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EFFECTS OF ULTRASOUND ON MALIGNANT AND BENIGNANT CELLS IN VITRO

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ABSTRACT

In developing uses for ultrasound in medicine, progress has been much faster with diagnostic applications than with therapeutic ones. Ultrasound is, however, widely used in physiotherapy. We studied the effect of ultrasound on a strain of fibroblast cells and two malignant cell strains, using ultrasound doses applied in physiotherapy. The damage in the benignant cells after the lowest intensity used (0.2 W/cm² for 4 minutes) was the same as in the malignant cells and about the same in all cell strains at the intensities of 0.4 and 0.9 W/cm². Dividing cells and the largest cells were especially sensitive to ultrasound. It is concluded that further studies in this field are needed.

The biological effects of pulsed ultrasound techniques have been extensively studied and it seems at present that there is no hazard in the use of ultrasound for diagnostic purposes[10]. The use of ultrasound irradiation in physiotherapy, on the other hand, involves the intensities much higher than those used in diagnostics and adverse effects have to be taken into consideration[11]. Attention should be paid to these problems and further studies concerning the biological effects of ultrasound are needed[10].

The action mechanism of ultrasound in biological structures is composed of the direct effects resulting from the conversion of the energy of vibration into mechanical and thermal energy and the indirect reaction of the organism to these stimuli. The effects of ultrasound have to be measured by indirect methods since the energy entering the tissues and the duration of radiation are the only parameters that can be accurately determined. In the study of the biological effects of ultrasound different methods have been applied[2,3,4,5,8].

The aim of the present study was to examine the effect of therapeutic doses on cells *in vitro* and to determine whether the effect of ultrasound on normal benignant cells was different from that on malignant cells.

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MATERIAL AND METHODS

A strain of fibroblasts called LLC, was used as benignant cells, and two cell strains, called SAL and VRT, differentiated from subcutaneous metastasis of human melanoma tumours, were used as malignant cells. All the cell lines were established, and the cell lines of SAL and VRT were typical melanoma cells; the SAL produced melanin, and the VRT-cells from an amelanotic malignant tumour, produced a pigment of other origin, as revealed by special staining and electron microscopy[6]. At the beginning of the ultrasonic irradiation experiments, the LLC-cell line was on its 88 passage, the SAL-cell line on its 45 passage and the VRT-cell line on its 54 passage; at the completion of the experiments the cells were on their 95, 53 and 62 passages *in vitro*. The cells were cultured by routine culture methods and the growth medium and the cell numbers used were the same used throughout the series[1]. For stabilization, the cells were incubated at 37 °C for 3–4 hours before irradiation. The number of control tubes was the same for each treatment of 10–14 tests tubes/dose and the number of experiments was twenty. The doses of therapeutic ultrasound given were 0.2, 0.4 and 0.9 W/cm^2 using one megacycle, and the duration of treatment was 4 minutes. The test tubes containing the cells were immersed in a water tank. The water was stabilized at 37.0 ± 0.5 °C. For the experiments, test tubes containing the cells were placed in the water tank two at a time at a distance of 15 mm from the radiation releasing ultrasound transducer, which was moving back and forth in the water tank during the experiments (Figure 1).

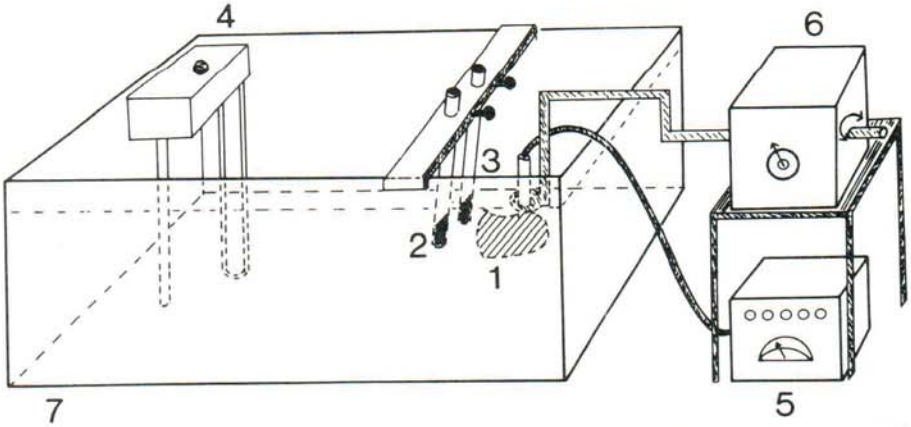

FIG. 1–The experiment arrangement: 1. Head of the ultrasound transducer; 2. and 3. Immersed test tubes; 4. Thermostat; 5. Ultrasound apparatus; 6. Equipment for moving the transducer arm back and forth and 7. Water tank (60 × 100 cm, height 50 cm).

RESULTS

The cells were examined on the day following the treatment and on the third day. When possible, cell counts after four to seven days of growth after the treatment were determined, too. If the cell growth was very fast, as in the VRT--cells, counting was no longer possible after the fourth day of growth. Figures

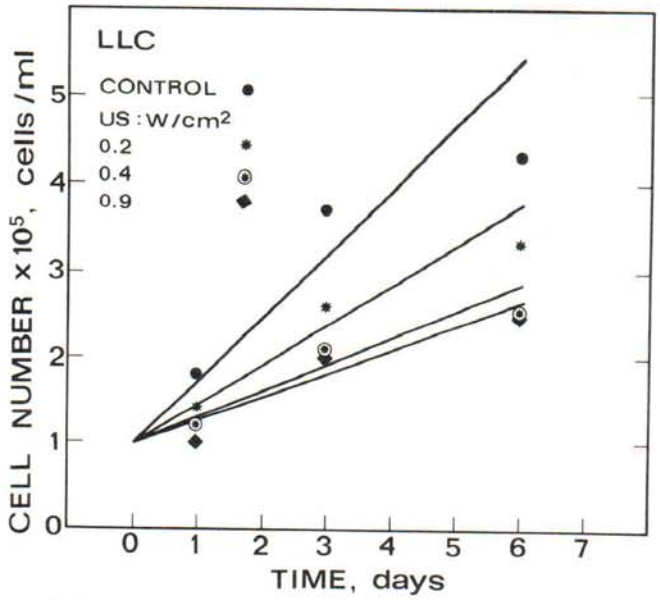

FIG. 2 – Effect of ultrasound on the growth of non-malignant (LLC) cells after ultrasound treatment with different intensities during 4 minutes.

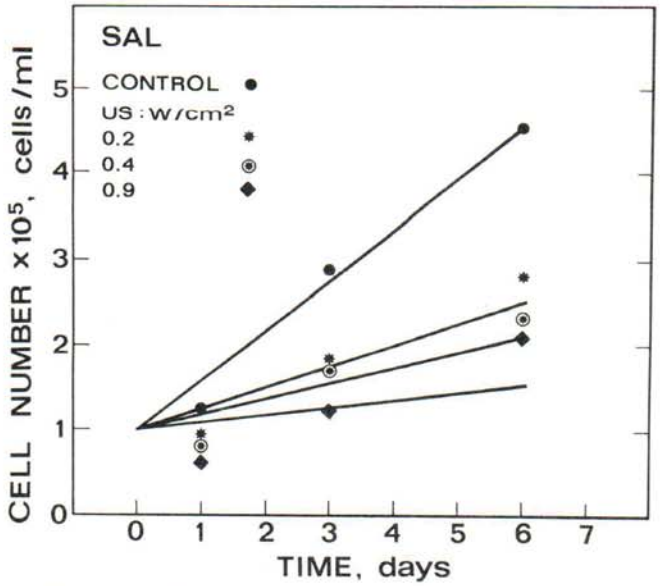

FIG. 3 – Effect of ultrasound on the growth of malignant cells (SAL) after treatment for 4 minutes with different intensities.

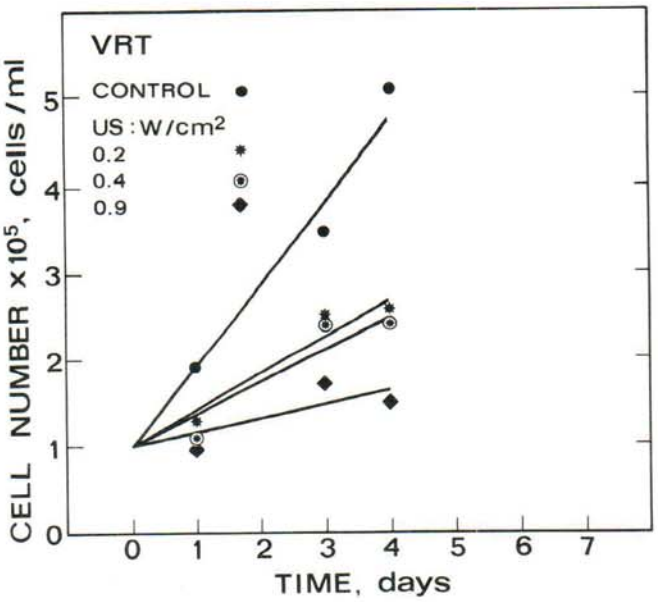

FIG. 4 – Effect of ultrasound on the growth of malignant cells (VRT) after 4 minutes of treatment with different intensities.

2–4 show the effect of ultrasound on the fibroblast and the two malignant cell strains. Control studies were always performed parallelly with the irradiation experiments.

Although the effect of ultrasound was not significant until on the third day, many severely damaged cells could already be seen at the first count. No recovery could be found in the damaged cells during the time of investigation; the inhibition of growth was irreversible in all the treated cells except in the control cells. It is noteworthy that the damage in the non-malignant LLC-cells after the lowest intensity of 0.2 W/cm² was the same as in the malignant cells, and about the same in all cell strains at the intensities of 0.4 and 0.9 W/cm². Destruction was almost total in the fastest growing malignant VRT-cells. In the VRT-cells, the lowest value was already found on the fourth day after the treatment. The reduction of cell numbers was partly caused by immediate cell lysis and cell death. As an overall feature in the examination of cells, it was found that the dividing cells and the largest cells were especially sensitive to ultrasound and therefore inactivated fastest; the number of these kinds of cells clearly reflected the inhibition of growth especially in the VRT-cells. The effect on the cells correlated with the duration of the treatment a longer treatment having a greater effect. The cytotoxic effect of ultrasound has been shown to be non-thermal by other investigators[7]. No recovery could be found in the malignant cells, whereas

a slow recovery was a prominent feature in the non-malignant cells during the time of investigation. The effect of cell killing by ultrasound should be considered in the treatment of local malignant tumours, especially when combined with other kinds of irradiation[9].

DISCUSSION

The increasing use of ultrasound in physiotherapy would seem to call for further studies in this field.

The doses of ultrasound used in this study on a strain of fibroblast cells and two malignant cell strains, was about the same as doses applied in physiotherapy. In this study the damage in the benignant cells after the lowest intensity used (0.2 W/cm² for 4 minutes) was the same as in the malignant cells and about the same in all cell strains at the intensities of 0.4 and 0.9 W/cm². Dividing cells and the largest cells were especially sensitive to ultrasound.

The fact that no recovery could be found in the malignant cells should be considered in the treatment of local malignant tumours, especially when combined with other kinds of irradiation.

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