Gold Nanoparticle-Assisted Delivery of TNF-α in Thermal Treatments of Cancer

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Abstract:

Gold nanoparticles are seen as a promising drug carrier for anti-cancer treatments because of their size, non-toxicity, high binding capacity, inertness and stability in the human body. The effects of the gold nanoparticle-based delivery of the inflammatory cytokine, TNF-α, to breast cancer and prostate cancer cells in combination with freezing and laser-induced heating were examined. Specifically, cell viability, clonogenicity, and temperature changes were studied.

Introduction:

Gold nanoparticles are considered potential anti-cancer drug carriers for a number of reasons. Gold is non-toxic, inert, stable, and has a high binding capacity [1]; furthermore, the nanoparticles, at 33 nm in diameter, are suitably sized for laser heating [4] as well as passive uptake by the leaky vasculature of tumor tissue [1]. Gold consequently should be able to deliver drugs selectively to cancer cells. In this research, we investigated gold's potential to deliver the inflammatory cytokine tumor necrosis factor-alpha (TNF-α) to augment thermal injury selectively in tumor cells. Both freezing and laser heating were used to induce thermal injury.

TNF-α triggers an inflammatory response, causing injury both to the cells directly and the blood vessels providing them nourishment [3]. By administrating the drug using gold as a carrier, the cancer cells should become more sensitive to the thermal treatments so that less extreme parameters will be necessary, minimizing damage to the healthy tissue surrounding the tumor. To determine the effectiveness of gold nanoparticle-assisted delivery of TNF-α in conjunction with thermal treatments, two different cell types were examined. Prostate cancer cells (LNCaPs) in suspension were used for studying viability after freezing and laser heating. Breast cancer cells (SCKs) as monolayers were used for studying the effect of freezing on clonogenicity.

Experimental Procedure:

LNCaPs Viability Assay with Freezing

LNCaPs were cultured in media as previously reported [2]. 1 μg/ml of native gold or gold-TNF-α was added to fresh media. Cells were incubated at 37°C with the treated media for 4 hours. Cells were detached and suspended in media. The LNCaPs were then frozen at an end temperature (ET) of -10 or –20°C using the directional solidification stage (DSS) with no hold time (HT). Viability was measured by incubating the cells with a Hoechst-propidium iodide (PI) dye for 15 minutes, after which the number of live and dead cells was counted under a fluorescent light microscope.

SCK Clonogenic Assay with Freezing

SCK cells were plated and incubated for 24 hours before being cultured with treated media (1 μg/ml of gold, gold-TNF-α, or TNF-α) for 4 hours. The treated media was then replaced with fresh media and the cells were frozen using the ethanol bath at ET –10°C with zero HT. The cells were allowed to thaw for 15 minutes at room temperature. The flasks were then left in the 37°C incubator for a week, after which the cells were stained with crystal violet and colonies were counted.

LNCaPs Temperature and Viability Assay with Laser Heating

LNCaPs were incubated at 37°C with 3.33 μg/ml Au-TNF-α-treated media for 24 hours. The cells were trypsinized from the flasks, counted, and then diluted in media until a 100 μl sample contained ~1 million cells. The samples were then irradiated for varying durations (45-75s) with a laser (532 nm, Nd:YAG) at 20 Hz corresponding to an energy density of ~ 0.4 J/cm^2. Temperature was measured before and after laser treatment using a thermocouple. Viability was also assayed before and after treatment using the Hoechst-PI dye.

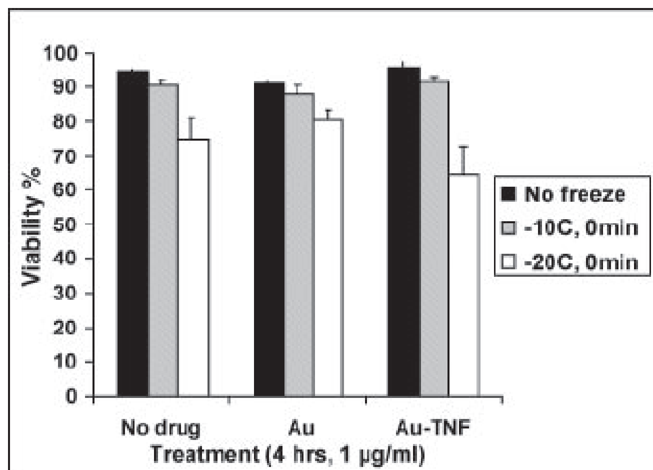

Figure 1: Viability of freeze-treated LNCaPs (N=2-6).

Results and Conclusions:

The viability results for the freeze-treated LNCaPs are detailed in Figure 1. Lower ET decreased viability. The results for the control LNCaPs and the gold-treated LNCaPs are not significantly different, confirming that native gold is non-toxic. In contrast, the LNCaPs treated with gold-TNF-α at -20°C had lower viability than the LNCaPs in the other drug treatment groups.

The SCK clonogenics data is depicted in Figure 2. Freezing significantly lowered SCK survival. The results for the control and gold-treated cells were similar, again indicating that the gold itself is not cytotoxic. Cells treated with gold-TNF-α had the lowest viability, suggesting that the gold is improving TNF-α uptake by the SCKs.

Figures 3 and 4 illustrate the results for the laser heating work. The gold-TNF-α-treated cells overall were more responsive to the heating treatment, experiencing a greater change in temperature and having lower viability than the control cells under the same laser parameters.

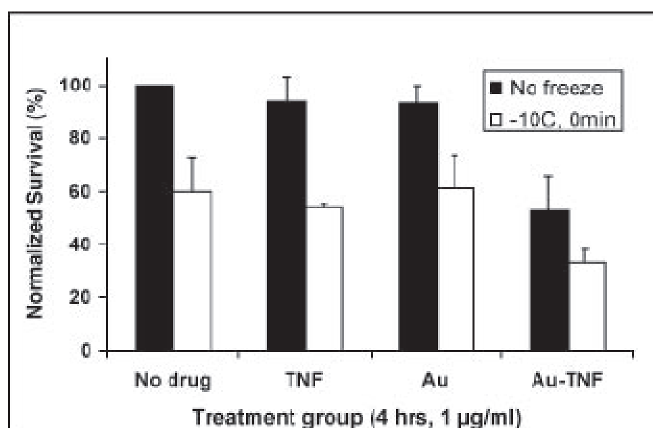

Figure 2: Freeze-treated SCK clonogenics (N=7).

Lengthening the duration of the lasing treatment generally resulted in lower viabilities and greater temperature changes, with an exception occurring for the viability of the gold-treated cells with 45s laser treatment. More experiments should be performed since the data reported is only for N = 1.

Future Work:

Further work on optimizing the freezing parameters and drug dosage by evaluating cell viability in suspension can be performed. More runs for the laser heating experiments should be performed. The effectiveness of laser treatment on different cell types in suspension with varying parameters could also be analyzed before work is moved on to systems *in vivo*.

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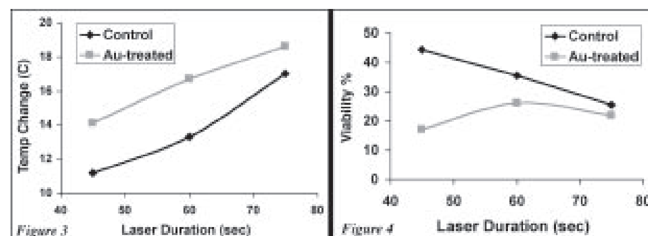

Figure 3, above left: Temperature change of laser-heated LNCaPs (N=1). Figure 4, above right: Viability of laser-heated LNCaPs (N=1).