MRI ANALYSIS OF THE EFFECT OF HALOFUGINONE - A NOVEL ANTI-CANCER DRUG ON METASTATIC RAT BRAIN TUMOR MODEL

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Abstract:

Halofuginone (HF), is a potent specific inhibitor of collagen type α1(I) gene expression, of extracellular matrix deposition and of cell proliferation. It was published previously that HF has a potent anti-tumor and anti angiogenic effect *in-vivo,* in systemic solid tumor models. The goal of this study was to evaluate the ability of HF to inhibit tumor growth in a metastatic rat brain tumor model (sarcoma). Using MRI we show here, non-invasively, the suppressing effect of HF on tumor progression, and on tumor vascularizton.

Introduction:

Halofuginone (HF), a low molecular weight (MW=495) quinazolinone alkaloid, is a potent specific inhibitor of collagen type α1(I) gene expression, of extracellular matrix deposition and of cell proliferation. It inhibits vascular tube formation and accordingly was found to be an inhibitor of both angiogenesis and tumor growth in *in-vitro* models. *In-vivo* it has a potent anti-tumor and anti-metastatic effect in systemic solid tumor models (1,2). Brain tumors are usually aggressive and highly angiogenic. The goal of this study was to evaluate the ability of HF to inhibit tumor growth in a metastatic rat brain tumor model. By using MRI we followed non-invasively the effect of HF on tumor progression and vascularization. We demonstrate that treatment with HF reduced tumor growth and angiogenesis of an intracranially implanted malignant sarcoma in rats.

Methods:

Animal model: Fischer rats were inoculated by a stereotactic technique with 10^5 cells of malignant sarcoma (metastatic model) on day 0. HF was given orally once a day starting on day +6 (when a macroscopic tumor is already present). Two doses were evaluated: 0.2 and 0.4 mg/kg/d. On day 14 tumors were excised and their 3-dimensions were measured for calculation of tumor volume. Animals were observed for signs of toxicity and for weight loss. Survival was evaluated after randomization to treatment with either vehicle or HF.

MRI: MRI experiments were performed on a horizontal 4.7 T Bruker Biospec spectrometer, using an actively RF decoupled surface coil, 2 cm in diameter, and a bird cage transmission coil. Rats (3 rats/group) were imaged on days 12,14 and 19 after cell inoculation. Tumor volume was determined from SE images. Tumor vessel functionality (VF) and maturation (VD) were determined from GE images (TR/TE=200/13 ms; slice thickness-0.8mm; FOV=3.5cm; 256X128 pixels; 4 averages) acquired during the inhalation of air, air-CO_2, and carbogen, as described previously (3). Mean VF values were calculated from the entire tumor and were divided by the mean values from region with the same size taken at the contralateral side.

Results:

On day 14 tumors were excised (n=10 per group) and mean tumor volume of vehicle treated animals (controls) was 49.4±8.4 mm^3. Treatment with HF induced dose related inhibition of tumor growth: 88% inhibition in the 0.2 mg/kg/d dose (p=0.0005), and 94% inhibition at the dose of 0.4 mg/kg/d (p=0.0001). All animals lost weight during the observation period. Weight loss ranged between 9-13% of initial body weight and did not differ significantly controls and HF treated animals. No other drug related toxicity was observed. HF treatment significantly prolonged survival with increase in life span of treated animals at doses of 0.2 and 0.4 mg/kg/d (142% for 0.4 mg/kg dose; p=0.001).

MRI analysis confirmed the delay in tumor growth in HF (0.4 mg/kg/d) treated rats in comparison to control rats (Figure 1). This was accompanied by a lower vascularization in HF treated tumors as determined from VF maps (Fig.2). In HF treated rats tumor vascularization was inhibited by 40% on day 14 and by 47% on day19 (Figure 2; VF).

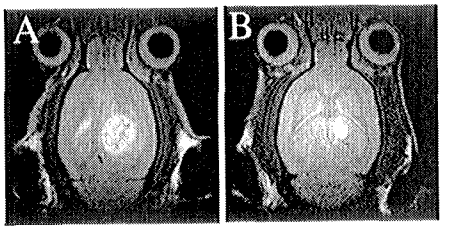

Figure 1: Sarcoma tumors in rat brain on day 14 after cell inoculation. Coronal SE image of (Left) control rat and (Right) HF treated rat.

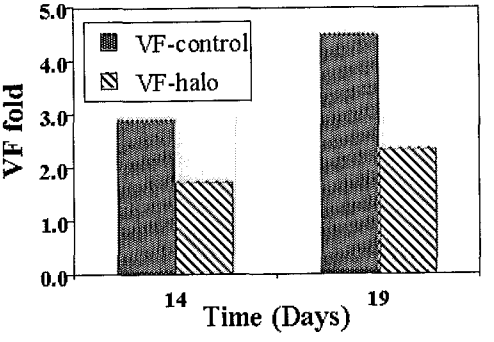

Figure 2: Tumor vessel functionality (VF) normalized to contralateral region, as a function of time after cell inoculation.

Conclusions:

HF, as a novel inhibitor of angiogenesis, is effective as a single modality therapy in metastatic brain tumors. Growth inhibition is dose dependent, and could be demonstrated even when treatment is given at a time that a macroscopic tumor mass has already developed. HF significantly prolonged rat survival. From MRI analysis we show here that HF significantly inhibits tumor growth and reduces tumor angiogenesis.

References:

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