

INDUCTION OF APOPTOSIS BY ULTRA-LOW INTENSITY ULTRASOUND FOR CANCER TREATMENT

T.Saliev^{*1}, K.Tachibana², P.A.Campbell³

¹Centre for Life Sciences, Nazarbayev University, Astana, Kazakhstan; ^{*}timur.saliev@nu.edu.kz; ²Department of Anatomy, Fukuoka University School of Medicine, Fukuoka, Japan; ³Carnegie Physics Laboratory, University of Dundee, Scotland, UK;

INTRODUCTION.

Ultrasound has demonstrated an ability to induce a range of bio-effects at the cellular level, including sonoporation [transient mediation of drug delivery] (see scheme below), lysis [direct cell destruction] [1] and apoptosis [programmed cell death] [2]. However, the role of ultrasound with ultra-low intensity in contributing to these effects has, until recently [3-5], received relatively minor attention in the literature. The purpose for the present study is to quantify the biological effects of ultra-low intensity and its ability to induce the apoptosis in cancer cells, mainly characterized via cell viability and apoptosis assays, and including scenarios with ultrasound-contrast agent presence.

MATERIALS AND METHODS.

The cell culture (cancer blood cells) will be exposed to low and ultra-low intensity ultrasound fields by using a commercial KTAC-4000 device (Fig. 1).

The irradiation of cell culture will be performed at different time periods. The ultrasound parameters will be also varied (incl. wave-shape and ultrasound duty cycle) as to investigate the correlation between them and induced bio-effects. After sonication the cells (in all groups) will be immediately collected and subjected to analysis for viability, level of apoptosis and mutagenic activity.

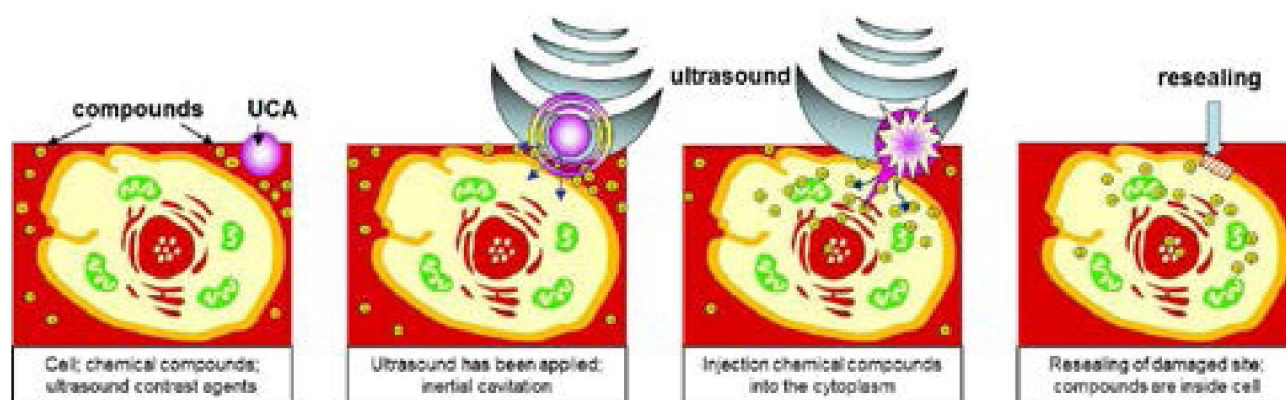


Figure 1. Schematic of the experimental setup for irradiation of cells with ultrasound.

The viability studies will be conducted by counting cells in a hemocytometer (C-CHIP DHC-N01 INCYTO) before and after sonication. The detection of apoptosis will be quantified by exploiting flow cytometry, performed using fluorescein isothiocyanate (FITC)-labeled annexin V which has a high affinity to phosphatidylserine (an indicator of apoptosis). Acquired data will be compiled and analyzed. All experiments will be repeated at least three times each (in order to achieve good statistical significance).

SUMMARY OF THE PROPOSED PROJECT:

- The project seeks to find a way for targeted induction of apoptosis in cancer cells trying to avoid massive cell lysis, which is highly undesirable due to its toxic effect.
- Novel modulator of apoptosis will be tested in combination with low and ultra-low intensity ultrasound.
- Testing of mutagenic potential of low and ultra-low intensity ultrasound will be conducted.
- New types of acoustic lenses will be examined to improve parameters of acoustic field. Obtained data will be used as a basis for developing of new devices (next project/s).
- The effect of combination of commercially available ultrasound contrast agents and modulator of apoptosis (bortezomib) will be studied.
- The in-house fabrication of lipid-based micro-bubbles (LBM) with incorporated drug (bortezomib) will be performed. The bio-effects of newly fabricated LBM will be studied and compared against the effects induced by standard ultrasound contrast agents.

FUTURE DIRECTIONS.

The results of the project will be used as a basis for development of the device for sonication of the blood of patients with blood cancer.

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