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Effect of pulsing electromagnetic fields on DNA synthesis in mammalian cells in culture[1]

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Summary. DNA synthesis in Chinese hamster V79 cells was significantly enhanced when they were exposed to weak, pulsing electromagnetic fields generated by specific combinations of the pulse width (25 μs), frequency (10, 100 Hz) and magnetic intensity (2×10^{-5}, 8×10^{-5} T). Conversely the DNA synthesis of cells in the fields at 4×10^{-4} T was repressed to 80% of that in controls not exposed to the fields.
Key words. Chinese hamster V79 cells; electromagnetic fields; DNA synthesis.

Weak, pulsing electromagnetic fields were shown to have the ability to stimulate bone healing[3–5] as well as other biological processes including DNA synthesis[6], cell proliferation[7] and cellular transcription[8]. However, the mechanism underlying the modifications of a variety of biological processes by magnetic fields is still largely unexplained. In the present study an attempt has been made to determine the optimal conditions of weak, pulsing electromagnetic fields for the enhancement of DNA synthesis in cell cultures, particularly the pulse width, frequency and magnetic intensity, if any.

Materials and methods. Chinese hamster V79 cells (approximately 2×10^4 cells) were inoculated into 35-mm plastic dishes containing 2 ml Eagle's minimum essential medium supplemented with 10% fetal bovine serum (Gibco) and 0.3 g/l L-glutamine, and were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air. After 24-h incubation, (methyl-^{3}H)thymidine (78.2 Ci/mmole) (New England Nuclear) was added to the cultures to give a final concentration of 0.05 μCi/ml. Then the cultures were divided into control and experimental groups. The experimental group was placed between a pair of 10 by 10 cm Helmholtz coils placed horizontally in a CO_2 incubator, and received various conditions of pulsing electromagnetic fields[9]. The controls were in a separate incubator and were not perturbed by the magnetic fields. Both of the groups were incubated for two days in the presence of (^{3}H)thymidine and the labeled cells were harvested by trypsinization followed by a brief centrifugation. After addition of cold 10% trichloroacetic acid (TCA), the cells were allowed to stand on ice for 15 min. The lysate was centrifuged to separate the TCA-insoluble materials from the TCA-soluble ones, and radioactivity incorporated into the TCA-insoluble fraction was measured in ACS II scintillation cocktails (Amersham). Statistical analysis was performed using the t-test.

Results. Three independent series of experiments were carried out to determine the effects of pulse width, magnitude and frequency on the DNA synthesis in cell cultures. In the first series, one frequency (100 Hz) and one intensity (2×10^{-5} T) were used for all experiments while varying the pulse width. Of the pulse widths tested so far, only a 25-μs pulse was found significantly to enhance the DNA synthesis; the amount of (^{3}H)thymidine incorporated into the DNA increased by up to 30% ($p < 0.001$, t-test) as compared to the control cultures not exposed to the fields. No significant difference in the DNA synthesis was observed between the experimental cultures exposed to 6-, 10-, 50-, 75-, and 125-μs pulses and their respective controls not in the magnetic fields (fig. 1).

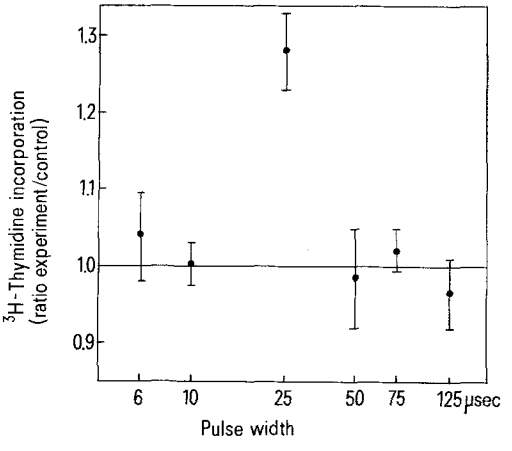

Figure 1. Effect of pulse width on DNA synthesis in Chinese hamster V79 cells exposed to the magnetic fields that are generated with 2×10^{-5} T pulse repeating at 100 Hz. Incorporation of (^{3}H)thymidine into the TCA-insoluble fraction was measured and the ratio of the mean value of experimental cells to that of controls was expressed. Mean values for the experimental cells are given ±SD.

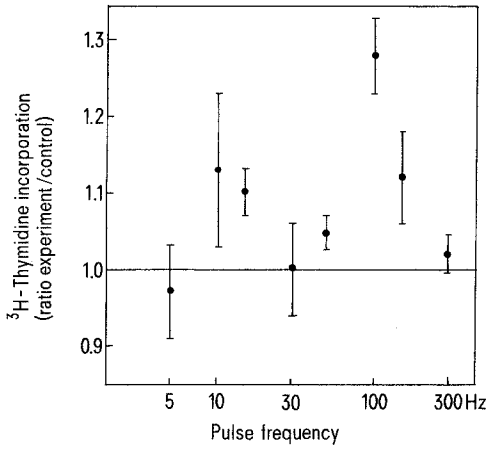

Figure 2. Effect of pulse frequency on DNA synthesis. The experimental cultures were exposed to a 25-μs pulse of 2×10^{-5} T at various frequencies. Mean values for the experimental cells are given ±SD as in figure 1.

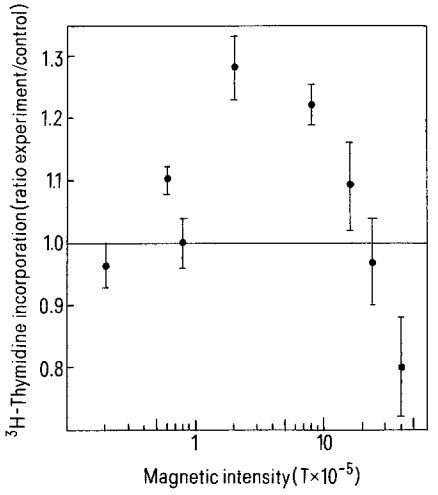

Figure 3. Effect of magnetic intensity on DNA synthesis. V79 cells were exposed to a 25-μs pulse repeating at 100 Hz. Mean values of the experimental cells are given ±SD as in figure 1.

In the second set of experiments, the cells in the experimental incubator were subjected to magnetic fields at various frequencies between 5 Hz and 300 Hz. The pulse magnitude and width used in this series of experiments were 2×10^{-5} T and 25 μs, respectively. A stimulatory effect on the DNA synthesis of identical magnitude was only obtained with the pulse frequencies of 10 Hz or 100 Hz (fig. 2). The incorporation of (^{3}H)thymidine increased by up to 13% ($p < 0.01$) at 10 Hz and about 30% ($p < 0.001$) at 100 Hz, but no significant change in the DNA synthesis was seen at other frequencies examined when compared to the controls not in the fields.

In separate series of experiments to examine the effect of magnetic intensity on the DNA synthesis, the cells were kept in the fields at various intensities of a 25-μs pulse that repeated at 100 Hz. In the range from 2 to 8×10^{-5} T, the DNA synthesis was significantly enhanced (fig. 3). The mean value of the incorporation of (^{3}H)thymidine into the DNA increased to about 1.3-fold ($p < 0.001$) of the controls at 2×10^{-5} T and to about 1.2-fold ($p < 0.01$) at 8×10^{-5} T. Magnetic fields which had an intensity less than 10^{-5} T failed to influence the cellular DNA synthesis. Conversely an inhibitory effect could be observed in magnetic fields with an intensity that was greater than 2×10^{-4} T, and the incorporation of (^{3}H)thymidine decreased to 80% ($p < 0.01$) of the controls at 4×10^{-4} T.

Discussion. The present work showed that the DNA synthesis in Chinese hamster V79 cells was enhanced when the cells were exposed to the magnetic fields generated by specific combinations of the pulse frequency (10 Hz, 100 Hz), magnetic intensity (2×10^{-5} T, 8×10^{-5} T) and pulse width (25 μs), indicating that enhancement of the DNA synthesis strongly depends on the pulse characteristics, especially the pulse width and frequency. The results disagree in part with the previous result that the DNA synthesis in human fibroblasts is enhanced when exposed to pulsing electromagnetic fields for a wide range of frequencies (15 Hz to 4 kHz) and amplitude (2.3×10^{-6} to 5.6×10^{-4} T)[6]. It seems likely that the difference between the two systems may be due to the differential sensitivities between the two cell lines to the magnetic fields. Dependency of the cellular response on the pulse characteristics was shown in a study in which the two pulses in clinical use, the repetitive single pulse and the repetitive pulse train, produced different results from each other with respect to the cellular transcription in dipteran salivery gland cells[8].

The magnetic intensity of the fields which affected the cellular DNA synthesis in the present study is of a similar level to that of the geomagnetic field (approximately 5×10^{-5} T). An unexpected result is that the magnetic intensity of 4×10^{-4} T, that is 5–20 times greater than those effective for enhancement of the DNA synthesis, is inhibitory. This suggests that the direction of the influence of the magnetic fields; i.e. whether they stimulate or inhibit cellular DNA synthesis, is largely dependent upon the magnetic intensity, rather than the pulse frequency or width. To estimate the effects of pulsing electromagnetic fields on a variety of biological processes, it seems indispensable to consider the cell types involved, and possible differences in the sensitivity of each biological process to magnetic fields.

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