

DCE-MRI of polyamidoamine dendrimer-nanoparticle permeability in the RG-2 Rodent Malignant Glioma Model

H. Sarin¹, S. H. Fung¹, A. Kanevsky¹, C. Regino², T. Barrett², R. P. Lewis¹, M. W. Brechbiel², E. H. Oldfield³, and J. A. Butman¹

¹Diagnostic Radiology Department, National Institutes of Health, Bethesda, MD, United States, ²National Cancer Institute, National Institutes of Health, Bethesda, MD, United States, ³Surgical Neurology Branch, National Institutes of Health, Bethesda, MD, United States

INTRODUCTION: Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) allows for real-time measurement of brain tumor vascular parameters. Gadolinium-chelated polyamidoamine (Gd-PAMAM) Dendrimer-nanoparticles are macromolecular contrast agents of highly defined sizes (1). Larger and higher generation dendrimers have ample additional reactive surface functional groups that can be labeled with malignant glioma targeting and anti-neoplastic agents. Although there is sufficient evidence to suggest that intravenously administered Gd-PAMAM Dendrimers as large as Generation 6, with approximate diameter 8 nm, are permeable to the blood-tumor-barrier (BTB) of “extra-cranial tumors,” there is a relative paucity of information on the permeability of Gd-PAMAM Dendrimers to the BTB of brain tumors (2). Therefore, in this work we sought to characterize the permeability of a spectrum of Gd-PAMAM Dendrimers to the RG-2 rodent glioma BTB. The generations of Gd-PAMAM Dendrimers that we investigated were G2 (3 nm), G4 (6 nm), G6 (8 nm), and G8 (13 nm).

METHODS:

- Animal model** – The brains of 20 adult male Fischer rats were stereotactically inoculated with cultured RG-2 glioma cells and then imaged on post-implantation days 11-12. The respective contrast agent was injected through a femoral venous canula inserted prior to the imaging session. The protocol was approved by the NINDS Animal Care and Use Committee.
- MRI technique** – Images were acquired on a 3.0 T MR scanner (Philips Intera; Philips Medical Systems; Andover, MA) using a 7-cm small animal solenoid coil. During scanning, rats were kept under isoflurane anesthesia. Two imaging sessions were performed in each rat, the first with injection of Gd-DTPA, and the second with infusion of a Gd-PAMAM Dendrimer. During the first session, a low flip angle baseline 3D FFE sequence (TR 8.1 ms, TE 2.3 ms, FA 3°, ST 1 mm, 16 slices, FOV 76.8 mm, MTX 256X256) was followed by dynamic scans with FA 12° every 20 s for 15 minutes. For the second session, the dynamic scans were conducted for 40 to 85 minutes in order to capture the slower kinetics of the Gd-Dendrimers relative to those of Gd-DTPA. For acquisition of the dynamic scans, either 0.25 mmol/kg of Gd-DTPA or 0.03 mmol/kg of Gd-PAMAM Dendrimer were infused through the femoral venous canula with a 1 min delay and at a constant rate over 1 min using a Harvard micro-infusion pump. The animals were euthanized immediately following the second imaging session.
- Analysis** – For each rat, all data were co-registered to the initial scan of the first session using 3Dvolreg (AFNI <http://afni.nimh.nih.gov/>). ROIs were selected on the confluence of sinuses and the enhancing tumor identified on the Gd-DTPA images. These ROIs were applied to the data from both sessions to obtain signal of plasma (Sp(t)) and signal of tumor (St(t)). Conversions to contrast agent concentrations in plasma (Cp(t)) and tumor (Ct(t)) were made by calculating the T1 for each ROI using the two flip angle data, and by using the previously measured relaxivity of 4.94 mM⁻¹s⁻¹ for Gd-DTPA and 9.6, 12.5, 13.8, and 12.8 mM⁻¹s⁻¹ for G2, G4, G6, and G8 Dendrimers respectively at 3 T (~22° C). Data was excluded if motion correction was unsatisfactory.

RESULTS: Representative curves for Cp(t) and Ct(t) for Gd-DTPA and the various generations of Gd-PAMAM Dendrimers investigated are shown below in the figure. Both Cp(t) and Ct(t) for the higher Dendrimer generations G6 (N = 3) and G8 (N = 3) were step functions. This is consistent with Dendrimer confinement to the intravascular compartment, i.e. no redistribution in the body, minimal renal clearance, and no leakage into the glioma extravascular space. For G2 Dendrimers Ct(t) diverged from Cp(t) thus indicating that the Dendrimer permeated the glioma BTB. For G4 Dendrimers Cp(t) was predominantly a step function with a slight “bump” at the beginning possibly reflecting redistribution. For G4 Dendrimers two different patterns of enhancement were observed in Ct(t). The first seen in four out of nine gliomas was a step function mirroring that of Cp(t), indicating no accumulation of Dendrimers in tumor tissue. The second seen in five out of nine gliomas was a slow linear rise of Dendrimer concentration within tumor tissue, indicating that the tumor was permeable to these G4 Dendrimers.

DISCUSSION: In the rat RG-2 malignant glioma model blood-tumor-barrier permeability to contrast agent is dependent on the agent’s size. G2 and G4 Gd-PAMAM Dendrimers cross the BTB into the tumor interstitium whereas G6 and G8 Dendrimers do not. These results support the use of higher generation Gd-PAMAM Dendrimers to accurately assess blood tumor volume and the use of lower generation Dendrimers along with Gd-DTPA to assess tumor permeability. Furthermore, lower generation Gd-PAMAM Dendrimers may be used as carriers of therapeutics into malignant gliomas.

REFERENCES:

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