# Evaluation of Eventual Risks of Unseeded Inertial Cavitation for Enhancing the Delivery of Chemotherapies

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***Abstract*** ***Acoustic cavitation is widely used to improve drug delivery in tumors. In this study, we evaluate the safety of using unseeded inertial cavitation (without external nucleation agents) for this application. We show that unseeded inertial cavitation (UIC) does not degrade doxorubicin molecules or alter its cytotoxicity. Moreover, histopathologic analysis shows only slight or reversible moderate effects on healthy tissues. Finally, UIC does not promote metastatic spreading. Thus, the particular conditions used in this study provide the possibility of safe UIC concerning these three points.***

***Index terms - Biophysics, Therapy monitoring, Ultrasound.***

## I. INTRODUCTION

Local drug delivery in tumors can be achieved with acoustic cavitation. While microbubbles are widely used to act as cavitation nuclei, this study focuses on unseeded inertial cavitation (UIC). This requires high pressure levels, beyond ten megapascals. Acoustic cavitation can act at several levels in tissues. Indeed cavitation can induce pores in cells [1] or damaging them [2]. Tissues can also be damaged. This is even the aim of histotripsy which disrupt tissues with cavitation [3]. At the tumor level, perfusion can be affected [4]. Studies also report metastatic spreading due to ultrasound [5]. Thus, the sine qua non of using UIC is to assess its safety. Firstly, effects on the drug molecular structure and cytotoxicity were assessed. Then, we evaluated damages on healthy tissues. Finally, we verified the absence of metastatic spreading.

## II. MATERIALS AND METHODS

### II.1. Ultrasonic setup

The ultrasonic setup is composed of two confocal transducers. The selected conditions consisted in 40-ms pulses of 1-MHz center frequency with 250-Hz pulse repetition frequency. At the focal point, the peak negative pressure (p-) is 13 MPa and the peak positive pressure (p+) is 20.5 MPa. The setup allows cavitation occurrence of cavitation both *in vitro* and *in vivo* in shallow tissues.

### II.2. Drug integrity

Eventual effects on drug molecular structure are evaluated by liquid chromatography and mass spectrometry (LC-MS) analysis. The cytotoxicity is evaluated by exposing MDA-MB231 carcinoma cells to sonicated or not doxorubicin solutions for various doxorubicin concentrations between 0.01 and 3 µM. MTT assays are performed to evaluate the resulting cytotoxicity.

### II.3. Evaluation of toxicity on healthy tissues

Healthy rats were sonicated in the thigh (skin, muscle, nerve, bone) and in the liver (vein). Thirteen rats were used. Six were euthanized 72h post-ultrasound and the seven remaining 2 weeks post-US. Histopathologic analyses were performed by independent histopathologists (NAMSA, Chassieu-sur-Rhône, France). Injuries were scored between 0 (no damage) and 4 (severe damages).

### II.4. Test on metastatic tumors

20 Balb/c mice were injected with 4T1 syngenic mammary carcinoma cells and divided in two groups. At D15, one group received an US treatment. At D25, mice were euthanized and tumors were collected. Lung and bone marrow were also collected, allowing the metastasis to be counted.

## III. RESULTS

### II.2. Drug integrity

Both sonicated and non-sonicated doxorubicin samples have a retention time of 2.99 minutes. The corresponding molecular mass is 543g/mol for both solutions. Thus we can assume that doxorubicin molecules are not degraded.
For the three doxorubicin concentrations that induced cytotoxicity (0.3, 1 and 3 mM), the percentages of difference of cytotoxicity between non-sonicated doxorubicine and sonicated doxorubicin are respectively 5.39 %, 2.91% and -2.13%. These values are in the range of the standard deviation observed in this experiment. Thus, we can consider that the sonications did not alter the doxorubicin samples.

### II.2. **Evaluation of toxicity on healthy tissues**

Histopathologic analysis revealed that no severe damage was observed on healthy tissues. Most of the observations consisted in reversible moderate damages to the skin (crust). Only negligible findings were observed on other tissues (muscle, nerve, bone and vein).

### II.2. **Test on metastatic tumors**

One non-treated mouse died during the experiment. In the non-sonicated group, 3 out of 9 mice (33%) had medullary metastasis and 7 of them (78%) had lung metastasis. In the US-treated group, these proportions were respectively 20% and 40%.

## IV. **DISCUSSION CONCLUSION**

In this study, we evaluated eventual risks of using unseeded inertial cavitation for delivering doxorubicin in tumors. Firstly, the effect of doxorubicin does not appear to be degraded by US exposures. Doxorubicin is a relatively small molecule is not representative of all drugs used in oncology. However, it is widely used in cancer therapy.
Secondly, we showed that UIC only induced slight or reversible injuries. These injuries were mostly on skin. It is highly probable that this is due to cavitation events at the skin. Finally, we demonstrated that UIC did not promote metastatic spreading. The present study permits to assume UIC as safe for these particular US parameters and regarding these three points. This consisted in a single UIC exposure. However, future treatment strategy is based on repeated exposures, one for each drug administration. The impact of the treatment periodicity remains to be evaluated.

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