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Vascular Tortuosity is Culprit to Abnormal Perfusion in 9L Gliosarcoma Tumor

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Vascular Tortuosity is Culprit to Abnormal Perfusion in 9L Gliosarcoma Tumor

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Introduction

The measurement of vessel attributes, such as vessel density, morphology and function, is very appealing for assessing tumor quantitatively and non-invasively. Dynamic susceptibility contrast MR perfusion imaging is an established technique that measures cerebral blood volume (CBV), cerebral blood flow (CBF), and mean transit time (MTT), and may reflect such vessel attributes. This study explores the underpinnings of abnormal perfusion by comparing MRI measurements of tumor perfusion to quantitative calculations of vessel tortuosity in a 9L rat brain tumor model treated with the steroid dexamethasone.

Materials and Methods

A total of ten Fisher 344 male rats (Sprague Dawley; Harlan, Indianapolis) were inoculated intracerebrally with 9L gliosarcoma cells (MGH Contrast Media Laboratory, Boston, MA). Four 9L rats were treated with 3mg/kg per day of dexamethasone for five days prior to imaging, four other control 9L rats were not treated, and two normal rats were included. These rats were later scanned and then sacrificed at 14 days after tumor cell inoculation. All procedures with animals were performed according to the institutional guidelines for use of laboratory animals and the NIH Guide for the Use and Care of laboratory Animals. MR examinations were performed on a 3T Bruker Medspec system (Ettlingen, Germany). Echo planar imaging (EPI) was used for acquiring coronal images of multiple sections with a temporal resolution of 1 second. The MR imaging protocol included a dynamic simultaneous GE/SE EPI sequence (TR:1s, TE(GE/SE):10.3ms/76.6ms FOV=3.5cm, SL=2mm, Matrix=64X64), and a post-contrast-enhanced T1-weighted sequence (TR:450ms, TE:119ms, FOV=3.5cm, SL=2mm, Matrix=256X256). The contrast agents MION (0.217ml/kg, Center for Molecular Imaging Research, Charleston, MA) and Gadodiamide (0.4ml/kg, Omniscan, Nycomed, Princeton, NJ) were injected through a catheter into a femoral vein during the GE/SE sequence and prior to the T1 sequence, respectively.

Programs developed in house with Red Hat Linux, and AFNI were used for post-processing of the MRI data. Tracer kinetics analysis was used to compute the perfusion parameters as described previously (1). Briefly, The arterial input function (AIF) was determined from arterial branches in normal contralateral tissue. By using singular value decomposition, the intravoxel tissue residue function was derived by deconvolving the tissue concentration time curves with the AIF. The maximum point of the residue function was determined as the cerebral blood flow (CBF). Cerebral blood volume (CBV) measurements were obtained by simply integrating the area under the $\Delta R2$ time curve. The calculation of mean transit time (MTT) required knowledge of CBF and was formulated using the central volume theorem, which states that MTT=CBV/CBF. Regions of interest (ROI) were drawn as: control tumor (Tumor_CTR), control contralateral site (Contra_CTR), dex-treated tumor (Tumor_DEX), and dex-treated contralateral site (Contra_DEX). Statistical analysis was performed using GraphPad Prism version 4.0a for Mac OS X. The Kruskal-Wallis statistical test followed by Donn's post test was performed to compare the CBF and MTT measures of untreated control rats to dexamethasone-treated rats.

To study the vascular tortuosity of 9L tumor, microfocal x-ray angiography was used to study 3 additional rats, also inoculated with 9L gliosarcoma cells. Of these 1 was treated with dexamethasone according to the same protocol described above. For these studies, both carotid arteries were catheterized and infused with a barium sulfate medium for X-ray contrast. Details of the cone-beam micro-focal X-ray computed tomography imaging and reconstruction were previously described (2). Morphometric analysis was performed on the reconstructed images using the Tree module of Analyze 6.1 (3). The Tree analysis module generates a skeleton of the vessel structure and computes branching angles, segments lengths, and cross-sectional area, which depict the vessel attributes.

Results and Discussion

A nonparametric test showed that SE CBF values were significantly different between tumor region of dex-treated rat and corresponding contralateral site (*figure 1, p>0.05*), thereby suggesting a normal perfusion in dex-treated tumors. Perhaps, the effect of dexamethasone in this scenario was a remodeling of microvessels that led to an efficient perfusion similar to the contralateral side. In contrast, CBF values were lower in tumor regions of untreated control rats in comparison to corresponding contralateral site (*p<0.01*), thereby suggesting a reduced perfusion in tumor region relative to contralateral site. In the case of MTT, the GE result indicated abnormally long MTT in untreated tumor compare to corresponding contralateral site (*figure 1, p<0.001*). Dex-treated group showed no significant difference between the tumor region and its corresponding contralateral site (*figure 1, p>0.05*). Figure 2 provides x-ray images of vessel trees obtained from untreated control and dex-treated rat brains. The yellow oval encircles the estimated tumor region. Tortuosity, defined as the branching angle-to-length ratio in units of degrees/mm, was determined for these regions. An increased vessel tortuosity and density was found in the tumor regions of untreated rats, as illustrated by the histogram (*figure 2.C*). This hypervascular tumor exhibits an increase in the total branched vessels as compared to the same anatomical region of opposite hemisphere (*figure 2*). Perhaps, this metric provides an indirect measure of vigorous vessel sprouting.

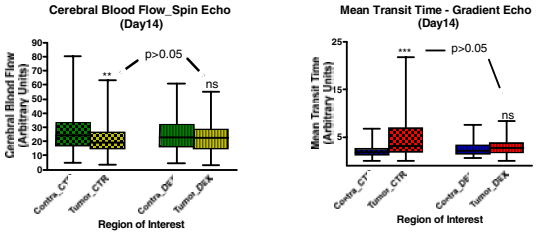

Figure 1 (CBF and MTT): Box and whisker plot of data as minimum, 25 percentile, median, 75 percentile and maximum values. P<0.001 (***), p<0.01 (**), p<0.05 (*), and p>0.05 (ns). Gradeint echo (GE), Spin echo (SE)

Summary of Findings

In summary, measures of cerebral blood flow and mean transit time are potential non-invasive markers for assessing pathological states of vessel attributes in brain tumor. This study demonstrates that quantitative vessel morphometrics are indeed reflected in DSC-MRI perfusion parameters, which have demonstrated a possible indication of normalization in vascular function after dexamethasone treatment.

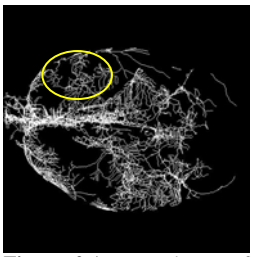

Figure 2.A: Vessel tree of Control rat brain.

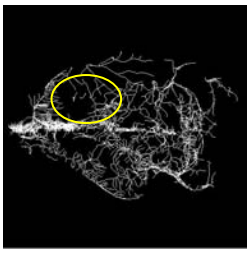

Figure 2.B: Vessel tree of Dex treated rat brain .

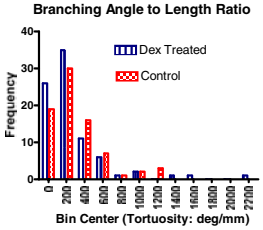

Figure 2.C: Histogram of on angles to length ratio.

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