Tumori, 98: 523-531, 2012

Cell damage of hepatoma-22 cells exposed to continuous wave ultrasound

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ABSTRACT

Aims and background. The cellular response of hepatoma-22 cells to ultrasonic irradiation and the potential cause for the action were evaluated.

Methods and study design. Hepatoma-22 cells were subjected to ultrasound irradiation at a frequency of 2.17 MHz and a spatial average intensity of 1.6 W/cm^2 for variable periods of time, and several biological parameters were analyzed. The terephthalic acid (TA) dosimetry method was used to evaluate the efficacies of irradiation parameters on the acoustic cavitation activity by monitoring hydroxyl radical (OH) production. Lactate dehydrogenase (LDH) leakage was assayed to investigate cell membrane integrity. The polarization value of fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) was measured to monitor plasma membrane fluidity. The malonaldehyde content in cells was determined to reflect lipid peroxidation. Trypan blue exclusion was used to detect cell viability. Additionally, electron microscopy was used to observe morphological changes. The generation of intracellular reactive oxygen species, mitochondria swelling and the loss of mitochondria membrane potential were also investigated.

Results. The results showed that 1) the concentration of ·OH production by ultrasonic irradiation in air-saturated cell suspensions increased as ultrasound exposure time increased; 2) compared with control, lactate dehydrogenase leakage, the polarization value of 1,6-diphenyl-1,3,5-hexatriene, malonaldehyde content and cell lysis were significantly elevated when cells were treated by ultrasound for 60 s; 3) cytotoxicity by ultrasound irradiation was also accompanied by an increase in production of intracellular reactive oxygen species and dissipation of mitochondria membrane potential as well as by mitochondria swelling.

Conclusions. Presently available information indicates that the plasma membrane and mitochondria are the main targets for ultrasound treatment, and free radicals formation such as ·OH due to ultrasound cavitation may play an important role in mediating these cellular response processes. Moreover the mechanical effect might also be involved in inducing cell damage because there was significant mitochondria membrane potential loss and no visible ROS detection when cells were exposed to ultrasound for 30 s.

Key words: cellular response, continuous ultrasound, hepatoma-22, instant cell damage.

Acknowledgments: This work was supported by the Natural Science Foundation for Youth (No. 81000999, 10904087), the Fundamental Research Funds for Central Universities (GK201102020), the Research Fund for Doctor Station, and the Ministry of Education (20100202110006).

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Received November 11, 2011;
accepted February 24, 2012.