# Thermosensitizer for Hyperthermic Treatment of Tumors

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**Statement of Purpose:** Local or regional hyperthermia has been used clinically for many years in the treatment of cancer. Mild regional heat has shown synergistic effects with chemotherapy and radiation, and focused energy via methods such as radiofrequency ablation (RFA) has been a successful targeted tumor treatment. Although effective, the approach can often be incomplete due to recovery of cells from heat shock. Thus, the goal of this project was to develop a thermosensitizing technique that would make cancerous cells more susceptible to heat-related injury and improve the outcome of focused hyperthermia in the form of RF ablation.

**Keywords:** Hyperthermia, thermosensitizer

**Methods:** DHD/K12/TRb rat colorectal carcinoma cells, which originated from dimethylhydrazine-induced colon adenocarcinoma in BDIX rats, were exposed to 0, 1 and 7 % (w/w) sensitizer solution under 37 or 43ºC heat in a circulating water bath for 30, or 60 min. In order to see if heat injured cells were able to recover and proliferate, a clonogenic survival assay was conducted. The number of colonies was counted after cells were fixed with methanol and stained with Giemsa and May-Grünwald stains. To test the heat sensitizing effects *in vivo*, BD-IX rats inoculated with bilateral subcutaneous tumors via injection of cell suspension were treated with intralesional sensitizer (28.1 mg/kg), followed by radiofrequency ablation at 80ºC for 2 min (n=6) 15 min later. In another treatment group the same agent was given via IV (3.3 mg/kg) and ablated 72±7min later (n=7). Finally, a group of rats received RFA alone at 90ºC for 3 min (n=14). Tumor diameters were measured with calipers weekly for two weeks. Treatment efficacy was assessed using % of tumor volume changes and coagulation necrosis. Statistical analysis for the clonogenic survival assay was done with the Tukey multiple-comparison test; otherwise it was done with two-tailed unpaired student T-test (*$P \leq 0.05$ is defined significant). Data are presented as mean or mean ± SEM

**Results/Discussion:** *In vitro* results from the clonogenic survival assay (Figure1)demonstrated that with the sensitizer, cell proliferation was significantly reduced in a time dependent manner compared to control with 33% reduction at 37ºC for 60 min when exposed to 1% sensitizer and 46% reduction for the 7% sensitizer exposure. Most importantly, at 43ºC the agent caused significantly less colony formation compared to control (84% reduction for 1% and 94% reduction for 7% after 30min exposure time; while under the same temperature, 60min exposure time, 1% solution caused 92% reduction and 7% caused 100% reduction). Morphology of stained cells suggested the agent induced permanent injury to cells upon hyperthermia. *In vivo,* tumor volume changes (Figure2) indicate that treatment with the sensitizer administered either IV (tumor volume decreased by 36.8±25.6%) or locally (reduction of 40.3±27.7%) resulted in significant tumor regression compared to tumors receiving RFA-only (volume increased by 92.0±39.4%). These results suggest that in the presence of the sensitizing agent, cancer cells are more susceptible to permanent heat injury. *In vivo* results have also indicated that in the presence of this agent, inflammatory effects upon injury may be reduced. The results have led to our further hypothesis that the underlying thermal sensitization mechanism may be mediated by an alteration of the function or the production of heat shock proteins. Currently, studies are underway to investigate this mechanism.

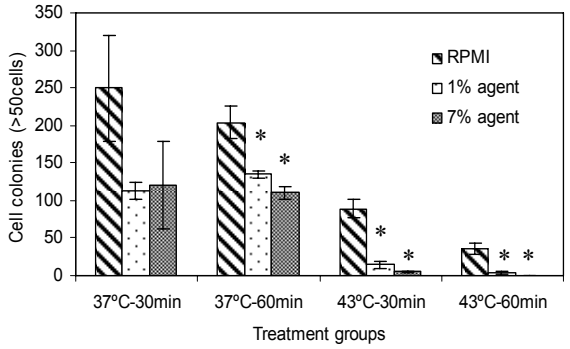


**Figure1.** Results of clonogenic survival assay (*indicates significance ($p<0.05$) compared to RPMI control)

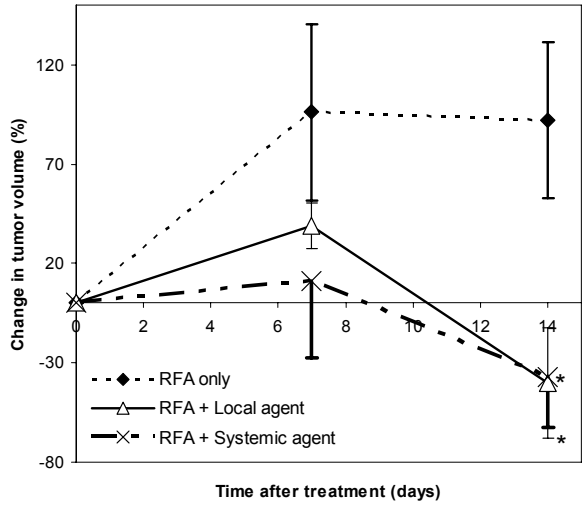


**Figure2.** *In vivo* % tumor volume changes (* $P<0.05$ compared to control).

**Conclusions:** Results conclusively indicate that the agent evaluated in this study can sensitize cancer cells to heat both *in vitro* and *in vivo*, and can be potentially applied clinically for ensuring a complete eradication of cancers with hyperthermia treatment.

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