Use of Cerebral Blood Volume as a Potential Surrogate Marker of Vascular Normalization in 9L Gliosarcoma Tumor

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Introduction

Promising concepts and evidence have purported that certain antiangiogenic drugs can transiently normalize the abnormal morphology and function of tumor vasculature. It has been shown that following antiangiogenic drug treatment, a specific time window exists during which vascular normalization occurs (1). Normalization of tumor vessel morphology and function can lead to an improved delivery of cytotoxic drugs (for chemotherapy) and increased oxygen level (for radiation therapy) in the tumor microenviroment. Therefore, identifying the optimal timing for administration of antiangiogenic drugs with chemotherapy or radiation therapy is crucial to maximizing treatment efficacy for such combined therapeutic regimens. Despite multiple advances in medical imaging, noninvasive monitoring of such therapeutic events has not been prudently investigated. In initial studies (1,2) it was demonstrated that dynamic susceptibility contrast (DSC) MRI methods could be used to assess the morphologic and functional changes in tumor vasculature in response to steroid treatment. The treated tumor vessels were more like those of normal brain. It was therefore posited that DSC-MRI can be used as a noninvasive indicator of vascular "normalization". In this study, this concept is further explored by performing DSC with correlative independent measures of vascular morphometry.

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). Cerebral blood volume (CBV) measurements were obtained by simply integrating the area under the $\Delta R2$ time curve. It has been demonstrated with simulations and measurements that CBV measures from a gradient echo (GE) sequence reveal the total vasculature whereas CBV measures from a spin echo (SE) sequence reveals microvessels. 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 CBV measures of untreated control rats to dexamethasone-treated rats.

Immunohistochemistry for endothelial cell marker CD31 was performed by indirect immunoperoxidase stain. Photomicrographs were taken on a light microscope with a Nikon Model E-400 SPOT Insight Color Camera. Computer-assisted morphometric analysis of tumor vessels was performed on these images by using MetaMorph version 6.2 to compute the number of vessels and their respective cross sectional area. An unpaired student's t-Test was performed to compare the mean vessel area of untreated control rats and dexamethasone treated rats.

Results and Discussion

CBV measurements made in both hemispheres of a normal rat's brain showed very little variability in data (*p>0.05, figure 1*). The gradient echo CBV measures in contralateral side of dex-treated rat did not differ significantly from the CBV measures in corresponding tumor region (*figure 1.B, p>0.05*). This implies that dexamethasone was profoundly effective on the total vasculature in the tumor regions of rats' brain. This could simply be a reflection of vascular normalization as a result of dexamethasone treatment. On the other hand, the gradient echo CBV measures of tumor regions in control rat significantly increased relative to contralateral side (*figure 1.B, p<0.001*). This increase of CBV in untreated control rats could be associated with increased total vasculature in the tumor. Results from spin echo sequence showed no significant difference (*figure 1.C, p>0.05*) between tumor region and contralateral site in CBV measures of dex-treated rats as well as untreated control rats. Assuming that spin-echo CBV reveals microvessels, one can speculate that the microvessels were not significantly affected by either tumor growth or dexamethasone treatment. We examined the architecture of CD31-immunopositive vessels to assess tumor vasculature, and subsequent changes to dexamethasone treatment. CD31-immunopositive vessels were sparse in treated tumors. However, vessel morphology was strikingly altered in untreated control tumor, with apparently larger area of CD31-positive cells as illustrated in *figure 2*. Dexamethasone seems to reduce the total cross sectional area and diameter of vessels in the tumor region as obtained from the CD31 stain.

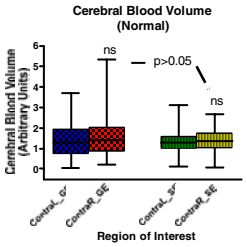

Figure 1.A: CBV Normal rats

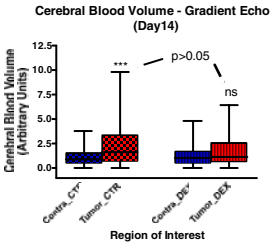

Figure 1.B: CBV Gradient Echo

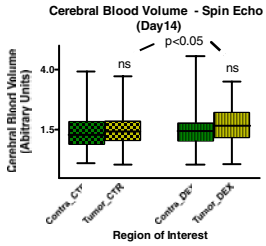

Figure 1.C: CBV Spin Echo (SE)

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).

Summary of Findings

A surrogate perfusion marker, cerebral blood volume, tends to elucidate normalization of vascular morphology at 14 days after the intervention of dexamethasone therapy. This result was validated, in part, by qualitative assessment of anti-CD31 histologic stain. Dexamethasone treatment causes significant changes in vascular morphology within tumor region, in terms of vessel diameters, possibly inhibiting progression to a dilated vessel stage. Whether dexamethasone actually causes normalization, it a regression from a dilated to normal vascular stage, needs to be addressed with a longitudinal study within the same rat before, after and during therapy.

References

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2. Quarles et al. *Techn. in Cancer Res. & Treat.* 4(3):245-249 (2005).

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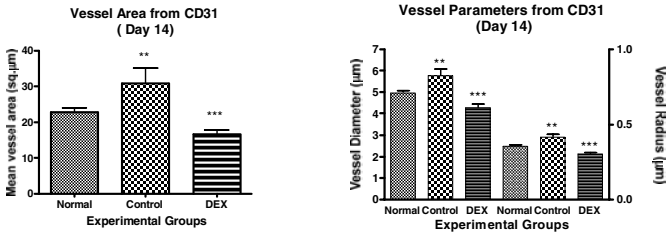

Figure 2: A bar graph from the mean vessel area **(Left panel),** diameter and radius **(Right panel)** of histological tissues in control group and dexamethasone treated rats. P<0.01 (**) and p<0.001 (***)