Longitudinal Monitoring of Vascular Normalization with MRI Perfusion Imaging of 9L Gliosarcoma Tumor

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Introduction The goal of this study was to determine the utility of DSC (dynamic susceptibility contrast) and DCE (dynamic contrast enhancement) imaging to track tumor vascular normalization and optimize therapeutic timing. Vascular normalization is an emerging concept that may explain the success (or failure) of combination therapies. The idea is that the characteristically chaotic tumor vasculature becomes more similar in morphology and function to that of normal vasculature. This leads to improved blood flow and tumor oxygenation, which enhances the efficacy of radiation therapy and the delivery of chemotherapy and other drugs to the tumor as demonstrated by recent clinical [1,2] and preclinical [3,4] studies. Despite this promise, there have also been concerns regarding whether the improved perfusion of tumors will enable rather than inhibit further growth. This in fact may explain why some studies showed an antagonistic response when anti-angiogenic therapy was combined with conventional therapies. Consequently, how to best monitor and use the knowledge of tumor angiogenesis to optimize treatment schedule for angiogenic therapy alone or in combination with radiation therapy or chemotherapy is a primary motivation for the proposed research.

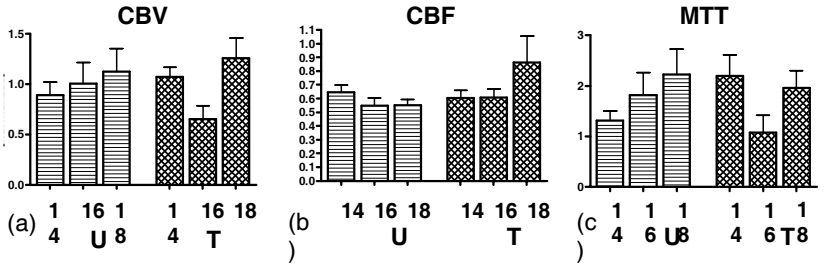
Materials and Methods A total of 24 Fisher 344 male rats (Sprague Dawley; Harlan, Indianapolis, IN) were inoculated intracerebrally with 9L gliosarcoma cells. Twelve 9L rats were treated with dexamethasone (i.p. 3mg/kg/day, American Regent Laboratories Inc., Shirley, NY) for five consecutive days, beginning on day 10 post-tumor cell inoculation, and twelve others were treated with equal volume of saline.

MR image acquisition was performed on a 3.0T GE Signa Excite system using a home-built quadrature birdcage rf coil. The following general acquisition parameters were used for all experiments: FOV=4cm; 4 slices, slice thickness=2mm, spacing=0.2mm. For DSC imaging, a GE-EPI sequence was used (TR=1s, TE=34.5 ms, matrix=64x64) to acquire images before, during and after injection of 2.5mgFe/kg i.v. of the intravascular susceptibility contrast agent MION (Center for Molecular Imaging Research, Charleston, MA). Programs developed in-house with Red Hat Linux and AFNI were used to create image maps of CBV (cerebral blood volume), CBF (cerebral blood flow) and MTT (mean transit time), as described previously [5]. Next, for determination of T1 maps, three sets of images were acquired with a fast-SPGR sequence, each obtained at different flip angles, 2^0, 10^0 and 35^0 (TE/TR=4.5ms/34s;). Then a DCE-MR image series again using fast SPGR (flip=35^o) was acquired approximately every 8 seconds. Gadodiamide (0.2mmol/kg, Omniscan, Nycomed, Princeton, NJ) was injected during the ninth acquisition of the DCE-MR series. Finally, a high-resolution, post-contrast T1w spin echo image was acquired (TE/TR: 12 ms/450 ms; matrix=256 x256; NEX=16). Determination of K^{trans} from the DCE data was performed using the TOPPCAT (T-One weighted Perfusion imaging Parameter Calculation Toolkit) plugin to ImageJ [6].

Immunohistochemistry for endothelial cell marker CD31 (indirect immunoperoxidase) was performed on 6m thick slices of the extracted rat brains. Photomicrographs were taken on a light microscope with a Nikon Model E-400 SPOT Insight Color Camera (Diagnostic Instrument Inc., Sterling Heights, MI). Determination of the number of vessels and their respective cross-sectional areas was performed using MetaMorph version 6.2 (Universal Imaging Co., Downington, PA). An unpaired student's t-test was used to compare the mean vessel area of dex-treated and saline (SA) treated rats. The tissue sections were also processed for localization of VEGF or VEGF receptor (FLK-1) with digital epifluoresence microscopy (Nikon Eclipse E600, Fryer, Huntley, IL) using filters specific for tetramethylrhodamine isothiocynate (TRITC, Sigma) or fluorescein isothiocynate conjugated with anti-rabbit IgG (FITC, Sigma). Histological images were acquired with a SPOT Advanced software.

Results and Discussion The DSC parameters (Fig 1) either increased (CBV, MTT) or remained unchanged (CBF) for untreated brain, while in treated brain there was CBV and MTT were minimized at day 16, but then reverted to an abnormally high blood volume by 18th day post-inoculation. This phenomenon is proposed as vascular normalization effect (or window of opportunity for the administration of adjuvant therapy) due to antiangiogenic therapy with dexamethasone. While K^{trans} was less for treated vs untreated tumor it did not show a similar normalization effect since K^{trans} continued to decrease over time (Fig 2).

Figure 1.



CD-31 immunoreactive vessels were particularly conspicuous in untreated tumor regions in comparison to dexamethasone treated tumor regions. In both untreated and treated groups, vessels were colocalized with dense nuclei as ascertained by the hematoxylin counterstain. Consistent with the DSC CBV data, morphometric analysis revealed an increased vessel area in untreated tumors (N=4, n=412) and dexamethasone treated rats (N=4, n=415) at the 18th day post-inoculation of tumor cells (Fig 3). Consistent with the idea that K^{trans} is a index of vascular permeability, increased expressions of VEGF and FLK-1 were observed in untreated tumor as compared to treated tumor. This result also is consistent with the fact that dexamethasone suppresses vascular permeability in rat 9L brain tumor model by inhibiting the effects of tumor-derived permeability factors, and by inhibiting the production of vascular permeability factor by tumors [7].

Figure 2.

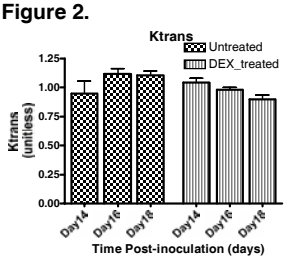
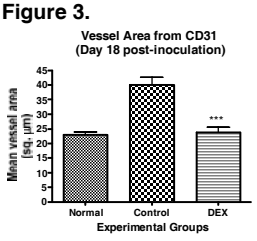


Figure 3.



Conclusion Collectively, these results suggest that both DSC and DCE MRI perfusion parameters can be used to noninvasively track vascular changes consistent with tissue markers of the same. The fact that some but not all parameters showed a normalization effect will be better understood once these changes are related to whether or not combining therapies during the normalization window improves tumor response to treatment, a topic of studies underway.

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Acknowledgements

This work was supported by the Government Aid in Area of National Need (GAANN[1]) grant and by the National Institute of Health or National Cancer Institute (NIH/NCI) grant RO1 CA082500.