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Effect of Different Ultrasound Frequency Sweep Pattern on Leukemic Cells

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Extended Abstract

The membrane subjected to low intensity ultrasounds becomes a mechanical oscillator in an acoustic field (Krasovitskia B et al., 2011). We conjecture the optimal frequencies for obtaining membrane damage, apoptosis and inhibition of proliferation are related to the geometrical and mechanical characteristics of membrane. If we subject a same cell line, human myelomonocytic lymphoma U937, to variable frequency, the probability of drastically interfere with the mechanical and dimensional characteristics of the cell increases, compared to that at a fixed frequency. In this regard, it should be specified that in the same cell culture we found a high heterogeneity in cell sizes. We evaluated the mechanical and biological effects induced by different sonication condition submitting U937 cells at various sweep frequencies and at fixed frequencies with different burst rate. The cells were stressed by pulse wave ultrasound with increasing and decreasing frequency between 400 and 620 KHz, at 0.5 Hz, 10 Hz, 50 Hz burst rate and 50 % duty. As previously shown (Buldakov et al., 2009) low value of Burst Rate determines stirring of the solution and interrupts the surface reducing the possibility for standing wave formation, and the effects are more random and less researchable. For high values of burst rate (10 Hz and 50 Hz), the effects related to the ultrasound streaming can be disregarded and standing waves established. In this case the higher mortality observed at variable frequencies may be related to sound wave frequency and the amplitude. The voltages used were 35 V and 60 V and the durations were 90 s and 180 s. We obtained the decreasing mode was better than increasing mode for 10 Hz and 50 Hz burst rate, while at 0,5 Hz the increasing mode give better results at duration 180 s. We found a dependence by duration: the survival rate was lower for 90 s duration and at 0.5 Hz the trend was reversed: decreasing mode was better than increasing mode, but after 180 sec the survival rate was lower. At 35 V the difference in behaviour between increasing mode and decreasing mode was not significant and the mortality was lower to 35 volts than to 60 V.

This data were compared with data at fixed frequency at extreme values and the centre of the frequency range (400 KHz, 510 KHz, 620 KHz) at 10 Hz Burst Rate, 180 sec duration, and 60 V voltage. At fixed frequency we found that survival rate was always higher than at sweep frequency. On closer inspection, however we observed that there was also a dependence by intensity. In fact the lowest values of the survival rate corresponded to the highest power values. The survival rate at 400 KHz (0.0360 V) and at 510 KHz (0.0720 V) was almost 100%, while at 620 KHz (0.1280 V) failed at 47 %, while 31% survival rate detected at increasing sweep 10 Hz Burst rate (average 0.1370 V). These results have been confirmed by tests at 0.5 Hz burst rate, with fixed frequencies. The mortality became comparable with that at variable frequency only for values of the power three times higher than the average power

measured in the interval of sonication at variable frequency. This leads us to assume that the death of the cells depends on the frequency and intensity of the ultrasound.

Sonication produces a stiffening of some cancer cell (Conneely M et al., 2012) and the metastatic potential (Swaminathan V et al., 2011; Xu W et al., 2012) is related at cells stiffness. Consequently, we expect that the cancer cell proliferation (Lejbkowicz F, Salzberg S, 1997) is inhibited by ultrasound. We found for 10 Hz burst rate, decreasing frequency, 180 s duration, 60 V voltage, 20% survival rate and in the cell cycle, we found more cell in G1 than in the controls and in increasing mode, immediately after sonication and after six hours of incubation. So we can conjecture to have inhibited the cell hyper proliferation. The results seem to lead us toward a non-invasive and effective purging of leukemic cells.

Keywords: Increasing frequency, decreasing frequency, burst rate, immediate lysis, hyper proliferation, cell cycle

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