Pre-clinical evaluation of anti-angiogenic agent RO0281501 on R3327 AT prostate model using lactate MRS and DCE-MRI

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Introduction

Pre-clinical development of agents targeting endothelial cell receptors with angiogenic inhibitors are ongoing. The present study was designed to study the anti-angiogenic effect of RO0281501 in the R3327 AT rat prostate tumor model using MRS lactate measurements and DCE-MRI.

Methods

The rat prostate tumor model R3327 AT was selected to study the anti-angiogenic inhibition of RO0281501 (generously provided by Roche Pharmaceutical). Animal studies were conducted in compliance with approved institutional protocols approved by the animal care committee. R3327-AT cells were subcutaneously implanted in Copenhagen rats in the thigh region (n=4 animals followed for drug treatment) versus control (n=5 animals followed for vehicle for RO0281501). Tumor volume measurements (X * Y * Z * π/6: X, Y and Z are length, width, thickness of the tumor), MR lactate determination and perfusion studies were conducted on day 0 (prior to treatment) and at 24hr, day 7, & day 14 after continuous daily treatment with oral RO0281501**.** MRS and DCE-MRI experiments were performed on a Bruker 4.7 T, 40 cm bore animal scanner. A 2 turn volume coil with a 25 mm diameter was used. Lactate editing was achieved using the Selective Multiple Quantum Coherence (Sel-MQC) sequence (1). Frequency selective 15 ms single-lobe Sinc pulses were employed for Sel-MQC editing. The ZQ $\rightarrow$DQ coherence transfer pathway was selected in Sel-MQC experiments using a phase cycling gradient scheme with g1:g2:g3 = 0:-1:2 with duration $\delta_1 = \delta_2 = 2$ ms, $\delta_3 = 4$ ms, and an amplitude of 24 G/cm. 512 data points were collected with 8 averages, TR=2 s and spectral width of 2500Hz. A matrix size of 16×16, FOV=40 mm (2.5 x 2.5 mm in plane resolution) was used. 2D CSI lactate maps were obtained from a 5 mm slice in the tumor region which was coregistered with a 5 mm thick T2-weighted image. Quantitation of tumor lactate is done by comparing its signal intensity with standard lactate solution by using substitution method (2). Dynamic contrast enhanced (DCE) MR images (3 sagittal slices, 2 mm thickness; 0.2 mm slice spacing, 6 sec temporal resolution, pulse sequence Gradient Echo Fast Imaging (GEFI), TR/TE-36.4ms/3ms; 96 X 96 matrix, 125 time points, NEX=2), were obtained after injection of Gd-DPTA (0.2 mM/Kg; Magnevist, Berlex Laboratories)) intravenously (2 minutes after start of the scan)). DCE-MRI data were analyzed to evaluate the rate constant, Ak_{ep} (based on a two-compartment model proposed by Hoffman et al (3)). In the DCE-MRI analysis, the tumor perfusion parameter Akep was calculated by drawing a ROI around the whole tumor, and subsequently analyzing the rim and core separately.

Results and Discussion

In **Fig 1** on day 0 the average tumor volume of study group animal and control group animals is 940 mm^3 and 1031 mm^3 respectively. After 24hr of treatment, there was an 18 % tumor inhibition (compared to control). By day 7 the tumor inhibition of the treated group animals was 40 % (compared to the control group). Control tumors continued to grow beyond day 7 and became necrotic before day 14, hence these tumor bearing animals were sacrificed . The tumor doubling time (TDT) for the control group was 3 days (4 and other reports (5)), whereas the TDT increased to 7-8 days in the treated cohort.

In **Fig 2** reduction of whole tumor average Akep value at 24 hr in both the treated and control group are not significant ($p > 0.05$) compared to their base line point (day 0). The tumor rim average Akep value at 24hr was significantly reduced compared to baseline values ($p < 0.05$) in the treated group , whereas in the control group, the reduction in Akep is not significant. Tumor rim Akep comparison between the treated and control group at 24hr was significantly different ($p < 0.05$). On day 7, reduction of tumor rim and whole tumor Akep value was significant ($p < 0.05$) both in the treated and control groups compared to their baseline point, although the changes in the core were not significant compared to baseline. On day 7, differences between treated and control groups approached statistical significance ($p<0.09$). These results indicate that the anti-angiogenic effect of the drug can be evaluated by DCE-MRI. The anti-angiogenic property of this drug appears to be more effective at the rim of the tumor and further studies measuring changes in vascularity and apoptosis are ongoing.

Representative 2DCSI lactate spectra of study group animals at pre (day 0) and post (24 hr, day 7 and day 14) treatment are shown in **Fig 4**. **Fig 3** shows that lactate was detected prior to treatment (day 0: 7.37 mM) and is significantly ($p < 0.05$) decreased to 2.28 mM at 24 hrs of treatment. At 24 hr post treatment, lactate has poor SNR in 3 animals (out of 4 study group animals). On day 7, with increased tumor volume the average tumor lactate significantly increased to 11.09 mM (compared to day 0 and 24 hrs). At the highest tumor volume on day 14 the tumor lactate noticeably decreased to 6.94 mM ($p < 0.05$) compared to day 7.

Fig. 1 R3327 AT Tumor growth inhibition

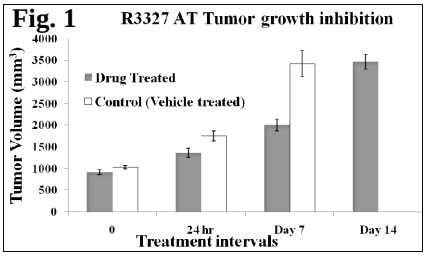


Fig. 2 Tumor rim DCE-MRI kinetics

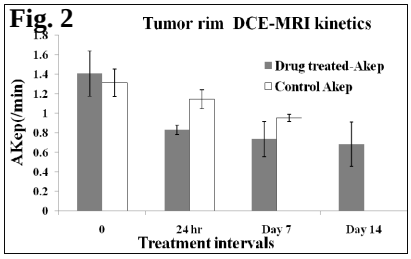


Fig. 3 Average tumor lactate in Roche Drug treated animal

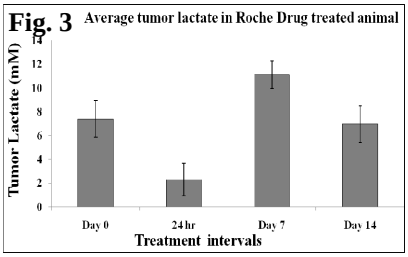
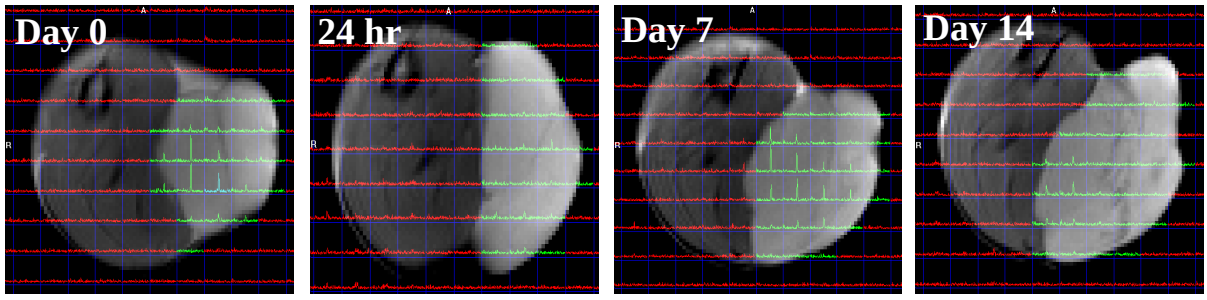


Fig. 4



Conclusion

Treated animals demonstrated an 18 % tumor growth inhibition at 24hrs post treatment compared to control and 40 % of tumor inhibition on day 7 compared to control group. At 24hrs after starting treatment, reduction of tumor rim Akep in the treated group is significantly different from its baseline and as well from control. The drug also induces a decrease in lactate at 24 hours after starting therapy, a potential early marker of response to treatment.

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