Application of a biodegradable, macrocyclic, polydisulfide-based contrast agent for monitoring tumor angiogenesis using dynamic contrast enhanced MRI

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Purpose:
The Na^+-K^+-$2Cl^-$ (NKCC1) cotransporter is ubiquitously expressed in most tissue types, aiding in the regulation of cell volume and intracellular ionic strength [1]. Several studies have shown that the inhibition of this transporter is associated with a decrease in the growth and invasiveness of tumor cells, in addition to the prevention of anti-apoptotic resistance in the presence of chemotherapeutic agents such as temezolomide [2-3]. The purpose of this study was to determine if Bumetanide, an antagonist of the NKCC1 cotransporter, is able to affect angiogenic development in colon cancer xenografts. To investigate any potential vasculature remodeling, we employed dynamic contrast enhanced MRI (DCE-MRI) techniques using a newly synthesized polydisulfide macrocyclic Gd(III) chelate contrast agent (shown in Figure 1). In addition to its high kinetic stability, this agent possesses a polydisulfide backbone that facilitates biodegradation for the safe use of macromolecular agents for DCE studies.

Methods:
Athymic nude mice bearing HT29 flank colon tumors were treated with a daily IP injection of Bumetanide at a dose of 5mg/kg. The administered therapy continued for 21 days. All mice were imaged pre-treatment, and then again 1 day, 1 week, 2 weeks, and 3 weeks into the therapy. At each time point, the mice were intravenously injected with 0.1mmol/kg of the contrast agent, and imaged for 30 minutes on a 7T Bruker system using a T1-weighted 3D FLASH gradient echo sequence. Upon completion of the therapy, each mouse was injected with 60mg/kg of the hypoxia marker pimonidazole, and then sacrificed for IHC analysis.

Results:
DCE images were analyzed using the adiabiatic approximation to the tissue homogeneity (AATH) model to observe changes in vessel permeability (PS), blood flow (F), and plasma volume fraction (vp). The average blood flow within the tumors did not significantly change when comparing Bumetanide- and saline-treated mice after 3 weeks. However, both PS and vp experienced a greater reduction in response to the bumetanide drug during the treatment. Such changes in the vasculature can be noticed in the parametric mappings shown in Figure 2. Histological analysis revealed that the greater reductions in PS and vp in the Bumetanide-treated tumors corresponded with a more hypoxic tumor, which was corroborated by the reduction in CD31 expression and therefore the decline of vessel growth and perfusion (Figure 3).

Discussion:
This study showed that DCE-MRI using the biodegradable polydisulfide macrocyclic contrast agent was effective for evaluating the progression of tumor angiogenesis due to the correlation between the reduction in vascular permeability parameters and CD31 expression. Bumetanide is able to induced changes in the vascular network of tumors by decreasing the permeability and volume fraction of blood vessels in xenograft colon cancer lesions. It was also discovered that a decrease in PS and vp contributed to a more hypoxic tumor with a smaller vascular network. In addition, by validating the changes observed from the DCE imaging with histology, the contrast agent developed for this study can potentially be widely used to non-invasively analyze the efficacy of other anti-angiogenic therapies for personalized medicine. The ability of the disulfide bonds in the backbone of this contrast agent to degrade upon intravenous administration is beneficial for DCE imaging because it enables the use of macromolecular agents without the potential to cause toxic side effects. Macromolecular contrast agents are typically preferred over smaller Gd-based molecules for DCE imaging since they can selectively extravasate through leaky tumor vasculature, and not through intact normal vessels, thus improving the accuracy of the model used for analysis.

Conclusions:
The macrocycle polydisulfide contrast agent used in this experiment has the ability to effectively monitor the anti-angiogenic behavior of Bumetanide. Reductions in the permeability and plasma volume fraction of tumor blood vessels directly correlate with a decrease in CD31 expression.

References:
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[2] Haas BR and Sontheimer H. Cancer Res. 2010; 70: 5597-5606.
[3] Algharabli J, et al. Cell Physiol Biochem. 2012; 30(1): 33–48.

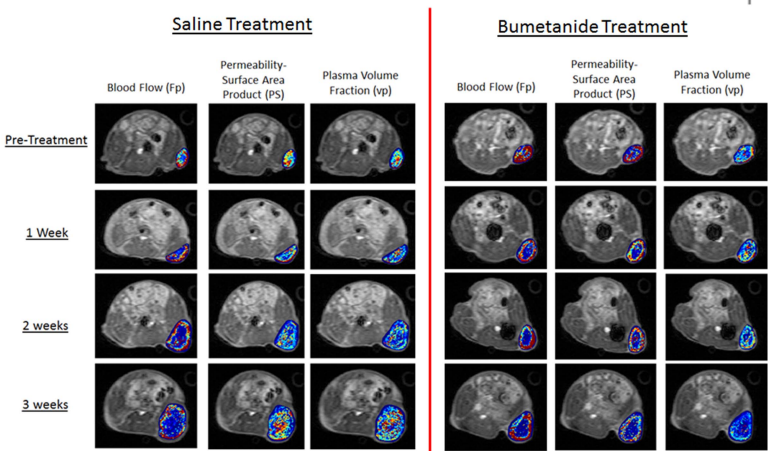

Figure 2. Parametric maps of blood flow (Fp), permeability surface area product (PS), and plasma volume fraction (vp) using the AATH model reveal physiological spatial differences of the vascular network induced by the Bumetanide therapy.

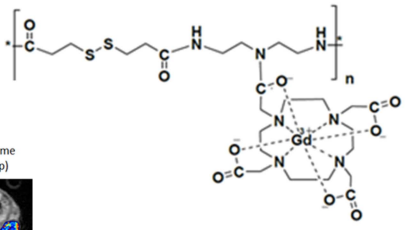

Figure 1. Structure of macrocyclic polydisulfide contrast agent.

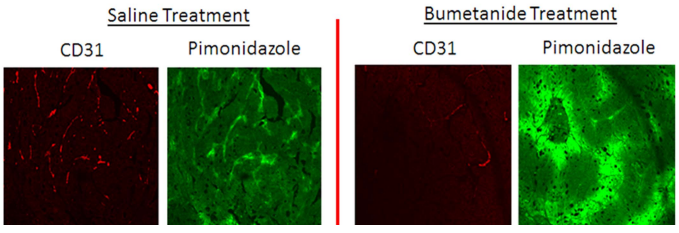

Figure 3. Tumor sections stained for CD31 (red) and for the pimonidazole hypoxia marker (green). This figure shows that the Bumetanide contributes to a greater hypoxic tumor microenvironment than that achieved by the saline therapy. This is evidenced by the pockets of intense pimonidazole staining. It is also apparent than an increase in tumor hypoxia corresponds with a decrease in vasculature, as evidenced by the reduced CD31 expression.