis in 2 cases. That is because a BV map reflects the increase in the blood volume, which includes the blood volume increase caused by microvessel density increase- or inflammatory response-induced local congestion. Therefore, a BV map cannot reveal the newly formed blood vessels without a blood volume increase.

The perfusion imaging software provided with the scanner that we used employs slope algorithms, which cannot provide a permeability surface map. Permeability surface refers to the one-way transmission rate of contrast agent entering into the intercellular space via the capillary endothelium, which reflects the permeability of blood vessels. It is mainly used for assessing tumor angiogenesis, which can avoid the aforementioned errors.\(^{15}\)

Magnetic resonance can provide more precise anatomical images than CT. However, in the modern three-dimensional treatment planning system, dose calculation and dose distribution are closely related to the CT values of the input tissue structures because CT images reflect changes in the tissue density. Computed tomography images are the basic images used for design and planning. Computed tomography perfusion images are directly integrated into conventional CT scan images, and there is no problem of registration accuracy. Moreover, MR perfusion imaging can only provide relative values of perfusion parameters, but CT perfusion can provide the absolute values of perfusion parameters. Therefore, CT is more suitable for this study than MR.\(^{14}\)

Early perfusion imaging could only obtain the image one slice at a time, which limited its application in radiotherapy planning. We used a 64-slice CT scanner that had a thickness of one-time acquisition of 4 cm. If the jogging technique is used, the z-axis length can be up to 8 cm long. The latest 320-slice CT scanner can completely cover the length of 16 cm in the direction of the z-axis during one scan, and can perform whole brain perfusion imaging. This technical development has made it possible to use perfusion imaging for delineation of the target volume in radiotherapy.\(^{21}\)

Our data showed that when contrast-enhanced CT was used to assess \( \text{GTV}_{\text{pathology}} \), the major axis length was less than \( \text{GTV}_{\text{pathology}} \) in 12 cases, and the minor axis length was less than \( \text{GTV}_{\text{pathology}} \) in 15 cases. However, when a BV map was used, the major axis length and minor axis length was less than \( \text{GTV}_{\text{pathology}} \) in only one case. Therefore, if a BV map is used to delineate the target area for brain tumor, there is a 95% certainty that \( \text{GTV}_{\text{pathology}} \) will not be missed. When contrast-enhanced CT was used to assess \( \text{GTV}_{\text{pathology}} \), the extent needed to be expanded by 81.8 to 124.5%. Using a BV map, the extent needed to be expanded by 8 to 12% (Table 2). Obviously, a BV map of functional CT can better assess the anatomical target area for brain tumors than traditional contrast-enhanced CT.

The generalization of our findings is limited by the fact that only VX2 tumor cells were used in the study. Further investigation is needed to find out whether using other cell lines would produce similar results.

**CONCLUSION**

A BV map of functional CT is superior to contrast-enhanced CT for delineating the target area of VX2 brain tumor in rabbits. Since this animal model tumor is similar to human brain tumor it appears that using a BV map of functional CT has potential for delineating GTV of brain tumors in clinical practice.

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