MRI of Calu-6 lung tumors for the assessment of the anti-vascular activity of MN-029

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Introduction: Anti-vascular and anti-angiogenic drugs are currently of great interest in the treatment of cancer (1-2). These drugs selectively disrupt tumor blood vessels or inhibit their formation. MediciNova is developing a novel vascular disrupting agent (MN-029) that reversibly disrupts the tubulin cytoskeleton of proliferating tumor endothelial cells and shuts down tumor blood flow. For clinical trials and the further development of anti-vascular therapeutics, methods for evaluating the vascular response of tumors to treatment are urgently required. Dynamic Contrast-Enhanced MR imaging (DCE-MRI) and T2* techniques were used with success to assess the tumor response to anti-vascular treatments non-invasively showing the usefulness of biomarkers such as K^{trans}(3). In this study, we characterized the blood flow changes induced by MN-029 in Calu-6 human lung tumors using DCE-MRI and T2* techniques.

Methods: 10^7 human Calu-6 cells were injected subcutaneously in the right flank of 70 female Nude rats. When tumors reached a volume of 400 to 600 mm^3, the rats were randomized into 3 groups of 18 rats. One group of rats received an intravenous (IV) infusion of vehicle, one group received an IV infusion of 3 mg/kg of MN-029 and one group received an IV infusion of 10 mg/kg of MN-029. 3 rats per group were imaged before treatment and at 1H, 6H and 24H post-treatment, while 3 other rats per group underwent MRI before treatment and at 3H and 24H post-treatment. The other 12 rats per group were sacrificed successively at the same time points (3 rats per time point) after injection of FITC-Dextran, Hoechst and pimonidazole. All the MR acquisitions were performed in a 4.7T magnet (Bruker, Wissembourg). Multi-slice T2-weighted MR images covering the entire tumor were followed by multi-gradient echo T2* MR acquisitions (TR/TE/α=500ms/5,10,15,20,25ms/45°; FOV=70x70mm; Matrix=256x256, slice thickness=1mm). DCE-MRI was performed using a FLASH-2D sequence with a temporal resolution of 12.8s per image (TR/TE/α=100ms/3.3ms/70°; FOV=70x70mm, Matrix=128x128, slice thickness=2mm). T1-weighted pre-contrast images were acquired 1-minute prior to an IV bolus injection of 0.3 mmol/kg Gd-DOTA (GUERBET, France). 115 post-contrast images were acquired during 20 minutes. Images were processed under IDL 6.1 using in-house written software. Region of interest (ROI) analysis and pixel by pixel analysis were performed to determine R2* values and K^{trans} and ve values using the Tofts and Kermode pharmacokinetic model.

Results: The results showed a significant drop in tumor perfusion at 1H and 3H post-MN-029 treatment in the 3 and 10 mg/kg treatment groups of rats with K^{trans} values in the tumor rim dropping by 28% at 1H post-treatment in the 3 mg/kg group and by 67% and 72.6% at 1H and 3H post-treatment, respectively, in the 10 mg/kg group compared to pre-treatment values. In the tumor center, K^{trans} dropped by 53% at 1H in the 3 mg/kg group and by 90% and 73% at 1H and 3H post-treatment, respectively, in the 10 mg/kg group compared to pre-treatment values. Reduced perfusion was still apparent in the 10 mg/kg at 24H post-treatment. Significant differences were also noted compared to the vehicle group. R2* quantification showed a decrease in R2* at 1H for the 10 mg/kg group corresponding to vascular collapse and necrosis which reduced the overall amount of deoxyhaemoglobin concentration. Immunohistochemistry findings confirmed these results showing decreased perfusion of Hoechst dye in the 10 mg/kg group at 1H and 3H post-treatment and increased necrosis at 24H post-treatment.

Conclusions: The changes in K^{trans} and ve in Calu-6 tumors after MN-029 treatment are reflective of tumor blood flow changes. K^{trans} values for Gd-DOTA uptake into Calu-6 tumors could be a useful non-invasive marker of blood flow changes induced by vascular disrupting agents such as MN-029.

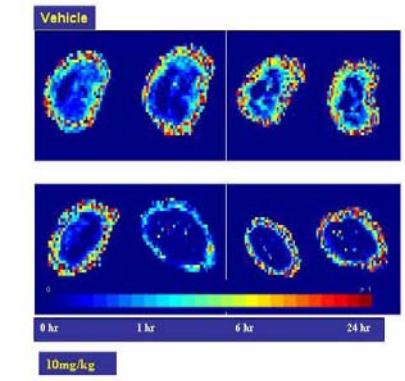

Figure1 :Ktrans maps within one tumor slice for a vehicle treated rats and a 10mg/kg treatedrat at 0,1h,6h and24h post-treatment

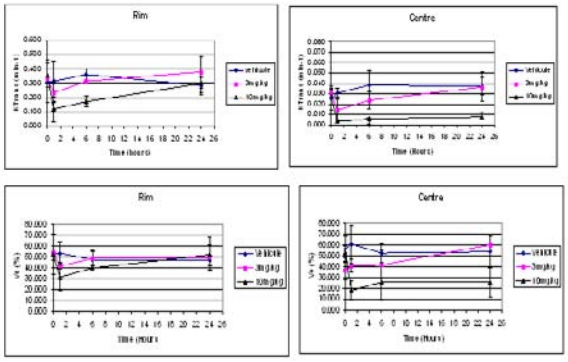

Figure 2. Evolution of Ktrans and ve as a function of time pre- and post- treatment in vehicle, 3 mg/kg and 10 mg/kg treated rats in the tumor rim and the tumor center.

References : (1) Micheletti G et al (2003), Cancer Research 63 1534-1537
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