Dose Response of Rat GH3 prolactinomas to 5,6-dimethylxanthenone-4-acetic acid (DMXAA) Assessed by Dynamic Contrast Enhanced MRI

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Introduction

The tumour vasculature is an attractive target for cancer therapy, as destruction of a single blood vessel results in the death of many tumour cells. Vascular disrupting agents (VDA) are a class of cancer therapeutic drugs that exploit the differences between normal vasculature and tumour vasculature to selectively damage the endothelial cells of established tumour blood vessels, culminating in vascular shutdown and tumour necrosis. 5,6-dimethylxanthenone-4-acetic acid (DMXAA) is a VDA which induces both direct anti-tumour effects in the form of endothelial cell apoptosis[1] and indirect anti-tumour effects through the induction of cytokines[2], and is currently in Phase II clinical trials. The majority of vascular targeted therapies do not induce any rapid tumour regression, so their clinical development requires the development and validation of clinically translatable, quantitative biomarkers of tumour blood vasculature and its response. To this end, we have investigated the dose-dependent effects of DMXAA in a rat tumour model using i) dynamic contrast-enhanced (DCE) MRI, ii) measurements of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), a plasma biomarker of DMXAA-induced anti-vascular activity in both rodent and human tumours[3], and iii) histologically assessed tumour necrosis.

Methods

GH3 prolactinomas were grown subcutaneously in the flanks of female Wistar Furth rats. DCE-MRI was performed prior to and 24 hours after treatment with vehicle (n=6), 100mg/kg (n=7), 200mg/kg (n=8) or 350mg/kg (n=7) DMXAA i.p. MRI was performed on a 4.7T horizontal bore Varian Unity Inova. Tumour data were acquired with a three-turn solenoid. A 9-turn solenoid was used to acquire AIF data from the large tail vessels[4]. Gradient echo MRI was initially used for the AIF acquisition, switching to a spin echo acquisition later in the study after it was found that R_2* effects were spoiling the first points of the [Gd] measurement. Accordingly, a previously measured (generic) AIF was used for analysis[4], and the individual rat AIF was used solely for acceptance testing/quality control of data. The probes were switched electronically under spectrometer control for interleaved acquisition of tumour and tail images. Baseline tumour T_1 data were acquired using an inversion-recovery FLASH sequence. Four slices were acquired from the tumour, matrix size 128x64, slice thickness 2mm, interslice gap 2mm, field of view 4x4 cm. A single slice of thickness 4mm and field of view 1cm was acquired from the tail. The repetition time for DCE-MRI of 120ms was the minimum dictated by the system's gradient duty cycle limits, giving 7.68 second time resolution. 32 scans were acquired prior to injection of contrast, and a further 180 scans after injection of 0.1 mmol/kg Omniscan by a syringe driver under spectrometer control. Flip angle mapping was used to reduce errors due to the non-uniform B_1 of the tumour coil[5]. The DCE-MRI data were analysed using the method of Tofts and Kermode[6] for the determination of K^{trans}, and an integrated area under the [Gd]-time curve method (IAUGC)[7]. After the post-treatment scan, laparotomy was performed and arterial blood taken from the aorta, and the plasma assayed for 5-HIAA by HPLC[3]. The tumours were excised and cut and stained with haematoxylin and eosin, and scored for necrosis on a scale from 1 (no necrosis) to 4 (extensive).

Results

Pre and post-treatment (350mg/kg) K^{trans} images are shown in Figure 1. Larger reductions were noted in the core of the tumour than in the rim. A paired t-test was used to compare the pre- and post-treatment median values for K^{trans} (Figure 2) and IAUGC in each group. A significant reduction in both K^{trans} and IAUGC was found only at the highest dose of 350mg/kg DMXAA. Plasma 5-HIAA significantly increased following treatment with 200 and 350mg/kg DMXAA (Figure 3). There was evidence of an increase in tumour necrosis following intermediate doses of DMXAA, significant following treatment with 350mg/kg.

Figure 1

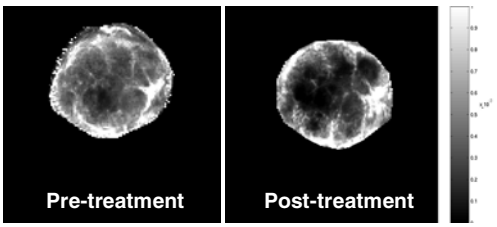


Figure 2

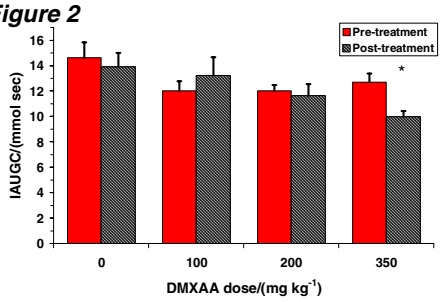
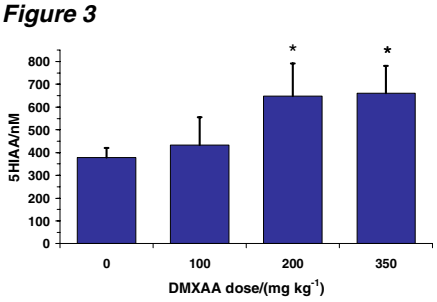


Figure 3



Discussion

DCE MRI revealed a significant reduction in K^{trans} and IAUGC of rat GH3 prolactinomas 24 hours after treatment with 350mg/kg DMXAA, but there was no evidence of dose response. A similar conclusion was reached in the Phase I trials of DMXAA[8]. Increased plasma 5-HIAA correlates with increased vascular permeability induced by DMXAA[9], and demonstrates biological effects at 200 mg/kg. DMXAA can both increase vessel permeability[9] and reduce tumour blood flow[2], effects which can respectively increase or decrease K^{trans} and IAUGC. The absence of any significant change, either up or down, in K^{trans} or IAUGC following treatment with 200mg/kg, despite the significant increase in 5-HIAA, thus raises concerns about the utility of established DCE MRI biomarkers to assess tumour response to DMXAA at doses well below the maximum biological effect, and emphasises the continued need to identify alternative MRI biomarkers of tumour response to vascular therapies.

References

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