Time-Dependent Anti-Vascular Effects of ABT-751 Assessed by Dynamic Contrast Enhanced MRI (DCE-MRI)

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Introduction

Tumor vasculature is a potential target for cancer therapy. Different strategies have been used to target tumor vasculature. Besides anti-angiogenic therapies, another approach was to target existing tumor vasculature by disrupting the integrity and the functionality of the tumor blood vessels. Combretastatin A-4 and ZD-6126, which are both tubulin-binding agents, cause tumor blood flow shutdown leading to tumor necrosis [1, 2]. ABT-751 is a similar type of anti–tubulin agent that has demonstrated potent antitumor activity against a broad spectrum of murine tumors and human xenografts including those that are resistant to vincristine or paclitaxel due to multidrug-resistant (MDR) phenotype. The compound possessed excellent oral bioavailability in human and is currently under phase II clinical development. In a recent study, we found that ABT-751, like combretastatin A-4 and ZD-6126, exhibited anti-vascular properties causing a rapid and drastic decrease in tumor perfusion following compound administration [3]. Such a decrease in tumor perfusion, if sustained, has the potential to enhance treatment efficacy by depriving nutrient supplies to tumor cells. However, the reduction in tumor perfusion could also prevent optimal compound delivery to the tumor from subsequent dosing. To further characterize the anti-vascular properties of ABT-751 and help design dosing schedules for the phase II clinical trials, we investigated the time-dependent tumor perfusion changes after a single dose treatment of ABT-751. Its microscopic effect on endothelial cell morphology was also assessed on the same time scale using HMVEC cells.

Methods

Female Fisher rats were injected subcutaneously with 9L glioma cells into the left hind limb. The experiments were performed when tumor volumes average 1 cm^3. On a 4.7T/40cm magnet, DCE-MRI perfusion measurements were conducted on a 3 mm cross section of the hind limbs covering muscle and the tumor tissues using T_1-weighted FLASH sequence immediately before, and 1 hour post ABT-751 or vehicle administration. Animals were allowed to recover from anesthesia then imaged again at 6 hours after the initial treatment. ABT-751 was administered through i.v. infusion at a dose of 30 mg/kg. Imaging parameters: TR/TE = 30/2 ms, field of view 6.4×6.4 cm^2, imaging matrix 128×64. Gd-DTPA was given bolus i.v. at 0.2 mmol/kg.

The dynamic signal intensity curves were first converted to curves representing dynamic changes of Gd concentration. The maximum Gd uptake rate and the area under the curve (AUC) were calculated on a pixel-by-pixel basis and then averaged over manually outlined regions of interest for muscle (from left, right hind limbs) and tumor (entire tumor, tumor rim, and non-rim) regions. Data from different regions were normalized to the right hind limb muscle to reduce inter-animal variations.

Human dermal microvascular endothelial cells (HMVEC-d) were plated and allowed to attach overnight. Cells were either treated at 0, 0.3, 1, 3 or 10 μM ABT-751 for 1 h on the following day. After treatment one set of cells was fixed and another set of cells was washed with PBS twice after the 1 h treatment, allowed to recover for 5 h in fresh medium, then fixed in formalin. Cells were stained for microtubules and microfilaments then imaged with a confocal imaging system for morphological evaluation.

Results

As shown in Figure 1 (n>6/group), no significant changes in Gd uptake were observed in either muscle or tumor tissues of the vehicle treated rats. The maximum Gd uptake rate in tumors decreased to 41±5% of the pre-treatment value 1 hour following the treatment of ABT-751, then recovered to 85±10% of the pre-treatment level at 6 hours. In contrast, the maximum Gd uptake rate in muscle was not different from that of the pre-treatment level at either 1 or 6 hours. When comparing ABT-751 induced Gd uptake changes in different tumor regions, no differential effects were observed between tumor rim and non-rim regions. Similar results were obtained from the analysis of the AUC data.

ABT-751 induced endothelial morphology changes at corresponding time points were evaluated *in vitro* using HMVEC cells. Following 1 h treatment, the cells became retracted. The degree of cell retraction increased in a dose-dependent manner with a clear loss of microtubules and increase in prominent peripheral microfilaments. Following 5-hour recovery, most of the cells became well extended and looked similar to untreated control cells.

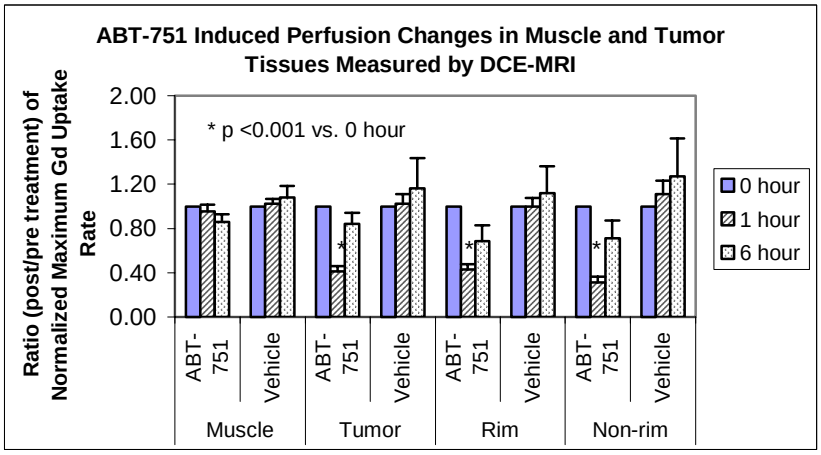

Figure 1

Discussion

In this study, a significant reduction in Gd uptake was observed only in tumors 1 hour after ABT-751 treatment. The results were consistent with our findings from previous experiments confirming the potent, selective anti-vascular effects by ABT-751. The reduction in Gd uptake was transient and reversible. The transient changes in endothelial cell morphology matched the time-dependent changes in tumor Gd uptake, suggesting that ABT-751 induced vascular disruption may be in part due to endothelial cell structure changes in tumors. Because ABT-751 compromises tumor vascular functions, consideration should be given to designing treatment schedules to ensure optimal drug delivery to the tumor when frequent dosing is required or combining with other drug treatments.

References

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