Short-term anti-angiogenic therapy improves tumor microvascular function: investigation with dynamic contrast enhanced MRI

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Introduction

Angiogenesis, the process of forming new blood vessels from the endothelium of existing vasculature, is fundamental for tumor growth, progression and metastasis. This tumor neovasculature is characteristically immature with incomplete endothelial linings resulting in a high permeability. Due to promising results in preclinical studies, it was believed that anti-angiogenic therapy eradicates tumor vasculature by depriving the tumor of oxygen and nutrients which are necessary for tumor growth and survival; but initially, no convincing clinical trial gave evidence for an efficient antitumoral therapy when using solely anti-angiogenic agents. However, they improved the efficiency of chemotherapy when combined [1]. This observation was unexpected as the efficiency of chemotherapy depends on tumor blood supply, which was assumed to be reduced by anti-angiogenic therapy; this triggered the hypothesis that a possible complementary effect of anti-angiogenic drugs is a "normalization" of tumor vasculature due to a reduction of immature and inefficient neovasculature by eliminating excess endothelial cells resulting in a more normal and conductive microcirculation for delivery of oxygen, nutrients and therapeutics [2]. As a result, many preclinical studies investigate newly developed anti-angiogenic compounds in terms of their therapeutic efficacy, but only a small number of them study the resulting functional changes in tumor microcirculation, and even fewer do so in established, highly vascularized tumors which are closer to clinical conditions. This, however, is necessary in order to identify the ideal time window for chemotherapy after the application of anti-angiogenic drugs. Therefore, aim of the present study was to investigate functional changes in tumor microcirculation in established, highly vascularized tumors in hamsters after short-term anti-angiogenic treatment by dynamic contrast enhanced (DCE) MRI.

Methods and Materials

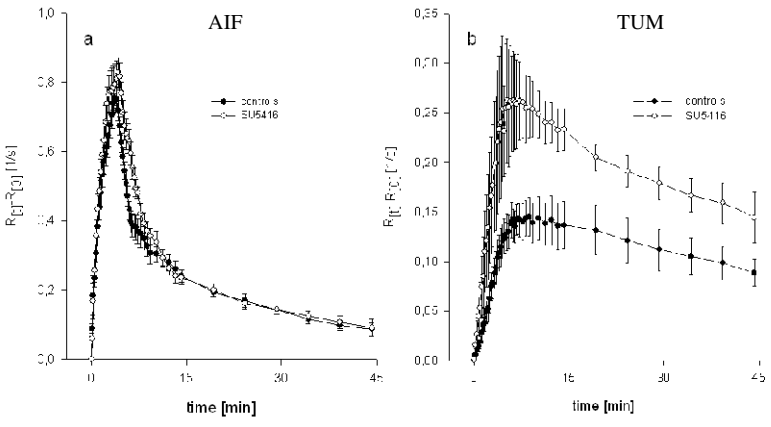
Approximately 5 x 10^6 cells of the amelanotic hamster melanoma A-MEL-3 were implanted subcutaneously at heart level into the right flank of male Syrian golden hamsters (40-60 g b.w., n=12) under i.p. anesthesia with ketamine (100 mg/kg b.w.) and xylazine (10 mg/kg b.w.). Six of the animals received an i.p. injection of the anti-angiogenic agent SU5416 dissolved in DMSO at a dose of 20 mg/kg b.w. on days 5, 6, and 7, the remaining six control animals received DMSO with equivalent volume and timing. Prior to MRI investigations on day 8, fine polyethylene catheters were inserted into the right jugular vein under i.p. anesthesia (as described above) for infusion of paramagnetic contrast agent (GD-DTPA). For MRI scans, hamsters were positioned in an animal handling system which was then inserted into a 9.4 Tesla MR microimaging system. During the MRI experiment inhalation anesthesia with 1% isoflurane in oxygen was applied at 1.5 l/min via a face mask, and temperature was kept constant at 28°C with a warm air heating system to prevent a drop in body temperature. The MRI experiment consisted of (a) a scout scan with 3 orthogonal slices, allowing for exact positioning of the slice for the DCE-MRI scan which had to intersect both heart and tumour, (b) 3 pre-contrast T_1 scans, and (c) a total of 47 post-contrast T_1 scans over 45 minutes after onset of Gd-DTPA infusion (at a constant rate over 4 min at a dose of 0.1 mmol/kg b.w.): 32 scans with 15 sec interval, then 4 scans with 30 sec interval, 5 scans with 1 min interval, and 6 scans with 5 min interval.

T_1 scans were acquired using an IR snapshot FLASH sequence with 24 consecutive snapshot FLASH images after inversion. FoV was 40 x 40 mm^2, with a matrix size of 128 x 64 zerofilled to 128 x 128, resulting in an in-plane resolution of 313µm, slice thickness 1.5 mm, bandwidth 120kHz. TE was 1.660ms, TR 2.636ms, resulting in a total acquisition time of 4.055sec per T_1 scan allowing for sufficiently accurate sampling of the fast relaxation dynamics in contrast enhanced tissue and blood with a minimum repetition time of 15 sec. From the 24 T_1-weighted snapshot FLASH images of each T_1 scan a T_1 map was calculated using a 3-parameter least square fit on a pixel-by-pixel basis [3]. In T_1 maps, ROIs for determination of T_1 in arterial blood and tumor were defined in the left ventricle and over the total visible tumor section, respectively.

Concentration-time curves C(t) for arterial blood (arterial input function, AIF) and tumor tissue (TUM) were determined from changes in T_1 relaxation rates $\Delta R(t)$ between pre- and post-contrast scans according to $\Delta R(t) = R(t) - R(0) = \alpha \cdot C(t)$ with α being the relaxivity of the contrast agent. Indicators for tumor blood flow and intratumoral blood volume are $d\Delta R/dt_{[0\text{-}4.5min]}$ and $\Delta R_{max,TUM}/\Delta R_{max,AIF}$, respectively, with ΔR_{max} being the maximum peak enhancement in tumor tissue (TUM) and arterial blood (AIF). The total tumor distribution volume of Gd-DTPA (intravascular and interstitial space) is indicated by AUC_{TUM}/AUC_{AIF} with AUC being the area under the concentration-time curve in tumor tissue (TUM) and arterial blood (AIF).

Results

Fig.1a shows the concentration-time curves of Gd-DTPA in arterial blood for control and treated animals; there is hardly any difference between both curves, in particular the slope $d\Delta R/dt_{[0\text{-}4.5min]}$ and the peak value $\Delta R_{max,AIF}$ are similar proving standardized infusion of Gd-DTPA and almost identical cardiac outputs. In contrast, concentration-time data of Gd-DTPA in tumor tissue (Fig.1b) clearly show different shapes with a 2-fold higher $d\Delta R/dt_{[0\text{-}4.5min]}$ in treated tumors compared to controls (P=0.026). Normalised peak enhancement $\Delta R_{max,TUM}/\Delta R_{max,AIF}$ was 60% higher in treated animals (P=0.026) indicating an increased tumor blood volume. AUC_{TUM}/AUC_{AIF} was 50% higher in treated animals (P=0.41) indicating an increased total tumor distribution volume of Gd-DTPA.



Conclusion and References

In summary, DCE-MRI experiments showed an overall increased tumor blood volume, and tumoral Gd-DTPA uptake in animals treated with an anti-angiogenic drug suggesting an increased tumor microvascular functionality.

As especially flow in immature vessel segments is chaotic with intermittent stagnation followed by high flow or even flow reversal in isolated segments [4], it can be concluded that overall tumour blood flow is critically determined by the proportion of immature and mature vessels within a tumor. Therefore, a reduction of microvessel density as found with intravital microscopy after anti-angiogenic treatment under identical treatment schedule does not necessarily implicate reduced tumor blood flow and contradict the observations of the present study, especially as the A-MEL-3 tumor exhibits a high angiogenic activity. Our findings rather seem to support the hypothesis that one effect of anti-angiogenic drugs is a "normalization" of tumor vasculature [2], with immature blood vessels with oscillating blood flow being pruned and hyperperfusion in remaining blood vessels. The latter is as well supported by intravital microscopy findings from our group showing an increased red blood cell velocity and increased blood vessel diameters after anti-angiogenic treatment.

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