Nitroxoline induces apoptosis and slows glioma growth in vivo

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TARGETED AUDIENCE: Scientist interested in preclinical glioma models, neuroradiologist and neuro-oncologist.

INTRODUCTION: High grade gliomas are invasive and lethal primary brain tumors with median survival time 12-16 months from diagnosis for grade IV tumors (glioblastoma)[1]. Multidrug resistance and inability to completely remove invading cells during surgical resection are main contributors to poor outcomes. Here we evaluated FDA approved antibiotic nitroxoline as potential chemotherapeutic agent for glioma using MRI and genetically engineered *Pten*/*Kras* mouse model. Nitroxoline was previously shown to be effective at inhibiting growth of breast and bladder cancer xenografts *in vivo*[2]. In addition, nitroxoline was found to be strong inhibitor of cathepsin B, a proteinase implicated in invasive properties of many human cancers[3]. As a compound with potential to suppress glioma invasion, nitroxoline has several advantages including a long history of human use (it has been prescribed for urinary tract infections for over fifty years in Europe[2]), minimal and tolerable side effects, and a favorable pharmacokinetic profile.

METHODS: A genetically engineered mouse with *Pten* deletion and *Kras*^{G12D} overexpression targeted to astrocytes (*mGFAP-Cre+*; *Pten* $^{lox/lox}$; *LSL-Kras* $^{G12D/+}$*, Pten/Kras in short)* was generated by Dr. Wu at UCLA. *Pten*/*Kras* mice spontaneously develop grade III glioma between 10 and 12 weeks of age. *Pten*/*Kras* mice were imaged between 10 and 12 weeks to establish presence of glioma and then randomly assigned to either nitroxoline (N=6, 80 mg/kg *ip.* nitroxoline suspended in soybean oil) or control (N=6, 0.2 ml *ip.* soybean oil treatment) group. MR imaging was performed on a 7T Bruker system. A multi-echo spin echo sequence was used to quantify transverse relaxation time (T_2) and for the tumor volume measurement (TR/TE 2000/7.26-101.64 ms, 14 echoes, 78^2 μm^2 resolution, 2 NAX). A diffusion-weighted echo planar imaging sequence (TR/TE 3800/22.03, with three b-value=0, 500, 1000, 3 diffusion directions and 156^2 μm^2 resolution, 2 NAX) was used to measure apparent diffusion coefficient (ADC). ADC and T_2 were calculated on a pixel-by pixel basis using ImageJ (plugin by Karl Schmidt). A post-contrast T1-weighted spin-echo dataset (1 mm thick slices, TR/TE 500/7.3 ms, 78 μm^2 resolution, 2 NAX) was acquired using 0.05 ml Gd-DTPA *iv.* injections to visualize if BBB is compromised within the tumor. At the end of the MRI studies, mice were sacrificed and tissue was processed for H&E and TUNEL-staining. TUNEL-positive cells were counted by pathologies blinded to the experimental design. A score between 0 and 4 was assigned for each section: 0 for no TUNEL-positive cells, 1: for 1–10%; 2: for 11–30%; 3: for 31–50%; and 4: for more than 50% TUNEL-positive cells. Statistical analysis was performed using paired t-test and two sample t-test for TUNEL scores, where *p*-value<0.05 was considered significant.

RESULTS: Significant inhibition in glioma growth was observed at 7 and 14 days following nitroxoline treatment, **Fig. 1 A**, **Fig. 2 A**, in contrast to control mice that experienced doubling in tumor volumes within 14 days, **Fig. 1 C**, **Fig. 2 B**. Compared to orthotopic xenograft glioblastoma models, this novel *Pten*/*Kras* model recapitulates many features of human glioma, including diffuse boundaries as observed on T_2-weighted images, **Fig. 1 C**, likely due to glioma cells invading into the neighboring brain parenchyma. No contrast enhancement with Gd-DTPA on T_1-weighted images, **Fig. 1 B, C** suggest that these are lower than grade IV glioma. Following nitroxoline treatment, there was a significant increase in ADC-values at 14 days compared to no change in ADC in control group, **Fig. 2 C**. No change in T2-values was observed at either 7 or 14 days, independent of treatment, **Fig. 2 D**. Upon histological examination, significantly more TUNEL-positive cells were found in nitroxoline treated mice (mean TUNEL score 1.625* for nitroxoline treated vs. 0.5 for control group, * *P*<0.05, two sample t-test). Histological evaluation of H&E stained section confirmed grade III glioma.

DISCUSSION: The two most common MRI parameters used in clinical setting, T_2 and ADC were measured in order to quantitatively determine treatment response profiles following nitroxoline. Since nitroxoline was mainly effective at suppressing glioma growth with ~15-20% of scattered apoptotic cells (as estimated based on TUNEL score) found upon histological examination, it is likely that this was not enough to cause change in T_2. In contrast, changes in ADC-values are more sensitive to alterations in cell density, and a small, but significant increase in ADC found after 14 days of nitroxoline treatment likely reflects slightly reduced cell density due to nitroxoline induced apoptosis. These findings taken together with documented history of clinical use as antimicrobial agent, makes nitroxoline a very good candidate for clinical trials for anti-glioma therapy.

Figure 1.

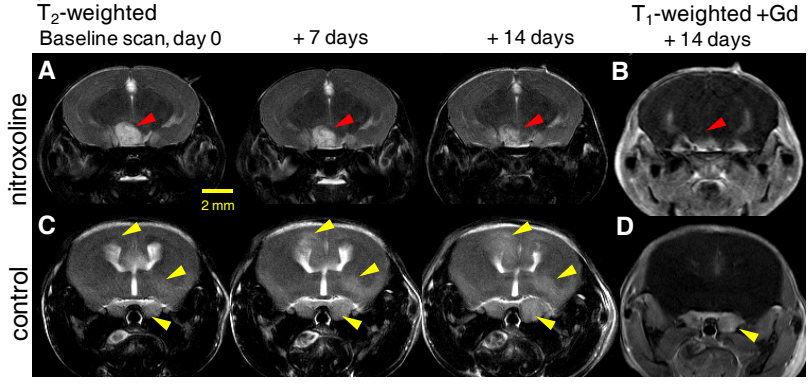

Figure 1. (**A**) Representative T_2-weighted images before, and at 7 and 14 days after nitroxoline treatment, along with (**B**) T_1-weighted post Gd-DTPA image. Red arrows point to the glioma location in nitroxoline treated mouse. Notice slight decrease in tumor size. (**C**) T_2-weighted baseline image and after 7 and 14 days of soybean oil (control) treatment, along with T_1-weighted post Gd-DTPA image (**D**). Yellow arrows point to the glioma location in control animal. Notice increase in glioma size. None of the observed glioma enhanced with Gd-DTPA.

Figure 2.

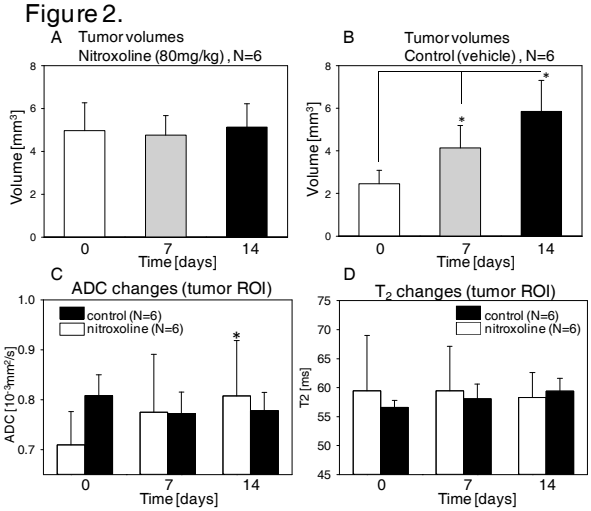

Figure 2. (**A**) Inhibition of glioma growth in nitroxoline treated mice, compared to (**B**) significant increase in tumor volume at 7 and 14 days in control group. (**C**, **D**) Quantification of ADC- and T2-values in nitroxoline treated and control mice. Notice increase in ADC-values in nitroxoline treated group. **P*<0.05 paired t-test.

REFERENCES: **1**. Johnson et al. J Neurooncol 2012; 107:359-64. **2**. Shim et al. J Natl Cancer Inst 2011; 102:1855-73. **3**. Kos J, Lah TT. Oncol Rep 1998; 5:1349-61.