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Nanoparticle Enhanced Thermal Therapies

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Abstract—**Thermal therapies such as hyperthermia, radiofrequency ablation, cryoablation, etc. have shown great potential and are gaining increasing clinical acceptance in the treatment of solid tumors. However, these treatment modalities are limited by the size of tumor that can be treated, incomplete tumor kill, and damage to adjacent normal tissues. To address these limitations, the concept of adjuvant-assisted thermal therapies has been proposed and tested to enhance the tumor destructive effects of thermal therapies. CYT-6091, a pegylated colloidal gold nanoparticle containing TNF-alpha bound to its surface, has been extensively investigated in our lab as an adjuvant to enhance thermal therapies. This paper describes our investigations of nanoparticle enhanced thermal therapies in various preclinical and translational models of solid tumors.**

I. Introduction

THERMAL therapy involves the transfer of heat energy into or out of bodily tissues to achieve a therapeutic result. In cancer treatment, thermal therapies are mainly used for eliminating solid tumors. Currently, there is tremendous interest in combinatorial therapies involving the administration of molecular adjuvants during or prior to thermal therapy for improved solid tumor destruction. Among the various adjuvants that have been tested, anti-angiogenic molecules targeting the tumor vasculature have had the most success in accentuating the thermal injury. Tumor necrosis factor alpha (TNF) is a multifunctional cytokine known for its antitumor properties. The current clinical use of TNF in cancer is limited to the regional treatment of locally advanced soft tissue sarcomas and metastatic melanomas and other unresectable tumors of any histology to avoid amputation of the limb [1]. TNF targets the tumor vasculature by inducing hyperpermeability and destruction of the vascular lining. The major drawback of this therapy is the systemic toxicity of TNF when administered at the required high doses for antitumor effects [2]. Thus, there is a need for achieving high local concentrations of TNF within the tumor while limiting systemic toxicity. Nanoparticles are an ideal delivery system for targeting TNF or any therapeutic small molecule to the tumor.

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Targeting cancer therapeutics to solid tumors is facilitated by nanoparticle (usually 20 – 100 nm) delivery systems capable of escaping phagocytic clearance by the reticuloendothelial system (RES) [3]. By design such delivery systems preferentially extravasate the tumor vasculature to accumulate drug within the tumor microenvironment while concomitantly reducing the accumulation of the drug in healthy organs [4, 5]. Consequently, these delivery systems may increase the relative efficacy or safety of a cancer therapy, and thus serve to increase the drug's therapeutic index.

CYT-6091 [6] is a multivalent drug that is assembled on nanoparticles of colloidal gold and designed to actively sequester recombinant human tumor necrosis factor alpha within solid tumors. For CYT-6091, receptor targeting is facilitated since TNF acts as both the ligand and the therapeutic. The drug is manufactured by covalently linking molecules of TNF and thiol-derivatized polyethylene glycol (PEG-THIOL) onto the surface of the colloidal gold particles. Each component of the multivalent drug serves a specific function to facilitate tumor specific drug delivery. The PEG-THIOL moiety serves to hydrate the colloidal gold nanoparticles and in doing so, shields the nanoparticle drug from detection and clearance by the RES. Unlike similar work in the area of laser technology, the gold nanoparticle in our work does not directly enhance the heating or cooling (thermal) effects of the therapy but rather enhances tumor destructive effects through the biological action of TNF on the tumor vasculature.

We have worked with several tumor models to determine the enhancement of the thermal injury zone within the tumor tissue as well as to understand the biodistribution and toxicity associated with nanoparticle usage.

II. Preclinical models

A. Hyperthermia - Preclinical

Previous work in our lab by Visaria et al [7] demonstrated that CYT-6091 in combination with hyperthermia significantly delayed tumor growth, reduced tumor cell survival, and reduced tumor blood perfusion in a hind limb tumor model of SCK murine mammary carcinoma. SCK mammary carcinomas grown in A/J mice were treated with 125 or 250 ug/kg CYT-6091 alone or followed by local heating at 42.5^{o}C using a water bath for 60 minutes, 4 hours after nanoparticle injection. Increases in tumor growth delay were observed for both CYT-6091 alone and heat alone, although the most dramatic effect was found in the

combination treatment. Tumor blood flow was significantly suppressed 4 hours after an intravenous injection of native TNF or CYT-6091. Tumor perfusion, imaged by contrast enhanced ultrasonography, on days 1 and 5 after treatment revealed perfusion defects after the injection of CYT-6091 alone and, in many regions, complete flow inhibition in tumors treated with combination treatment. The combination treatment of SCK tumors in vivo reduced the in vivo/in vitro tumor cell survival to 0.05% immediately following heating and to 0.005% at 18 hours after heating, suggesting vascular damage–mediated tumor cell killing. Thermally induced tumor growth delay was enhanced by pretreatment with TNF-coated gold nanoparticles (CYT-6091) when given intravenously at the proper dosage and timing. Figures 1 & 2 depict results obtained in a murine fibrosarcoma hind limb tumor model.

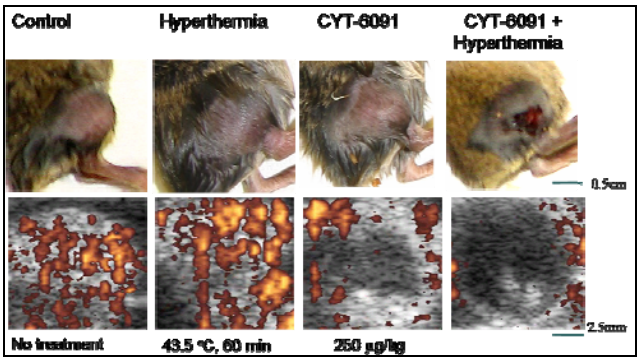

Fig. 1. Representative images of murine fibrosarcoma bearing hind limb of C3H mice and the corresponding tumor perfusion defects imaged using contrast enhanced ultrasonography, 7 days post treatments [8].

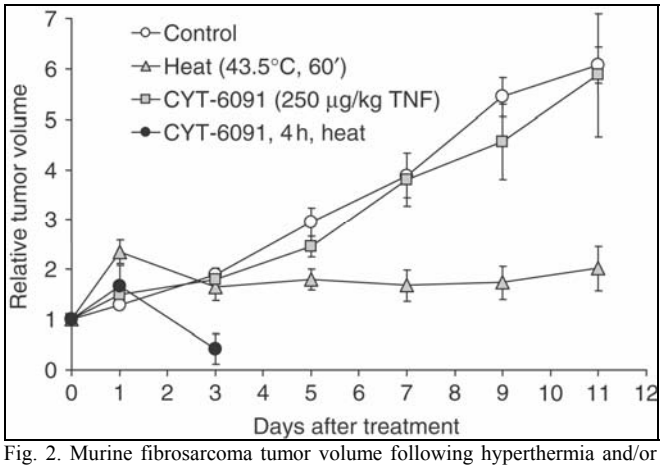

Fig. 2. Murine fibrosarcoma tumor volume following hyperthermia and/or CYT-6091 treatments. Results are expressed as mean ± SEM of 5-6 animals and normalized to pretreatment volumes [8].

B. Cryoablation - Preclinical

We have worked with a number of preclinical tumor models to document the enhancement of cryosurgical injury by CYT-6091. In the dorsal skin fold chamber (DSFC) and hind limb models we have shown the ability to destroy all LNCaP tumors within a cryosurgical iceball after topical or intravenous administration of native TNF and CYT-6091 (Fig. 3) [9].

Importantly, we observed that approximately 40% of the mice injected intravenously with a TNF dose sufficient to enhance cryoinjury died due to systemic toxicity whereas no systemic toxicity was observed with CYT-6091 administration (Fig. 4). Thus, the nanoparticle allowed us to deliver the same dose of TNF required to enhance cryoinjury without systemic toxicity.

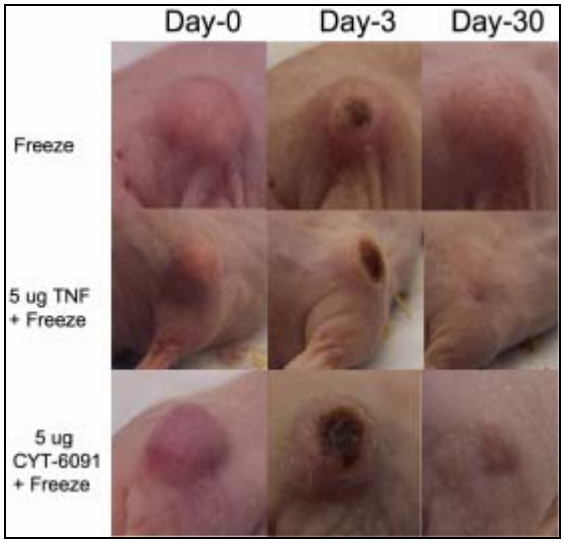

Fig. 3. Enhanced destruction, including complete remission, in an LNCaP mouse hind limb tumor model of prostate cancer by administration of native TNF and CYT-6091 4 hrs prior to freezing to the visible edge of the tumor [9].

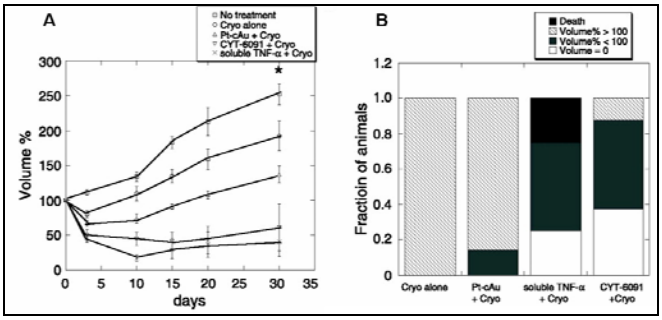

Fig. 4. Injury response of TNF-enhanced cryosurgery in the hind limb model. A. growth delay plot of hind limb tumor for cryosurgery with and without TNF pretreatment. A dose of 5 µg of soluble TNF or CYT-6091 was injected intravenously 4 hrs before cryotreatment. B. normalized response at day 30 after cryosurgery for various treatments. *Volume = 0,* no palpable tumor on the overlying skin. Animals reported as dead were found within a few hours after cryosurgery [9].

III. TRANSLATIONAL MODELS

Verification of the nanoparticle enhancement of thermal therapies in a translational model is necessary before clinical acceptance of the therapy. Our initial translational studies were conducted using VX2 tumors grown in rabbit kidneys as a model for renal cell carcinoma. No other model of renal cell carcinoma exists in an animal of sufficient size to be considered translational.

A. *Radiofrequency Ablation - Translational*

Administering CYT-6091 4 hours prior to radiofrequency ablation (RFA) yielded a significantly larger zone of central necrosis than RFA treatment alone on histological evaluation 3 days after the procedure. Overall, a 23% increase in ablation volume was obtained. This difference in ablation size was due to a replacement of partially ablated tissue at the periphery in the RFA only group by completely ablated tissue in the RFA plus CYT-6091 group. Control experiments with no RFA also showed a significant reduction in tumor volume after CYT-6091 treatment [10]. No metastases was observed in either group.

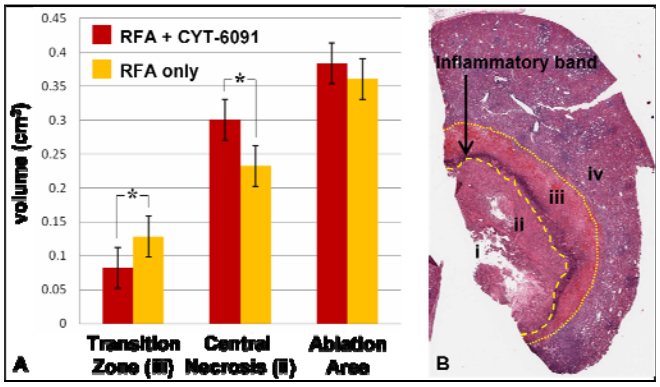

Fig. 5. A. Comparison of histological zones of injury after RFA treatment with and without CYT-6091 administration 4 hours prior to tumor ablation. B. Representative histological section of bivalved kidney treated with RFA showing (i) location of RF probe tract, (ii) central necrosis zone, (iii) transition zone, (iv) viable kidney tissue [10].

B. *Cryoablation - Translational*

We were also interested in discovering if the enhancement of the ablation size can be replicated using cryoablation in the same translational model. Cryoablation was achieved using the Seednet system (Galil Medical Inc., Yokneam, Israel) 1.58 mm diameter cryoprobes (IceRodTM, 15mm active region at tip) inserted directly into the tumor mass. Freezing was done until the iceball completely covered the tumor. Gross pathology of the cryotreated kidney tumors at day 3 revealed cystic, hemorrhagic lesions containing tissue that was inconsistent and falling apart easily. This precluded accurate sectioning of tissue for histological assessment and the day 7 time point was chosen for histological assessment. Excessive tumor growth into the ablation lesion at day 7 following cryoablation prevented accurate measurements in these groups; however, a *significant decrease in the rate of peritoneal carcinomatosis* (metastases) was obtained in the cryo plus CYT-6091 group compared to the cryoablation alone group (1/10 vs. 8/10, p=0.04) [11]. This was an unexpected, serendipitous finding and we are going back to preclinical mouse models to investigate this further.

IV. Summary and Future Directions

This work provided some of the first evidence for the enhancement of thermal therapies using a metallic nanoparticle drug delivery system in both pre-clinical and translational models. This work adds to the existing and evolving knowledge base for nanoparticle based thermal therapy adjuvants [12-15].

Although the concept of using nanoparticles and their adjuvant cargo to enhance thermal therapies is well established, relatively few have made it to clinical trials. This is in part due to a lack of basic science research in the field. Some of the key challenges for researchers in the field are: 1) selection of the appropriate nanoparticle and molecular adjuvant for the specific tumor type, 2) demonstration of safety and efficacy, and 3) timing of the nanoparticle administration prior to thermal therapy. Studies to address these challenges need to be carried out in robust preclinical and translational models to demonstrate safety and enhancement of the tumor destructive ability of the thermal therapy.

The rapid growth and advancement of nanoparticle technologies can allow these studies to provide added peri- and post-operative benefits such as improved real-time intraprocedural imaging of the tumor "kill zone", monitoring of local tumor recurrence, etc. Specifically, new advances in the nanotechnology field allows the design of multifunctional nanoparticles with multiple molecular adjuvants tagged onto a single nanoparticle and/or the design of magnetic resonance sensitive particles for visualization and localization within the tumor. Active collaboration between the nanoparticle research community and thermal therapy interest groups are required to achieve significant breakthroughs in the translation of these combinatorial antitumor therapies into the clinic.

Acknowledgment

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