

Nitrite Induces the Extravasation of Iron Oxide Nanoparticles in C6 Brain Tumors

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INTRODUCTION: Nitrite undergoes reconversion to nitric oxide (NO) under conditions characteristic of the tumor microenvironment, such as hypoxia and low pH. Nitric oxide is a known vasomodulator that has also been shown to selectively alter vascular permeability and cellular respiration in tumors [1, 2, 3]. This selective conversion of nitrite into NO in tumor tissue has led to the possibility of using nitrite to enhance drug delivery and radiation response [1,3]. In this work we propose to serially characterize the vascular response of brain tumor bearing rats to nitrite using contrast-enhanced (CE) R_2^* mapping.

METHODS: Rats (N=8) were inoculated with C6 tumor cells and imaged using a 4.7T MRI. Anatomical images were acquired using a fast-spin echo (FSE) imaging sequence. Serial imaging was carried out using a multi-gradient echo sequence to estimate the R_2^* : a) after the intravenous injection of an intravascular 30nm iron oxide based CA and b) after an intravenous bolus injection of nitrite (3 mg/kg). The imaging parameters were: FOV 40 x 40 mm, 5 slices, 1.5 mm ST, 128 x 128 matrix, NEX = 10, 7 echoes spaced 5 ms apart with echo times (TE) from 3 to 28 ms, a 24° flip angle, and a TR of 200 ms. Approximately 5-6 baseline images were acquired before injecting nitrite and imaging continued for 1 hr thereafter. The percent change in the R_2^* value was calculated using the following equation:

$$\% \Delta R_2^* = 100 \% * (R_2^*_{post-Nitrite} - R_2^*_{post-MION}) / (R_2^*_{post-MION}).$$

RESULTS: Figure 1 (top) shows a representative FSE anatomical image of a tumor bearing rat brain. The arrow indicates the location of the tumor foci. Figure 1 (bottom) shows the ΔR_2^* map 55 minutes after nitrite injection. Figure 2 shows a plot of the mean percent change in ΔR_2^* before and after nitrite injection. The normal tissue ΔR_2^* (blue) remains unchanged after the injection of nitrite, while the tumor ΔR_2^* (red) gradually increased for the duration of the study (max change ~ 15%). This indicates an increase in CA concentration specifically within the tumor tissue. The mean of the ΔR_2^* of the final 4 time-points (highlighted in Figure 2) in tumor and normal tissue were compared using a student *t*-test and were found to be statistically different ($p < 0.00001$). Follow-up studies (data not shown) in additional rats have revealed that these pronounced ΔR_2^* changes plateau approximately 1.5 hrs after nitrite injection and persist even after 6 hours.

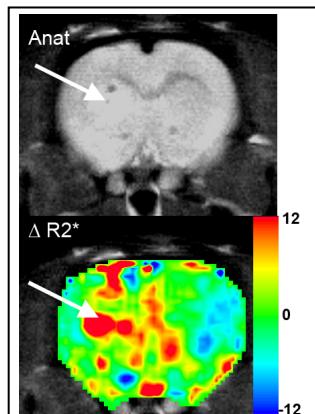


Figure 1: Top: Anatomical image with arrow indicating the location of the tumor. Bottom: Percent change in ΔR_2^* after nitrite injection.

DISCUSSION: These preliminary results indicate that nitrite induces tumor-specific vascular changes in C6 gliomas. These prolonged changes are not consistent with the more acute effects (~ 30 min) of nitrite on cellular respiration or hemodynamics previously reportedly [3], indicating they are likely due to increased vascular permeability and iron oxide CA accumulation within the tumor tissue rather than tumor vessel vasodilation. Histological studies are currently

underway to confirm CA extravasation and hypoxia selectivity. The substantial nitrite-induced extravasation of iron-oxide nanoparticles observed here has implications for the enhancement of nanotherapeutic drug delivery. Further, the dynamic contrast enhanced R_2^* mapping approach could be a valuable tool for monitoring tumor-specific vasoactivity and/or detection of drug accumulation within tumors.

REFERENCES: [1] Weyerbrock *et al*, J Neurosurg 99: 728-737, 2003, [2] Yin *et al*, Clin Cancer Res 2008, 14(12), 4002-4009, 2008. [3] Frerart *et al*, Clin Cancer Res. 14(9), 2768-2774 2008

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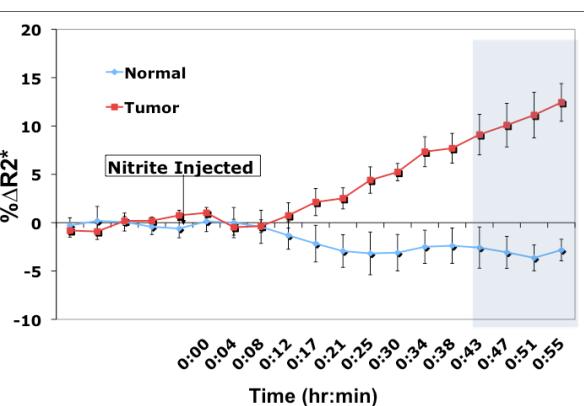


Figure 2: Percent change in ΔR_2^* in normal (blue) and tumor tissue (red) before and after nitrite injection.