OKN-007 decreases tumor necrosis and tumor cell proliferation and increases apoptosis in a pre-clinical F98 rat glioma model

Rheal A. Towner[1], Patricia Coutinho De Souza[1], Krithika Balasubramanian[2], Charity Njoku[1], Nataliya Smith[1], David L. Gillespie[3], Andrea Schwager[4], Osama Abdullah[5], Kar-Ming Fung[6], Debra Saunders[1], and Randy L. Jensen[3]

[1]Advanced Magnetic Resonance Center, Oklahoma Medical Research Foundation, Oklahoma City, OK, United States, [2]Radiology & Biomedical Imaging, University of California San Francisco, CA, United States, [3]Huntsman Cancer Insitute, University of Utah Health Sciences Center, UT, United States, [4]Neurobiology & Anatomy, University of Utah Health Sciences Center, UT, United States, [5]Small Animal Core Facility, University of Utah, UT, United States, [6]Pathology, University of Oklahoma Health Sciences Center, OK, United States

Target Audience: This study will be of interest to cancer researchers, particularly those investigating gliomas and potential therapies, as well as basic scientists who are correlating MRS and DWI data.

Purpose: Gliomas are the most common and lethal primary brain tumors in the adult, with a median survival time of 12 to 15 months for grade IV gliomas, glioblastoma multiforme. OKN-007 is an effective anti-cancer agent in rodent pre-clinical models for adult GBMs[1-3]. Here, we report the effects of OKN-007 on the necrotic tumor core and non-necrotic tumor parenchyma in the F98 rat glioma model assessed by ^{1}H-MRSI and DWI. Histological assessment for tumor necrosis, and immunohistochemistry (IHC) for cell proliferation and apoptosis were also conducted.

Methods: Twenty four F98 rat glioma-bearing rats were divided into two groups: OKN-007 treated (n=12) and UT (n=12). ^{1}H-MRSI was obtained in normal Fischer 344 rats (n=6), and untreated (UT) (n=7) and OKN-007 treated (n=8) animals. DWI was performed in each animal from each group and the ADC was calculated in the in the tumor parenchyma and tumor necrotic core in each slice. The percent tumor necrosis was also assessed in both T2W images and H&E tumor sections of UT and OKN-007 treated animal. Counts of nuclei per high power field in 5 high power fields provided a quantitative measure of cell density. Ki-67 IHC provided an index of the proportion of proliferating cells, and the apoptotic index was obtained by the IHC for active caspase-3. Microarray analysis was also performed on total RNA of the brain from OKN-007 treated and UT F98 rat gliomas.

Results: Based on the 1H-MRSI, the Lip5.3/Cr ($p<0.0001$), Lip0.9/Cr ($p=0.0012$), and Lip1.3/Cr ($p=0.0015$) ratios significantly decreased in OKN-007 treated group compared to UT F98 gliomas at the end phase of tumor progression (Fig. 1A). Morphological MR TW2 images also were used to assess the percent necrosis tumor volume in both groups, which was significantly higher ($p<0.01$) in the UT group (20.08% ± 2.00, n=10) compared to the OKN-007 treated group (11.96% ± 1.80, n=7) (Fig. 1B). The percentage of tumor necrosis compared to the total tumor area on H&E stained tumor sections were higher in the UT group (23.44, n=1) compared to the OKN-007 treated group (10.32, n=1). Furthermore, the Cho/Cr ($p=0.0004$) ratio significantly decreased in OKN-007 treated group compared to UT F98 gliomas at the end phase of tumor progression. ADC values at the necrotic tumor core for the OKN-007 treated group were significantly lower than the UT group ($p< 0.0001$) (Fig 1C). The OKN-007 treated group also showed significantly higher ($p< 0.0001$) ADC values for the non-necrotic tumor parenchyma in the treated group compared to the UT group (Fig. 1D). The OKN-007 treated group (7571 ± 251, n=10) showed significantly lower ($p<0.001$) cellularity compared to the UT group (8442 ± 139, n=10). The Ki-67 labeling index (Ki-67 LI) was significantly lower ($p<0.05$) in the OKN-007 treated group (53.25 ± 5.72, n=6) compared to the UT group (71.73 ± 5.31, n=7). The apoptotic index was significantly higher ($p<0.01$) in the OKN-007-treated group (27.09±2.25, n=5) compared to the untreated group (11.69±3.38, n=7).

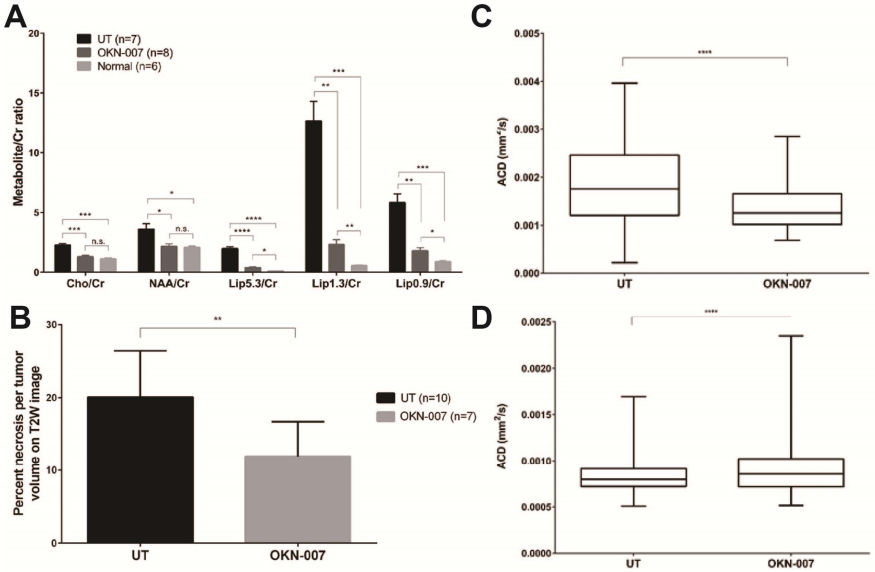

Figure 1. (A) Brain metabolite ratios measured by 1H-MRSI in normal rat brain, and UT and OKN-treated F98 rat gliomas. (B) Percent necrosis tumor volume in OKN-007 treated and UT F98 rat gliomas based on the T2W images. (C) ADC values at the necrotic core of the OKN-treated and UT F98 rat gliomas. (D) ADC values at the non-necrotic parenchyma of the OKN-treated and UT F98 rat gliomas.

Discussion: The 1H-MRSI data showed a statistically significant decrease in the vinyl protons (5.3ppm)/Cr, methylene (1.3 ppm)/Cr, and methyl (0.9 ppm)/Cr ratios which were observed in F98 rat gliomas following OKN-007 treatment compared to the untreated group. In brain tumors, the elevation of the lipid levels usually correlates with necrosis and are considered as important biomarkers in the diagnosis and monitoring the effects of treatment response. These spectroscopic findings were also confirmed by DWI, T2W, and histopathological data analyses. The possible explanation for this observation could be that OKN-007 affects necrosis by down-regulating the genes associated with Ca2+ channels, thereby reducing the intracellular Ca2+ levels. The effect of OKN-007 on genes associated with Ca2+ channels was confirmed by our microarray results wherein OKN-007 was shown to down-regulate two genes, MGP and MFAP4, which are directly related to Ca2+ channels. Furthermore, OKN-007 significantly decreased the Cho/Cr ratio compared to UT F98 gliomas at the end phase of tumor progression, due to a decrease in the tumor cell proliferation, which was also confirmed by our DWI and histopathological results. Our results corroborate with other studies[4] that confirmed the positive correlation of glioma choline signal with cell density and inverse linear correlation between glioma ADC and cell density.

Conclusion: Our results thus indicate that OKN-007 mediates multiple effects on different (tumor necrotic core and non-necrotic tumor parenchyma) regions of the tumor in F98 gliomas that can be detected in-vivo, indicating the efficacy of OKN-007 as an anti-cancer agent and its potential use in clinical trials.

References

1. Towner RA, Gillespie DL, Schwager A, et al. Regression of glioma tumor growth in F98 and U87 rat glioma models by the Nitrone OKN-007. Neuro Oncol. 2013;15(3):330-40.

2. He T, Doblas S, Saunders D, et al. Effects of PBN and OKN007 in rodent glioma models assessed by 1H MR spectroscopy. Free Radic Biol Med. 2011;51(2):490-502.

3. Garteiser P, Doblas S, Watanabe Y, et al. Multiparametric assessment of the anti-glioma properties of OKN007 by magnetic resonance imaging. J Magn Reson Imaging. 2010;31(4):796-806.

4. Gupta RK, Cloughesy TF, Sinha U, et al. Relationships between choline magnetic resonance spectroscopy, apparent diffusion coefficient and quantitative histopathology in human glioma. J Neurooncol. 2000;50(3):215-26.