Differential cytotoxicity and sonosensitization induced by sanazole (AK-2123) under aerobic conditions: Effects of cell type and acoustic conditions.

Marjane A. Hassag, Yukihiro Furusawa, Qing-Li Zhao, Ichiro Takasaki, Loreto B. Feril, Jr., Katsuhiro Tachibana, Nobuki Kudo, Masami Minemura, Toshiro Sugiyama, Takashi Kondo
(1) Department of Radiological sciences, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan. (2) Division of Molecular Genetic Research, Life Science Research Center, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan. (3) Department of Anatomy, School of Medicine, Fukuoka University, Nanakuma, Fukuoka 814-0180, Japan. (4) Laboratory of Biomedical Instrumentation and Measurement, Graduate School of Information Science and Technology, Hokkaido University, Sapporo 060-0814, Japan. (5) Department of Gastroenterology and Hematology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan.

Tel: +81-(0)76-434-7265, Fax: +81-(0)76-434-5190, E-mail: rommpharm@hotmail.com

Key Word: Apoptosis; pulse repetition frequency, sanazole; sonosensitization.

<Abstract>
The current study was undertaken to evaluate the effect of sanazole as a sonosensitizer at previously studied acoustic conditions of different pulse repetition frequencies using two cell lines representatives of solid tumors and hematopoietic cancers. Sanazole alone displayed selective cytotoxic effects towards solid tumor-derived cancer cells resulting in complete cell death after 24 hr treatment and enhanced the ultrasound-induced cell killing after 6 hr post-treatment. The enhancement seemed to be mediated by an additive increase in intracellular oxidative stress levels. Sanazole seems to be an efficient cytotoxic agent for the treatment of solid tumors and a promising sonosensitizer under aerobic conditions.

<Introduction>
Ultrasound (US) has been gaining solidarity over the past decades as a modality in cancer therapy where it has been shown to induce apoptosis in tumor cells [1], modify the therapeutic outcomes when combined with chemotherapeutics [2, 3] and permeate cells to drugs and high molecular weight moieties [4]. A common feature in ultrasound-induced bioeffects is the elevation of intracellular reactive oxygen species (ROS) [5]. ROS are known to be mediators to multiple and even contradictory pathways [6] including apoptosis induced by ultrasound. The use of sanazole, a well-known hypoxic radiosensitizer [7] and a cytostatic agent [8], as one-electron oxidant, can oxidize and fix free radical damage at cellular targets [9] and result in an enhancement in cell killing. Preliminary results proved the validity of the working hypothesis where an enhancement was observed even at ineffective dose of sanazole (5 mM) and at an acoustic intensity below the threshold for cavitation. Sanazole sonosensitization was sensitive to the acoustic conditions employed where an intensity-dependence was observed. These findings motivated us to seek an optimized sonosensitizing activity under different acoustic conditions of varying pulse repetition frequencies (PRF; from 0.5 to 100 Hz). Two cell lines were used, namely, U937 cells, as representative of hematopoietic lymphomas and HeLa cells, as representative of solid tumors.

<Methods>
The study was divided into two parts; the first part was devoted to evaluate the effect of sanazole on U937 and HeLa cells. For this study, concentration- and time-dependent experiments were performed and samples were analysed for apoptosis both flow cytometrically using double staining with fluorescein isothiocyanate (FITC)-Annexin V and propidium iodide and spectrophotometrically for DNA fragmentation. In the second part, sanazole at ineffective dose (5 mM) was combined with ultrasound at intensity 0.3 W/cm², duty factor 50% and PRF in the range from 0.5 to 100 Hz. The results from flow cytometric analysis were obtained at 6 hr incubation following the combined treatment. Changes in intracellular ROS were monitored with intracellular fluorescent probes immediately and at 3 hr following sonication.

<Results and Discussion>
The results revealed that sanazole exerts a differential cytotoxic effect depending on cell type. Thus, HeLa cells were completely eradicated after incubation with 10 mM of sanazole for 24 hr whereas only 20% of U937 cells were shown to be killed under similar treatment. The mechanisms involved in cell killing seemed to be different as indicated from DNA fragmentation analysis. Further experiments showed that the cell killing in HeLa cells is mediated through a caspase - independent pathway. From these set of experiments, an ineffective dose of sanazole, 5 mM, was chosen for a combination protocol with ultrasound. The flow cytometric data showed that the preincubation of cells with sanazole for 30 min resulted in an enhanced cell killing 6 hr
following acoustic irradiation. The enhancement was phase specific depending on cell type and PRF used. The analysis of intracellular ROS revealed that, using hydroethidine (HE) dye, which possesses some selectivity to superoxide anion (O₂⁻), sanazole treatment resulted in more induction of intracellular ROS in U937 cells compared to similarly treated HeLa cells. The combination treatment resulted in immediate additive rise in ROS except at 5 and 100 Hz in both cell lines, whereas after 3 hr, an additive increase was observed at all sonication conditions except at CW where the increase was significantly synergistic in U937 cells. On the other hand, the use of dichlorofluorescein diacetate (DCFH) dye, which detects the total intracellular oxidative stress [28], showed that the effect of sanazole treatment alone was more pronounced in HeLa cells compared to U937 cells. The effect of the combined treatment was additive in both cell lines except at CW where the increase in ROS in U937 cells 3 hr post treatment was significantly synergistic. As a conclusion, the observed enhancement in cell killing following the treatment with sanazole and US reflects that this combination might be promising in managing cancer. In such case, the systemic administration of sanazole followed by localized exposure of the tumor mass to ultrasound can guarantee optimized cancer eradication with minimal adverse effects. Moreover, the present results, which have been concerned only with non-thermal effects of ultrasound, pave the way for future studies to evaluate the use of high intensity focused ultrasound with sanazole, the thing that will add a thermal effect which has been proven previously to promote cell killing when used with sanazole- as a contributor in the final outcome while retaining a high degree of tumor targeting.

<References>