

## Apoptosis of Tumor Cells Induced by Weak Electromagnetic Fields *in Vivo*

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### Abstract

In order to study the possibility of apoptosis of tumor cells induced by weak EMFs *in vivo*, mice were inoculated with Ehrlich ascites cells and exposed to an electromagnetic field (1mT, 700 K Hz) for a long term. During the treatment growth curves of mice were measured. Comparisons have been made between the growth curves of treated and sham treated mice. After the treatments, apoptosis of Ehrlich ascites cells from treated and sham treated inoculated mice was analyzed by several methods, including flow cytometry, fluorescence microscopy and DNA gel electrophoresis. The results show that the apoptotic ratio of cells from treated cancer mice was significantly higher than those from sham treated mice ( $P < .05$ ) and the growth curves of cancer mice deviate from the theoretical curve of healthy mice but tend to it by the EMF treatments. The mechanism of the apoptosis induced by weak EMFs has been discussed in terms of interactions between external electromagnetic fields and DNA.

**Key words:** weak electromagnetic fields (EMFs), long-term exposure, apoptosis, Ehrlich ascites cells,

It has become generally accepted that electromagnetic fields (EMFs) cause various biological effects<sup>[1,2]</sup>. EMFs have been used for some clinical purposes, for instance, for promoting bone healing. Based on thermal effects, microwaves have been used in anti-cancer treatments successfully. However, up to now if weak EMFs can be used for cancer treatment and what is the mechanism for the treatment is still remained unknown. Apoptosis or programmed cell death plays a major role in many human diseases, including cancer. Tumors develop not only from abnormal cell proliferation, but also from inhibition of apoptosis. Promoting apoptosis of tumor cells is a new strategy for cancer treatment. Recently, apoptosis induced by weak EMF *in vitro* have been reported<sup>[3-5]</sup>. However, research reports concerning apoptosis of tumor cells induced by weak EMFs *in vivo* have not appeared yet.

In this experiment mice (Male,  $20 \pm 2$ g,) were inoculated with Ehrlich ascites cells. EMF treated mice were placed in a solenoid connected to a generator which produced a 700KHz, 1 mT alternating magnetic fields, the specific absorption rate (SAR) is about 0.3-0.5 mW/g. Sham treated mice handled under the same conditions as the controls. Healthy mice were taken as the controls of inoculated mice. In Group 1, healthy mice were exposed to weak EMFs for 10 days (6h/day) and sham treated healthy mice as the controls. After the treatments bone marrow cells and thymic cells from treated and sham treated mice were analyzed for characteristic apoptosis. In Group 2, inoculated mice were exposed immediately to EMFs for 10 days (6h/day) after the inoculation. In Group 3, cancer mice were exposed to EMFs on the 7th day after the inoculation and were treated continuously for seven days (6h/day). During the treatments weight of each mouse was measured every day and growth curves were made according to statistical means of the measurements.

Cells from treated and sham treated mice were collected, washed and stained in Propidium iodide (PI) and then directly analyzed by flow cytometry (FACScan, Becton Dickinson, San Jose, CA). Cells from treated and sham treated animals were collected and stained with a dye mixture of 10 $\mu$ g/ml AO and 10 $\mu$ g/ml PI, then examined under a Nikon fluorescence microscope to determine the cells undergoing nuclear changes in characteristic of apoptosis. Agarose gel electrophoresis of DNA from cells of treated and sham treated mice was run at 120mV for 1.5h. The gel was then observed under ultraviolet light and photographed.

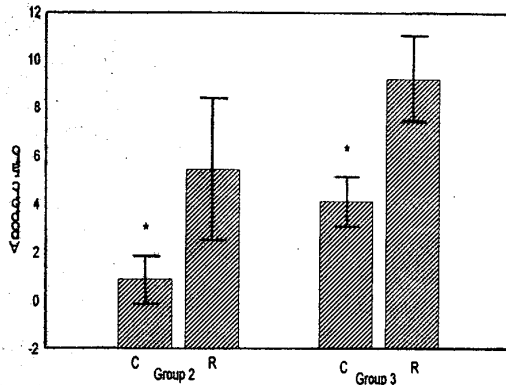
### Results

### Difference in growth curves

The results show that there is no difference in growth curves between treated and sham treated healthy mice and both fit well with the theoretical line according to the logistic growth model<sup>[6]</sup>. However, the growth curves of cancer mice deviate from the theoretical line. Moreover, the curves of sham treated mice deviate more than those of the treated mice, in other words the growth curves of treated mice are closer to the theoretical line.

### Changes in cell cycles analyzed by Flow cytometry

No apoptotic peak was observed in the sham treated control. Compared with the control, the number of cells in S- and G2-M phase decreased and the number of cells in G0-G1 phase increased from the treated mice. A distinct population of apoptotic cells in sub-G1 phase (apoptosis peak, AP) was identified. The statistical averages of apoptosis ratio of cells from treated and sham treated cancer mice were showed in figure 1.



**Figure 1.** Statistical averages of apoptotic ratio of cells from treated and sham treated mice in Group 2 (left, n=8) and in Group 3 (right, n=9) showing that the average apoptotic ratio in cells from treated Ehrlich ascites mice is significantly higher than those from sham treated mice ( $P < .05$ ). R for treated, C for sham treated.

### Characteristics assayed by agarose gel electrophoresis of DNA

The agarose gel electrophoresis of DNA from treated and sham treated mice in Group 2 shows that the chromatin DNA exhibited ladders, a characteristic feature of internucleosomal degradation of DNA by the treatments. Analysis of agarose gel electrophoresis of DNA from Group 3 obtained the similar results.

### Discussion

There were numerous hypotheses dealing with the mechanism of interactions between EMFs and living systems<sup>[7-15]</sup>. M.Blank<sup>[7]</sup> believed that direct interaction of EMF with DNA should be considered as a possible mechanism. Recently an interference model has been raised by Popp and Chang<sup>[8]</sup>. According to this model, caused by biological non-linearly polarizable double layer, like the cell membranes or the exciplexes (excited complexes) of the DNA, destructive interference establishes in the outside of the living matter, as a consequence, at the same time in the inside constructive interference takes place. The process is not passive absorbance but an active process where energy is accumulated and stored by constructive interference within the system. The results of the interaction does not only depend on the field but rather on the biological matter. Therefore it may happened that under certain conditions of the EMFs, tumor cell is more sensitive than normal cells since their DNA is in different excited states. This may be the reason why in our experiments apoptosis did not occur in bone marrow cells and thymic cells from healthy mice exposed to the same EMFs and the growth curves of exposed healthy mice still follows the theoretical line and does not deviate.

Although the mechanism of apoptosis induced by weak EMFs is to be elucidated, the experimental results show complexity and variety of EMFs effects and suggest that EMFs may have a valuable potential in anticancer treatment.

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