Multi-parametric MRI assessment of the anti-angiogenic effects of liposome-encapsulated glucocorticoids

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Introduction: Inflammation plays an important role in tumor progression and anti-inflammatory drugs have therefore been proposed as anti-cancer agents. However, the adverse effects caused by the high doses required for tumor growth inhibition are a major limitation. Liposome incorporated glucocorticoids (GC), a prominent class of anti-inflammatory agents, cause effective tumor growth inhibition at much lower doses than free drug, with little side effects [1]. The **aim** of this study was to test the hypothesis that liposomal GC therapy involves angiogenesis inhibition, using a multi-parametric MRI protocol.

Materials and Methods: Prednisolone phosphate-loaded liposomes (PLP-L; ø100nm) were prepared as in [2]. C57BL/6 mice were inoculated *s.c.* with 10^6 B16F10 cells. After pre-treatment MRI (day 0), mice received a single *i.v.* dose of either PLP-L (20mg PLP/kg; n=6) or saline (n=6). Post-treatment MRI was done on day 2, 4 and 6. Thereafter mice were sacrificed and tumors dissected for microscopy. MRI was done at 6.3T, using isoflurane anaesthesia, and included: (a) T_2-w spin-echo (TE/TR=35/4200ms; NA=4); (b) diffusion-weighted (DW) spin-echo (TE/TR=35/2000ms; b-value=0 or 400s/mm^2; NA=2); (c) T_2 mapping from spin-echo images with 16 different TEs from 9-144 ms (TR=2000ms; NA=2); (d) DCE-MRI, using T_1-w FLASH (TE/TR=3/80ms; α=50°; slices=8; NA=1) for 25min collecting 200 frames. A tail vein infusion line was used to deliver 0.3mmol gadoteridol/kg approx 50s after the start of DCE-MRI. For all scans: matrix=128×128; FOV=3×3cm^2; slice=1mm. Tumors were segmented on DW-images and their volumes calculated. ADC maps were calculated from DW-scans. DCE-MRI data analysis was restricted to pixels with signal enhancement ≥ 5 times the noise. DCE-MRI data were converted to [CA]-time (C_t) curves with the muscle reference method [3], using a pre-contrast muscle T_1 of 1285ms, muscle endothelial transfer constant (K^{trans}) of 0.11min^{-1}, fraction of extravascular extracellular space (v_e) in muscle of 0.20 and gadoteridol relaxivity of 3.7mM^{-1}.s^{-1}. C_t curves were fitted with the two-compartment Tofts model [4], using a golden section search. Pixels with v_e≥0.95 were discarded. The descriptive parameters time-to-peak (T_{peak}) and initial slope ($Slope_i$) were derived from the C_t curves. Microscopy was done on tumors dissected 6 days after therapy. 5-µm-thick sections were stained for endothelial cells (CD31) and cell nuclei (DAPI). Images were acquired at 200×magnification. Microvessel density (MVD) was estimated from the number of vessels in 5 vascular regions per tumor. Vessel normalization was probed by anti-actin staining, followed by CD31 co-staining. Data are presented as mean±SD. DCE-MRI indices were analyzed on the basis of median values, determined from parameter histograms. Paired t-tests were used to evaluate time effects of treatment compared to the baseline. Between-group comparison was done using repeated measures one-way Anova.

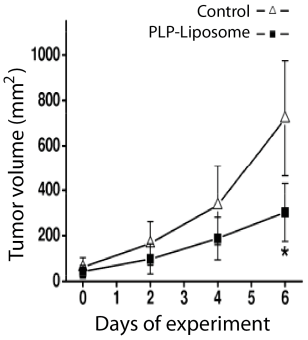

Figure 1

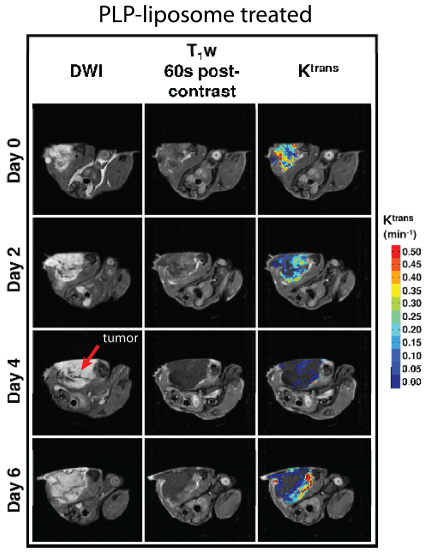

Figure 2

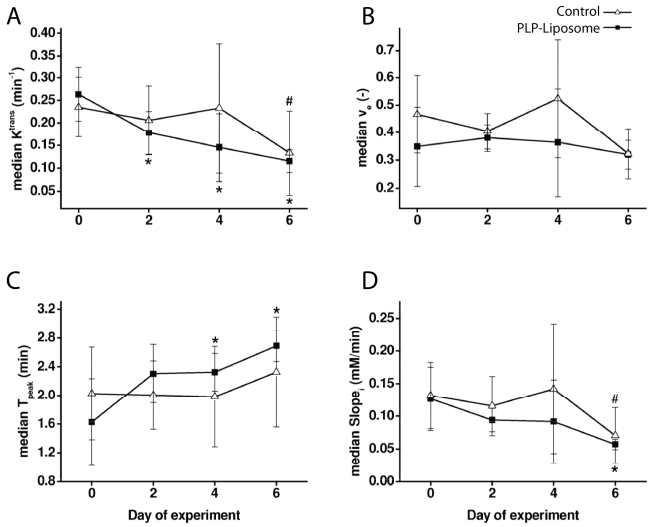

Figure 3

Results & Discussion: The growth-inhibiting effects of PLP-L were significant at day 6 (Fig 1). The median values of DCE-MRI (Fig 2) derived parameters showed large variations (Fig 3), indicative of substantial inter-tumor differences in vascular function. Therefore, baseline measurements were used to assess potential changes in vascular status. Median K^{trans} was significantly reduced from day 2 to 6 in the treatment group. T_{peak} was prolonged by therapy from day 2, while $Slope_i$ was significantly lowered only on day 6. In the control group, the above three DCE-derived indices were the same at day 0 to 4. At day 6, median K^{trans} and $Slope_i$ were significantly reduced compared to day 0, while T_{peak} was unchanged. v_e remained constant throughout, in both groups. The comparison between longitudinal data for both groups revealed no significant PLP-L treatment effect on any vascular index. ADC and T_2 mapping were used to study the effects of PLP-L therapy on tissue status. Neither parameter was significantly altered, indicating that PLP-L caused no gross necrosis. However, the control group showed a significant reduction in ADC from day 2, suggesting that normal tumor growth was accompanied by an increase in cell density. PLP-L had no significant influence on ADC or T_2 compared to the control group. Microscopy of day 6 samples showed that average MVDs were 34 and 58 vessels/mm^2 in PLP-L treated and control tumors, resp, however, no significant difference was found (p=0.05). Microscopy provided no evidence for PLP-L induced vascular maturation.

Conclusions: The present study yields a complex picture of the effects of liposomal PLP on tumor vascular status. No significant differences in DCE-MRI indices and MVD values were observed between treated and control tumors. Nevertheless, PLP-L caused a significant reduction in K^{trans} from day 2 post-therapy and prevented the ADC reduction seen in nontreated tumors. More specific indices of PLP-L treatment are desired to identify its mechanism-of-action. Potential candidates are tumor-associated inflammatory markers.

References: [1]. Schiffelers *et al.* Neoplasia 7: 118-27, 2005; [2]. Metselaar *et al.* Arthritis Rheum 48: 2059-66, 2003; [3]. Heisen *et al.* Phys Med Biol 55: 4871-83, 2010; [4]. Tofts *et al.* JMRI 10: 223-32, 1999.