**Doxorubicin delivery to rat tissues using polymeric micelles and ultrasound**

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**Statement of Purpose:** The purpose of this research is to assess the use novel polymeric micelles in ultrasonically-activated Doxorubicin® (DOX) delivery to tumors. This cancer therapy involves the exposure of the animal to localized ultrasound to investigate this novel drug delivery system. 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 rapidly dividing cells, causing some healthy tissue to die and making the patient ill. In order to alleviate these negative side effects of the drug, a novel drug delivery system which encapsulates doxorubicin within polymer micelles is being studied. Encapsulation prevents drug interaction with normal, healthy cells. Ultrasound can trigger the release of the drug from the micelles at a specific site. Localized release of doxorubicin limits the areas within the patient where the drug can take effect. While cell viability studies have been performed using this delivery system[1], 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.

**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[2]. DHD/K12/TRb rat colonic cancer cells were subcutaneously injected and grown in each upper 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/cm$^2$) in ultrasound-conducting gel on the skin over the tumor. Each rat was euthanized at 1, 6, 12, 24, or 48 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 and quantified using high-performance liquid chromatography and a fluorescence detector.

**Results / Discussion:** The analysis of the drug concentration/time profile in liver, heart, leg muscle, and tumor (leg) tissues (Figure 1) 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 2.4 mg/g, respectively) with almost complete drug clearance in the liver (0.01mg/g) within 24 hours after ultrasound application. However, the clearance in the heart was much slower (0.8mg/g at 24 hrs, 0.4mg/g at 48 hours). The drug concentration in muscle remained constant between 1 and 12 hours (0.8mg/g) and dropped to 0.2mg/g in 48 hours whereas the tumor concentration remained constant between 1 and 24 hours after ultrasound application (0.9mg/g) and dropped to 0.4mg/g at 48 hours, the same concentration as in the heart.

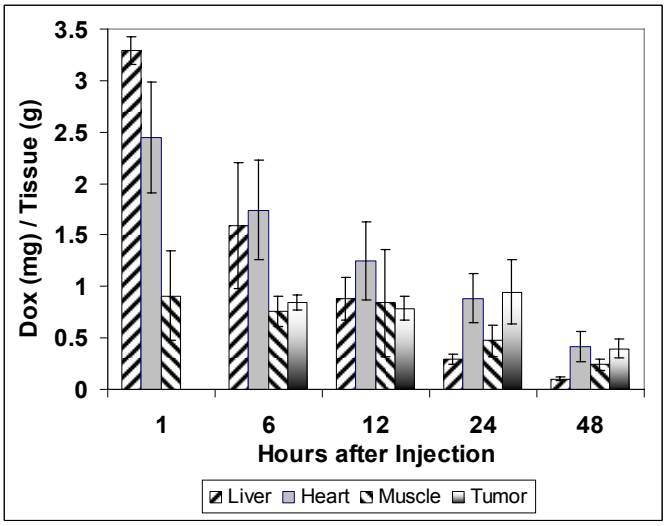


**Figure 1. Concentration of doxorubicin (mg/g) in four rat tissues (liver, heart, leg muscle, and tumor) at 1, 6, 12, 24, and 48 hours after ultrasound application to the tumor.**

Accumulation studies showed no significant accumulation of DOX in the liver, muscle, or tumor tissues, but showed possible significance (p=0.08) in the heart.

**Conclusions:** The results show that the drug is quickly removed from the liver, but remains longer in the heart, leg muscle, and tumor. Due to the rapid drug removal from the liver and minimal retention of the drug in the leg muscles, further studies with these tissues are unnecessary. While longer retention of drug in the tumor cells is effective in chemotherapy, it is detrimental in the heart. In addition, the accumulation of drug in the heart and not in the tumor causes further concern. Studies are currently being performed to measure the difference between drug amounts in ultrasonicated verses non-ultrasonicated tumors.

**References:**

1. Husseini GA. Cancer Lett 2000; 154:211-6
2. Pruitt JD. Macromolecules 2000; 33 (25): 9306-9