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Quantification of Doxorubicin Concentration in Rat Tissues using Polymeric Micelles in Ultrasonic-Drug Delivery

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Introduction

The triblock copolymer, Pluronic P105, has been found to be an ideal ultrasonically activated drug delivery vehicle because it forms micelles with hydrophobic polypropylene oxide cores that sequester hydrophobic drugs (Fig. 1). These micelles release their contents upon the application of low frequency ultrasound [1] such that drugs can be released specifically at the ultrasonicated region (Fig. 2). Such ultrasonically controlled release has been effective against cancer cells in vitro [2] and in vivo [3].

The purpose of this research is to assess the use of these novel polymeric micelles in ultrasonically-activated Doxorubicin® (DOX) delivery to tumors. This cancer therapy involves the exposure of the animal to localized ultrasound. Currently, one of the most effective therapies for cancer treatment involves the use of chemotherapeutic agents such as DOX. One of the major drawbacks of this therapy is that the drug attacks all rapidly dividing cells, causing healthy tissues to die. These localized treatment using micelles and ultrasound may alleviate the negative side effects of the drug on healthy tissues. Encapsulation prevents drug interaction with cells. Localized release of DOX by ultrasound limits the areas within the patient where the drug can take effect. While cell viability studies have been performed using this delivery system [2], no research has been performed in vivo to measure the pharmacokinetics of DOX using ultrasonic-drug delivery with micelles. This project's goal is to quantitatively measure the drug concentration profile with time in various rat tissues, including induced tumors; to determine the concentration-time difference (if any) between ultrasonicated and non-ultrasonicated tumors; and to determine if there is drug accumulation in the studied tissues.

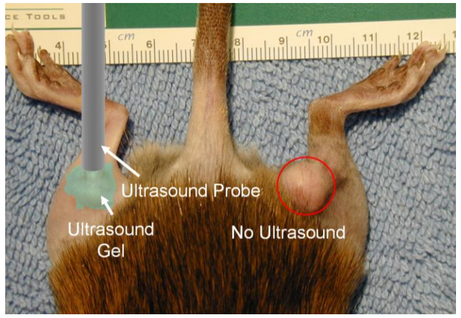

Figure 3. Rat with subcutaneous tumors grown on each leg. Ultrasound (20 kHz, 1 W/cm^2) was applied to one of the tumors and the other was the control.

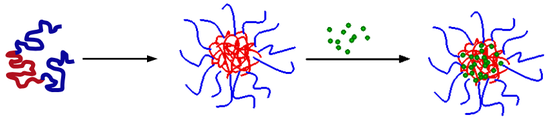

Figure 1. Pluronic P105 forms dense micelles whose hydrophobic core readily sequesters hydrophobic chemotherapeutic drugs.

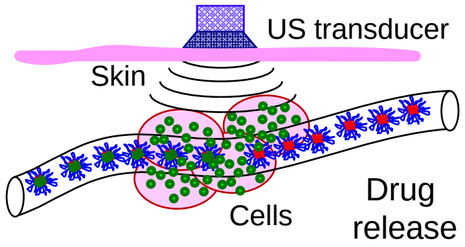

Figure 2. Micelles release the encapsulated drug at the targeted tissue upon application of ultrasound then quickly reform.

Methods

The drug carrying micelles were formed from Pluronic™ P105, a tri-block copolymer consisting of a central block of poly(propylene oxide) flanked by blocks of poly(ethylene oxide). These micelles were stabilized by polymerizing an interpenetrating network of a thermally responsive N,N-dimethylacrylamide within the core of the micelle [4]. DHD/K12/TRb rat colonic cancer cells were subcutaneously injected and grown in each lower leg of the BIDX rat model. All rats received an injection of micellar-encapsulated Dox at 2.67 mg/kg. Ultrasound exposure followed for a period of fifteen minutes to only one leg of the animal. Ultrasound was applied by a 20 kHz probe (1.0 W/cm2) in ultrasound-conducting gel on the skin over the tumor (Figure 3). Each rat was euthanized at 0.5, 1, 6, 12, 24, 48, 96, or 168 hours after ultrasound application. Some rats were given the drug/ultrasound treatment for four consecutive weeks before being euthanized 6 hours after the last treatment in order to test for accumulation effects. After euthanization, DOX was immediately extracted from heart, muscle, liver, and tumor tissue (both ultrasonically treated and untreated) and quantified using high-performance liquid chromatography and a fluorescence detector (Figure 4).

Results

The analysis of the drug concentration/time profile in liver, heart, leg muscle, and tumor tissues (Figures 5-7) showed an exponential decrease in the amount of DOX in the liver and heart. Initially, the majority of the drug was found in the liver and heart (3.3 mg-DOX/g-tissue and 4.3 mg/g, respectively) with almost complete drug clearance in the liver (0.01mg/g) within 24 hours after drug administration. The clearance in the heart was slower than the liver, but complete removal was observed one week after administration (Figure 7). The drug concentration in muscle remained constant between 1 and 12 hours (0.8mg/g) and dropped to 0.2mg/g in 48 hours. Accumulation studies showed no significant accumulation of DOX in the liver, muscle, tumor tissues, or the heart.

Comparing average doxorubicin concentration in solid tumors which did and did not receive ultrasound treatment (Figure 5) showed an increase in drug concentration in the treated tumor 30 minutes after injection, (ten minutes after ultrasound treatment); the concentration was almost twice that of the untreated tumor. After twelve hours, the drug concentration in the two tumors essentially became the same, which decreased linearly after two days. Drug still remained in the tumors one week after injection.

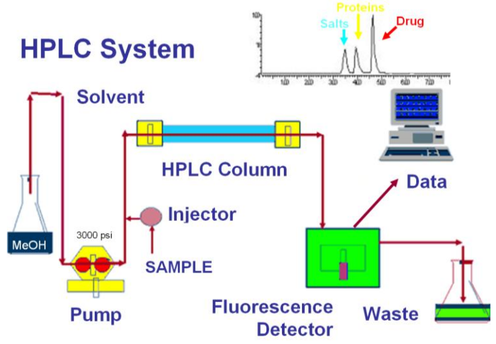

Figure 4. High Performance Liquid Chromatography Setup used to separate and quantify the amount of doxorubicin in a sample.

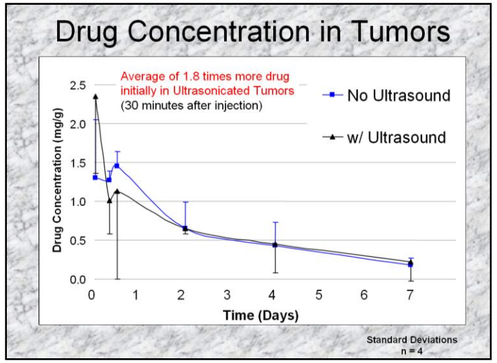

Figure 5. Comparing average doxorubicin concentration in solid tumors over a period of one week. Tumors which received ultrasound following the carrier injections showed almost twice as much drug as non-ultrasonicated tumors within the first thirty minutes.

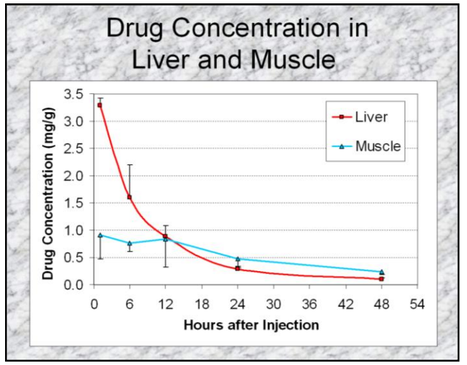

Figure 6. Mass of doxorubicin per mass of tissue over a period of one week for liver and muscle tissues. The liver showed high initial concentrations and rapid removal while the drug slightly entered the muscle tissues and slowly cleared.

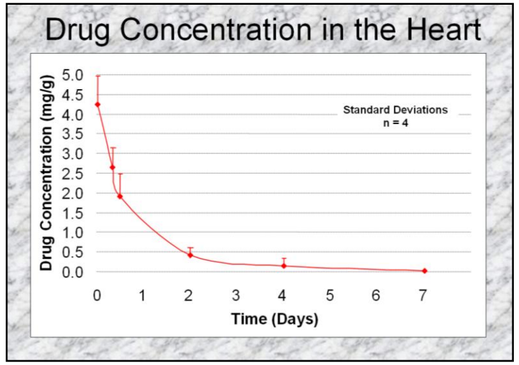

Figure 7. Mass of doxorubicin per mass of heart tissue over a period of one week. The drug is rapidly cleared from heart tissue and is completely removed one week after injection. This is important in order to minimize (or eliminate) the cardiotoxic effects of the drug.

References

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Conclusions

The drug is initially at higher concentrations in the blood-perfused oragans such as the liver and heart. However, the drug is quickly removed from these organs. Though the concentration of the drug in the tumors is initially lower than in the highly vascular organs, the drug is slowly cleared, leaving a measurable amount even after one week. While longer retention of drug in the tumor cells is effective in chemotherapy, it is detrimental in the heart. The micelles appear to protect the heart from long-term drug exposure.

The results from comparing doxorubicin concentration in ultrasonically-treated tumors to that in non-treated tumors show that ultrasound increases the amount of drug delivered to the tumor soon after its application. It is hypothesized that this is due to ultrasonically-increased membrane permeability.