

HYPERTHERMIA IN COMBINATION WITH METRONOMIC DOSED CYCLOPHOSPHAMIDE INDUCE THROMBOSPONDIN 1 (TSP-1) IN TUMOR VASCULAR ENDOTHELIUM

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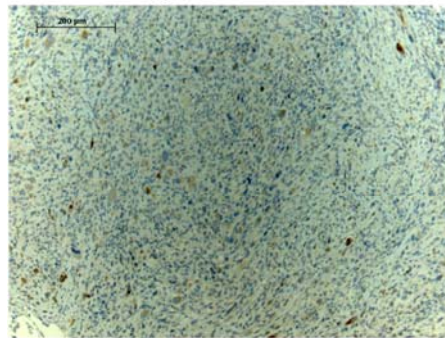
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Purpose. Metronomic dosed CTX has been shown to induce the antiangiogenic protein thrombospondin 1 (TSP-1) in endothelial cells. We have previously presented that both a metronomic dosage regimen of CTX ($p = 0.006$) and hyperthermia ($p < 0.001$), significantly delayed the time for the tumor to reach four times the initial volume in an experimental brain tumor model. A combination of the two regimens exhibited significantly better tumor control than the two modalities separately ($p < 0.001$). Complete tumor regression was seen in 6 % of the rats treated with CTX, in 12 % of the rats treated with hyperthermia alone and in 41 % of the animals treated with both CTX and hyperthermia. The objective of this study was to elucidate whether the enhanced effect observed by adding hyperthermia to metronomic dosed CTX was related to induction of TSP-1.

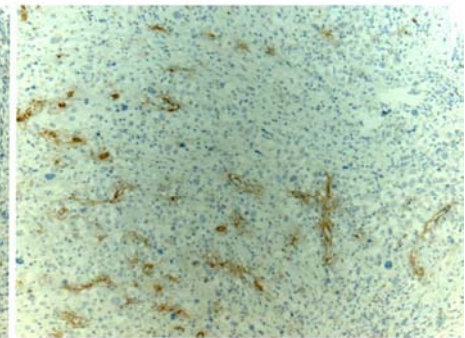
Materials and methods. We administered the combination of a metronomic regimen of the alkylating agent cyclophosphamide (CTX) combined with hyperthermia in the BT₄An aggressive rat glioblastoma-like tumor model, serially transplanted orthotopically on the right hind foot in BD IX^{HanFoss} rats. CTX was administered at doses of 35 mg/kg i.p. three times a week for two weeks and local hyperthermia at 44.1 +/- 0.1 °C was administered for one hour. From tumor samples taken within the first three hours after completed treatment day zero, we analyzed TSP-1 gene expression using RT-PCR, and TSP-1 protein by immunohistochemical (IHC) staining.

Results. IHC revealed a marked upregulation of TSP-1 in the endothelium of tumors receiving the combined treatment, and to a lesser extent in those treated with hyperthermia alone. There was no difference in the gene expression of TSP-1, but the vast majority of the cells in the samples were tumor cells, and therefore gene expression changes restricted to the endothelial cell are not detectable.

IHC staining of TSP-1



Control, 180 min



HT + CTX, 180 min

Conclusion. Metronomic dosed CTX induce TSP-1. A distinct upregulation of TSP-1 in the endothelium of tumors treated with hyperthermia combined with metronomic dosed CTX indicate that influence on TSP-1 is associated with the improved tumor response.