MRI guided anti-tumor therapy with liposomal prednisolone phosphate

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Introduction

High daily doses of corticoid drugs can effectively inhibit tumor growth [1]. Unfortunately the acute side effects make prolonged therapy problematic. In order to overcome this toxicity problem, a water soluble prednisolone disodium phosphate (PLP) was encapsulated into long circulating DPPC-based liposomes [2]. Treatment with these liposomes in a low dose and a weekly schedule resulted in increased drug accumulation in the tumor tissue and slow local release of the drug, leading to a strong tumor growth inhibition. Although it is anticipated that the anti-tumor effect is due to angiogenesis inhibition [1-3], the exact mechanism of this anti-tumor action remains unclear. Therefore, we decided to perform Magnetic Resonance Imaging (MRI)-guided therapy to bring further insight into the observed effects. In order to obtain a full picture of the disease during the course of treatment, several MRI methods were applied. T_2-weighted imaging was used to visualize the tumor and heterogeneity of its structure, i.e. necrotic sites. T_2 maps were generated in order to follow changes of T_2 relaxation time throughout the therapy. With diffusion MRI, which has been proposed as a method for early prediction of therapeutic efficacy in tumor treatment [4], we quantified the apparent diffusion coefficient (ADC) within the tumor. Moreover, we performed contrast enhanced T_1-weighted imaging using RGD-conjugated paramagnetic liposomes as a contrast agent. This liposomal formulation has been successfully used for in vivo imaging of angiogenesis [5, 6] by targeting the αvβ3 integrin, a receptor overexpressed on activated endothelium. Inhibition of angiogenesis leads to a reduction of tumor vasculature and expression of αvβ3 integrin. Therefore the contrast enhancement in the treated tumors is expected to be reduced compared to that of control group.

Materials and methods

Two liposomal formulations were used in this study. First, a long-circulating formulation based on dipalmitoyl-phosphatidylcholine (DPPC) and loaded with prednisolone phosphate disodium salt (PLP-L) was used as a therapeutic. To assure sufficient accumulation in the tumor, the particle size was chosen to be 100 nm. The other type of liposomes was 200 nm diameter RGD-conjugated paramagnetic liposomes. These are long-circulating distearoyl-phosphatidylcholine (DSPC)-based liposomes containing 25 mol% of Gd-DTPA-bis(stearylamid) (Gd-BSA), and were used as a MRI contrast agent on the last day of the experiment.

Male C57BL/6 mice (6-8 weeks of age) were inoculated subcutaneously in the flank with B16F10 melanoma cells (3 animals per experimental group). The therapy started at the time when tumors became palpable (Day 1). Then the pre-treatment MRI was performed after which the drug was administered. Liposomal prednisolone phosphate was intravenously injected at a dose of 20 mg/kg, whereas control animals received saline injection. Four days after the pre-treatment MRI, the first post-treatment MRI was performed (Day 5). During the second post-treatment MRI, on the last day of the experiment (Day 8), the paramagnetic contrast agent was intravenously administered. Tumor size was measured daily, and tumor volume was calculated according to the formula: $V = 0.52a^2b$, where a is the smallest and b is the largest superficial diameter.

MRI examination was performed on a 6.3 T scanner (Bruker Biospin) with a 3 cm birdcage coil. Pre-treatment MRI measurements (Day 1) and first post-treatment MRI (Day 5) included T_2-weighted imaging (TR/TE = 4200/35ms), T_2 mapping (TR/TE=2000/9ms, 16 echoes), diffusion MRI (TR/TE = 2000/35ms, b=400 s/mm^2) and T_1-weighted imaging (TR/TE=800/8.8ms). The second post-treatment MRI (Day 8) had the same measurement scheme but, additionally, paramagnetic contrast agent was administered, after which T_1-weighted images were generated every 10 minutes for at least 6 time points.

Results

Tumor size measurements and the anatomical T_2-weighted images indicated no regression of the tumor mass and continued tumor growth. Nevertheless, treatment with liposomal prednisolone phosphate affected tumor volumes compared to vehicle-treated control animals (Figure 1). Although T_2 maps showed, in the course of the therapy, an increasing tumor area having a shorter T_2, indicating enhanced necrosis, the same trend was observed for the control group (data not shown). ADC histograms (Figure 2) did not show any significant change in water diffusion, suggesting that the cellular composition of the tumor was not affected by the therapy. Interestingly, the ADC histograms of both groups were very similar, although the size of the tumors was very different. Contrast enhanced T_1-weighted imaging using RGD-conjugated paramagnetic liposomes showed a clear difference between the tumors treated with liposomal PLP and the control group (Figure 3). Compared to the control group, the treated tumors show a significantly stronger signal enhancement after contrast agent injection (Figure 4). Accumulation of contrast agent was found in the entire tumor volume. In a previous study [5], it was found that the contrast agent accumulated mainly in the periphery of untreated tumors, where angiogenic activity is most pronounced. We assume that the observed contrast enhancement is not due to a receptor-mediated uptake of the contrast agent but results from unspecific accumulation by increased vascular permeability.

Conclusions

Treatment of B16.F10-bearing mice with 20 mg/kg liposomal prednisolone phosphate significantly inhibited tumor growth compared to vehicle-treated control animals. On the basis of MRI parameters measured before and throughout the therapy it seems likely that the anti-tumor activity of liposomal prednisolone does not originate from induction of tumor cell damage or necrosis. Unexpected massive accumulation of liposomal contrast agent, possibly due to increased permeability of the tumor vasculature, did not allow us to specifically image angiogenic endothelium, with the present experimental protocol. In order to support the interpretation of our results, histological and immuno-histochemical evaluation is conducted.

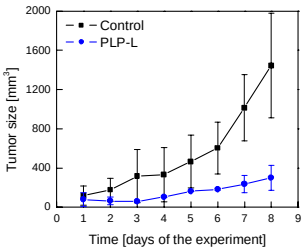

Figure 1 *Effects of liposomal prednisolone phosphate on tumor growth compared to vehicle-treated animals. Mean ± SD*

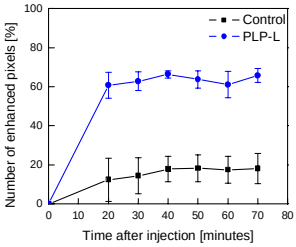

Figure 4 *The percentage of tumor area (mean ± SD) that showed significant signal enhancement after contrast agent injection*

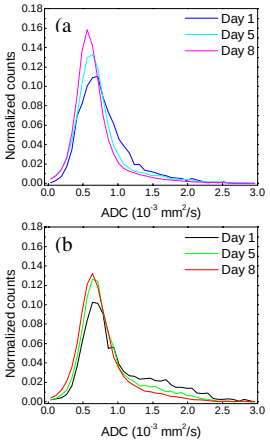

Figure 2 *ADC histograms of (a) control and (b) PLP-L-treated tumors from 3 time points (calculated for all animals)*

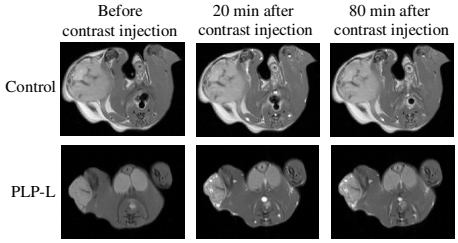

Figure 3 *Representative T_1-weighted MR images through the tumor of control and PLP-L-treated tumors acquired before, 20 min and 80 min after administration of the RGD-conjugated paramagnetic liposomes*

References

[1] Folkman J, Science, 1983
[2] Raymond M. Schiffelers, *Neoplasia*, 2005
[3] Manuela Banciu, *Journal of Controlled Release*, 2006
[4] Ross B.D., *Mol Cancer Ther*, 2003
[5] Mulder W.J.M., *FASEB J*, 2005
[6] Mulder W.J.M., *FASEB J*, 2007