Induction of Apoptosis in Human Leukemic Cells by Magnetic Fields

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Summary: When human myelogenous leukemic cell lines, HL-60 and ML-1, were exposed to 50 Hz electromagnetic fields (EMFs), nucleosome-sized DNA fragmentation (a biochemical marker of apoptosis) was induced as assessed by agarose gel electrophoresis. However, EMFs could not induce detectable DNA fragmentation in either human peripheral blood leukocytes or

polymorphonuclear cells. The minimum exposure period required for the onset of DNA fragmentation in leukemic cells was 1.0 h. Although adverse effects of EMFs on human health have been reported, the apoptosis-inducing action of EMFs on leukemic cells may suggest a possible medical application. [Japanese Journal of Physiology, 47, 307–310, 1997]

Key words: apoptosis, human leukemic cell line, *in vitro*.

The relationship between exposure to extremely-low-frequency (50–60 Hz) electromagnetic fields (EMFs) and human health is of increasing interest. Many epidemiological studies have revealed a positive correlation between exposure to EMFs and the incidence of several types of cancer, particularly leukemia, and brain tumors [1, 2]. However, a number of other studies have failed to discern any correlation between extremely-low-frequency EMFs and cancer incidence [3, 4]. The conflicting findings of such reports complicate the elucidation of the influence of EMFs on human health.

We report here our findings that EMFs can induce DNA fragmentation in HL-60 and ML-1 human myelogenous leukemic cell lines but not in normal human peripheral blood leukocytes.

HL-60 [5] and ML-1 [6] human myelogenous leukemic cell lines were grown in RPMI-1640 medium (Flow Laboratories, Irvine, Scotland) supplemented with 10% fetal bovine serum (ICN Biochemical Co., Ltd., North Ride, Australia) at 37°C in a humidified atmosphere of 5% CO₂ (Water-Jacket CO₂ incubator; Hirasawa Co., Ltd., Tokyo, Japan). The cells were diluted with fresh medium every 2 d to maintain cell densities at or below 1×10^6 cells/ml,

since high cell density (over 2×10^6 cells/ml) induces apoptotic cell death in such cells (Sakagami, personal communication).

Normal lymphocytes and polymorphonuclear (PMN) cells were prepared from healthy human heparinized peripheral blood by density gradient centrifugation through Lymphocyte Separation Medium (Organon Technika Corp., Durham, NC, USA) and Mono-Poly Resolving Medium (Flow Laboratories) at $25 \pm 2^\circ\text{C}$ as described previously [7]. After separation, the cells were resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum at a concentration of 1.25×10^6 cells/ml and placed in the Water-Jacket CO₂ incubator until used.

Alternating current magnetic treatment devices (Soken Inc., Tokyo, Japan) were used to produce EMFs. These devices were approved by the Ministry of Health and Welfare of Japan as AC magnetic treatment devices (Approved No. 55B-1533), and use an ordinary commercial AC power source (100 V, 50/60 Hz). The maximum magnetic flux density was 45 mT (450 Gauss) around the magnetic pole as assessed by a gaussmeter (Model No. 9200, F.W. Bell Inc., Orland, FL, USA), and the magnetic pattern was a sine curve. The device was designed to minimize the

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generation of heat and false thermal effects, a problem that often occurs in magnetic therapy.

Cell suspensions were introduced into each well of 96-well flat-bottomed microtiter plates (Nunc, Denmark) at 2.5×10^5 cells/well in a volume of 200 μ l. The plates were placed on the alternating current magnetic treatment devices. The density of the magnetic fields at the bottom of wells was 45 mT (450 Gauss). They were then inserted into a 5% CO₂ humidified atmosphere at 37°C (Air-Jacket CO₂ incubator; Shimaden Co., Ltd., Tokyo, Japan) for various periods. The plates were then detached from the devices and maintained for a further 3.0 h in the Water-Jacket CO₂ incubator. The density of stray magnetic fields inside these incubators was 1 μ T (0.01 Gauss). Control cell cultures were treated in a similar manner without magnetic exposure.

DNA fragmentation was assessed by agarose gel electrophoresis [6]. Briefly, cells (5×10^5) were pelleted and lysed in 20 μ l of a lysis buffer. The solution was incubated sequentially with 0.5 mg/ml RNase A (Amersham plc., Bucks, UK) for 1.0 h at 50°C and 0.5 mg/ml proteinase K (Amersham) for 1.0 h at 50°C. The resultant DNA preparations were directly applied to 1.8% agarose gel electrophoresis in TBE buffer containing 0.5 μ g/ml ethidium bromide at 100 V for 1.0 h. λ DNAEco T14I digest (Takara Biochemicals, Kyoto, Japan) was used as the DNA marker to estimate the size of DNA fragments. The DNA fragmentation patterns were examined in photographs taken under UV illumination.

The viable cell number was determined in triplicate by trypan blue dye-exclusion test at 3.0 and 24 h after EMF exposure, and the results were expressed as a mean percent $\pm$ SD of viable cells.

When HL-60 and ML-1 cells were exposed to EMFs, the chromatin DNA exhibited ladders, a characteristic feature of internucleosomal degradation of DNA (Fig. 1, upper panel). DNA fragmentation in these cells was apparent even when the leukemic cells were exposed to EMFs for only 1.0 h. Although this type of DNA fragmentation was observed in leukemic cells exposed to EMFs for up to 2.5 h, DNA fragmentation patterns in the cells exposed for 3.0 and 3.5 h were quite different and characterized by cleavage of DNA to approximately 20 kbp fragments. The reasons for this discrepancy are not clear at present. It is possible that during short-term (within 2.5 h) EMF exposure, endonuclease activities responsible for production of ladder type-DNA degradation was enhanced and resulted in internucleosomal DNA fragmentation. On the other hand, long-term EMF exposure (3.0 and 3.5 h) may reduce endonuclease activities to produce

oligonucleosome-sized DNA fragmentation, and an insufficient increase in enzymatic activity results in the production of large DNA fragments. Another possibility is that two quite different types of endonucleases are presented in leukemic cells: one type of enzymes has the property to produce large DNA fragments [8] and the other to produce nucleosomal DNA fragments [6]. In the induction of apoptosis, the former enzymes are activated first and the latter are then activated, resulting in the production of ladder-type DNA fragmentation. Therefore, the present results may suggest that long-term EMF exposure inactivates the latter type of enzymatic activity. However, further experiments are needed to prove this point. Data in Fig. 1 (upper panel) also show that EMF exposure did not induce significant nucleosomal DNA fragmentation in human peripheral blood lymphocytes or PMN. We further examined whether DNA fragmentation in leukemic cells is involved in cell death. Typical results of 1 out of 4 different experiments are shown in Fig. 1 (lower panel), and demonstrate that the viability of leukemic cells examined at 3.0 h after exposure was $87.8\% \pm 2.6$ or more. However, the viable cell number of both cell lines examined 24 h after exposure was significantly lower, and almost all cells exposed for more than 2.0 h died (Fig. 1, lower panel). These results strongly suggest that EMF exposure can first induce specific nucleosome-sized DNA fragmentation in leukemic cells, resulting in tumor cell death.

A number of *in vitro* studies have revealed that magnetic field (50 Hz, 22 mT) exposure for 1.0 h increases intracellular Ca²⁺ concentration in several types of tumor cells [9, 10]. Recently, several types of endonuclease have been reported to play important roles in apoptosis induction [6, 8, 11]. It is also reported that the endonuclease activity associated with the nuclear fraction of apoptotic leukemic cells was enhanced by Ca²⁺ [6]. Taken together, one can speculate that exposure to EMFs elevates intracellular Ca²⁺ concentration and enhances endonuclease activity, resulting in an induction of internucleosomal DNA cleavage. It is generally accepted that alternating magnetic fields produces induction current (eddy current) which can increase temperature, but the 50 Hz sine curve magnetic field used in this study cannot produce enough induction current to elevate temperature. Indeed, temperature measured at 1.0 h after EMF exposure, the minimum exposure period for the onset of DNA fragmentation, was 38.2°C in our experiments. Shiota reported that apoptosis in both HL-60 and ML-1 cells induced by thermal effects was observed when these cells were cultured at 43.0°C (not below) for 30 min [6]. Therefore, it is reasonable to speculate

HL-60 Cells

ML-1 Cells

Lymphocytes

PMN Cells

Fig. 1. Effects of a 50 Hz magnetic field on induction of DNA fragmentation and cytotoxicity in human leukemic cell lines HL-60 and ML-1, and human peripheral blood leukocytes. Tumor cells were exposed to the 45 mT magnetic fields for 0 (Lane 1), 1.0 (Lane 2), 1.5 (Lane 3), 2.0 (Lane 4), 2.5 (Lane 5), 3.0 (Lane 6), and 3.5 (Lane 7) h. Control cells were also treated in the same manner except for magnetic exposure (non-stimulation). Human peripheral blood leukocytes were exposed to the 45 mT magnetic fields for 0 (Lane 1), 1.0 (Lane 2), 1.5 (Lane 3), 2.0 (Lane 4), 2.5 (Lane 5), 3.0 (Lane 6), and 3.5 (Lane 7) h. After magnetic field exposure, cells were cultured for a further 3.0 h. Cells were then lysed, digested with RNase A and proteinase K, and assayed for DNA fragmentation. The cell viability was also examined at 3.0 (○) and 24 h (●) after exposure to the magnetic fields by trypan blue dye-exclusion test. Each value is a mean percent of viable cells from triplicate assays. Bars showing SD are omitted for clarification. PMN cells, polymorphonuclear cells; M, DNA molecular weight standards; EMFs, electromagnetic fields.

that apoptotic tumor cell death observed in this study is not due to hyperthermia. A more detailed study to investigate the precise mechanism(s) of EMFs in apoptotic cell death is underway in our laboratory.

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