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Exposure to Strong Static Magnetic Field Slows the Growth of Human Cancer Cells In Vitro

Raymond R. Raylman, Anaira C. Clavo, and Richard L. Wahl

University of Michigan Medical Center, Department of Internal Medicine, Division of Nuclear Medicine, Ann Arbor, Michigan

Proposals to enhance the amount of radiation dose delivered to small tumors with radioimmunotherapy by constraining emitted electrons with very strong homogeneous static magnetic fields has renewed interest in the cellular effects of prolonged exposures to such fields. Past investigations have not studied the effects on tumor cell growth of lengthy exposures to very high magnetic fields. Three malignant human cell lines, HTB 63 (melanoma), HTB 77 IP3 (ovarian carcinoma), and CCL 86 (lymphoma; Raji cells), were exposed to a 7 Tesla uniform static magnetic field for 64 hours. Following exposure, the number of viable cells in each group was determined. In addition, multicycle flow cytometry was performed on all cell lines, and pulsed-field electrophoresis was performed solely on Raji cells to investigate changes in cell cycle patterns and the possibility of DNA fragmentation induced by the magnetic field. A 64 h exposure to the magnetic field produced a reduction in viable cell number in each of the three cell lines. Reductions of 19.04 ± 7.32%, 22.06 ± 6.19%, and 40.68 ± 8.31% were measured for the melanoma, ovarian carcinoma, and lymphoma cell lines, respectively, vs. control groups not exposed to the magnetic field. Multicycle flow cytometry revealed that the cell cycle was largely unaltered. Pulsed-field electrophoresis analysis revealed no increase in DNA breaks related to magnetic field exposure. In conclusion, prolonged exposure to a very strong magnetic field appeared to inhibit the growth of three human tumor cell lines in vitro. The mechanism underlying this effect has not, as yet, been identified, although alteration of cell growth cycle and gross fragmentation of DNA have been excluded as possible contributory factors. Future investigations of this phenomenon may have a significant impact on the future understanding and treatment of cancer.

Key words: **magnetic fields, magnetobiology, human cancer cells, cellular growth, biophysics**

INTRODUCTION

Recently, the possibility of enhancing the effectiveness of radionuclide therapy of cancer by magnetically confining emitted electrons to tumors has been proposed [Raylman and Wahl, 1994]. In this technique, which is known as magnetically enhanced radionuclide therapy (MERiT), a strong static magnetic field is applied following accumulation of a radiolabeled tracer, such as monoclonal antibodies, in the tumor. Increases in radiation dose to small tumors due to magnetic confinement of electrons in a 10 Tesla (1 Tesla = 10000 Gauss) field are predicted to be between 50 and 70%. This concept has rekindled interest in the effects of strong magnetic fields on cancer cell growth. There are no consistent conclusions regarding the effects of a static magnetic field on tumor cell growth. Studies have shown some reduction in tumor growth resulting from the application of a static magnetic field [Mulay and Mulay, 1961; Barnothy, 1962, 1963; Butler and Dean, 1964; Ardito et al., 1984]. For example, Ardito et al. [1984] measured a minimal reduction of 4.1% in the proliferative rate of human lymphocytes exposed to a static magnetic field of 0.074 T for 48 hours. Tata et al. [1994] reported that 1 h exposure to a 11.6 Tesla static magnetic field resulted in as low as a 10% survival fraction (compared to appropriate control groups) in some mouse and human cancers. Several other researchers have found no effect on cell growth rate [Eiselein et al., 1961; Hall et al., 1964; Halpern and Greene, 1964; Greene and Halpern, 1966; Iwasaki et al., 1978; Chandra and Stefani, 1979; Frazier and Andrews, 1979; Short et al., 1992]. For instance, Short et al. [1992] found no change in

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Address reprint requests to Richard L. Wahl, MD, University of Michigan Hospitals, Room B1G412, Ann Arbor, MI 48109.

growth rate for two cell lines, human melanoma (PS1273) and human fibroblastoma (DMD-A), subjected to a 4.7 Tesla static magnetic field for 72 h. None of these studies considered the effects of prolonged exposure (>2 days) to a very strong magnetic field, such as 7 Tesla, on tumor cells. The goal of our investigation, therefore, is to determine the effects of a high static magnetic field on tumor cell growth.

MATERIALS AND METHODS

Cell Lines

Three human malignant cell lines, melanoma (HTB 63) [Fogh and Trempe, 1975], ovarian carcinoma (HTB 77 IP3) [Wahl et al., 1987], and lymphoma (Raji cells; CCL 86) [Hinuma and Grace, 1968], were obtained from the American Type Culture Collection (ATCC; Rockville, MD) and were used in the initial experiments. Cells were adapted to grow in CO_2-independent medium (Gibco, Grand Island, NY). This growth media was supplemented with 15% fetal bovine serum, 4 mM L-glutamine, 50 IU/ml penicillin G, and 50 μg/ml streptomycin sulfate. These supplements follow the recommended guidelines [Epstein and Barr, 1965]. Cell feeding was conducted on alternate days with a complete change of media. All cells were maintained in a humidified atmosphere at 37 °C (Forma, Marietta, OH) prior to use in the magnet. These three cell lines were chosen because they are representative of malignancies originating from three distinctly different types of cells; thus, the effect on a variety of cells exposed to a magnetic field can be determined. In addition, different types of cell growth are represented; Raji cells grow in suspension, whereas the carcinoma (IP3) and melanoma (HTB63) cells adhere to the cell culture plate walls. Hence, the affect of a magnetic field on cells that are from differing origins and that grow in different ways can be evaluated.

Cell Growth Cycle and Growth Rate Measurements

One milliliter aliquots of the growth media previously described containing 15×10^4 cells were placed in the central eight wells (eight wells span the uniform field volume of the magnet) of 24-well cell culture plates (three wells IP3, three wells melanoma, and two wells Raji). This starting number of cells was needed to obtain the approximately 10^6 cells necessary for flow cytometry 3 days after plating. The experimental group was positioned in the isocenter of a 7 Tesla superconducting solenoid magnet (Oxford Instruments, Oxford, United Kingdom). The isocenter of the magnet is a spherical region (~5 cm in diameter) where the field is highly uniform. The magnetic field of this device is constantly monitored and did not vary over the course of the experiment. The schematic in Figure 1 shows the positioning of the cells in the magnet. The control group was placed in the same room as the experimental group at a location where the magnetic field was less than 0.0005 Tesla (as measured by a Hall probe). Both groups were maintained at a constant 37 °C temperature with the use of circulating water blankets. At the end of a 64 h incubation period, four 1 μl samples were removed from each cell line. This process was repeated for the control group. The number of viable cells was determined by using the Trypan blue dye exclusion technique [Freshney, 1987] with an Olympus IMT-2 inverted microscope (Lake Success, NY). Viability was determined by calculating the ratio of viable cells to the sum of viable plus nonviable cells (as determined by Trypan blue exclusion). Statistically significant differences in the number of viable cells present in the experimental group (exposed to the magnetic field) and the control group for each cell line were determined with a Mann-Whitney U test. Cells remaining in the cell culture plates were harvested, fixed, and prepared for multicycle flow cytometric analysis. Because the IP3 and melanoma cells adhere to the cell plate walls, trypsin was used to remove these cells from the culture plate. Salmon red blood cells (SRBCs) were included as an internal standard for assessment of different phases of the cell cycle [Iverson and Laerum, 1987].

To determine whether the magnetic field had permanent effects on the cell growth (beyond the acute exposure period), a second set of experiments was performed. The experiment previously described was repeated utilizing only Raji cells. At the completion of the 64 h exposure, 1×10^4 cells from each group (experimental and control) were replated in 200 μl of growth media. It should be noted that the cells did not reach the plateau phase of growth during the 64 h exposure period (as determined by flow cytometry). In addition, cell

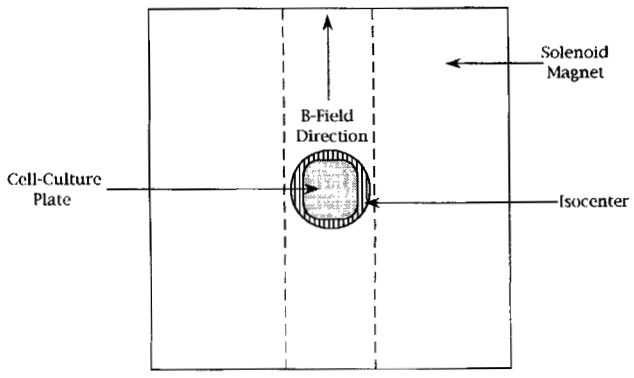

Fig. 1. Schematic drawing showing the positioning of the tissue culture plate containing the cancer cells in the magnet. The plate was centered in the constant field volume (isocenter) of the solenoid magnet.

viability was determined in the manner previously described. The number of viable cells in individual groups was then measured over each of the next 3 days by using the Trypan blue dye exclusion procedure. The amount of viable cells measured each day was utilized to calculate the number of cell doublings that occurred in consecutive days. This calculation was performed by dividing the cell number from two consecutive days and then determining the number of cell doublings that must have occurred to produce this result. The equation below summarizes the procedure:

$$2^n = \frac{P_2}{P_1},$$

where n is the number of cell doublings, and P_1 and P_2 are the number of viable cells measured on two consecutive days; each measurement of viable cells (P_1 and P_2) utilized 16 1 µl samples. The measurement P_1 precedes P_2, so that P_2 was always greater than P_1, because the plateau growth phase was not reached during the observation period.

Pulsed-Field Electrophoresis

To determine whether the magnetic field was inducing significant amounts of double-strand (ds) DNA breaks, Raji cells exposed to a 7 Tesla magnetic field were examined by pulsed-field gel electrophoresis. This technique is a standard method for measurement of DNA ds breaks induced in cellular DNA by agents such as ionizing radiation [Elia et al., 1991; Story et al., 1993]. In this experiment, 20×10^4 Raji cells in 1 ml aliquots were placed in the central eight wells of a standard 24-well cell-culture plate. Following a 64 h exposure to a 7 Tesla magnetic field (a control group was placed in the same room at a location where the magnetic field was less than 0.0005 Tesla) during which the temperature was maintained at 37 °C, the cells were removed and prepared for pulsed-field electrophoresis.

Qualitative pulsed-field electrophoresis analyses were performed, as described by Lawrence et al. [1993], using a CHEF DR III apparatus (Bio-Rad, Hercules, CA). Agarose plugs containing about 40×10^4 nuclei were loaded onto a 0.7% pulse-field grade agarose (Bio-Rad) and were run at 1.9 V/cm with a reorientation angle of 120 degrees. The switching interval was ramped linearly from 30 to 120 s over 27 h and then from 2 to 50 min for 46 h. The buffer, 0.5×45 mM Tris borate, pH 8.0, and 1 mM EDTA (TBE), was recirculated at 14 °C. *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* chromosomes (Bio-Rad) and λ phage DNA *Hind* III fragments (Gibco, Gaithersburg, MD), with DNA fragment size standards ranging from 125 base pairs to 5.7 megabases, were included in the samples. Gels were stained with ethidium bromide (7 µg/ml) for 15–20 min, destained in distilled water at 4 °C, and photographed.

RESULTS

All cell groups exposed to a 7 Tesla magnetic field for 64 h exhibited a significant reduction in the number of viable cells compared to the appropriate control groups ($P < 0.03$; Fig. 2). Specifically, the group of cells exposed for 64 hours to a 7 Tesla magnetic field contained $19.04 \pm 7.32\%$, $22.06 \pm 6.19\%$, and $40.68 \pm 8.31\%$ fewer cells than the control groups for the melanoma (HTB 63), ovarian carcinoma (HTB 77 IP3), and lymphoma (CCL 86, Raji) cell lines, respectively. Viability of both the control and experimental groups was greater than 98% (without the presence of cellular debris). Cell viability measured 3 days after removal from the magnet remained high (98%), even though absolute cell number was lower than the control group. Although cell loss to apoptosis produced by exposure to the magnetic field was not measured, if significant apoptosis had been induced, then a large number of cells would certainly have been expected to die by day 3 [Mirkovic et al., 1994], and the viability would be less than 98%. Therefore, these results indicate that the reduced number of cells measured in the experimental groups was most likely the result of slower cell growth and not cell death, programmed or otherwise.

Results from the multicycle flow cytometry are presented in Table 1. The percentages of the total number of cells in three phases of the cell cycle are shown for each of the cell lines. The only difference occurs in the ovarian carcinoma group exposed to the magnet, in which there are fewer cells in the G1-phase compared to the controls (with a commensurate increase in the number in S-phase).

When Raji cells were exposed to the 7 Tesla magnetic field for 64 h, they continued to exhibit slowed growth for at least 2 days following removal from the magnet. Results from this experiment are shown in Figure 3. The slower cell growth exhibited during the first day of the experiment is most likely due to the presence of a low number of cells (1×10^4) with respect to the amount of growth media (200 µl). As molecular metabolites and macromolecules are released into the medium and become available, cell growth increases. By day 2, the cells were growing at ~1 cell doubling per day. The experimental group also grew slowly during the first day, approximately 29% slower than the control group (Fig. 3). On day 2, unlike the control group, they did not reach the maximum growth rate. In fact, they grew 15% slower than the control cells. Not until the third day after magnet removal did these cells begin to grow at the same rate

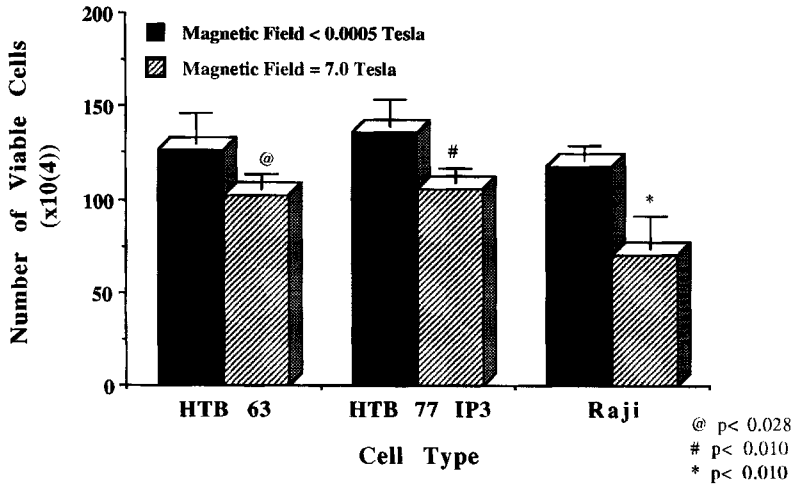

Fig. 2. Number of viable cells in the experimental group [M(+)] and control group [M(−)]. Results for three different cell lines are shown (mean ± S.D.; n = 4).

TABLE 1. Flow Cytometry Results for Three Human Tumor Cell Lines*

Magnet state	G1 (%)	G2 (%)	S (%)
HTB 63 (melanoma)			
M(−)	64.4	12.6	23.0
M(+)	67.0	12.6	20.4
HTB 77 IP3 (ovarian carcinoma)			
M(−)	65.4	1.0	33.7
M(+)	53.6	1.0	45.4
CCL 86 (lymphoma, Raji cells)			
M(−)	52.5	3.1	50.8
M(+)	49.1	2.8	48.1

*Results are given as percentage of cells in each phase of the cell cycle. M(−), control group; M(+), experimental group.

as the control group. Cell viability in all groups measured at all time points remained high (>98%). Thus, as previously stated, it appears that no significant apoptosis has been induced. Finally, results from the pulsed-field electrophoresis indicated that no increased numbers of DNA strand breaks were present in the cells exposed to the magnetic field compared to the control group.

DISCUSSION

To date, there is no clear consensus as to the effects of a static magnetic field on the growth of cancer cells. In this investigation, three human tumor cell lines were exposed to a very strong static magnetic field for an extended period of time. Our results indicate that the growth rate of magnet-exposed cells was lower than the

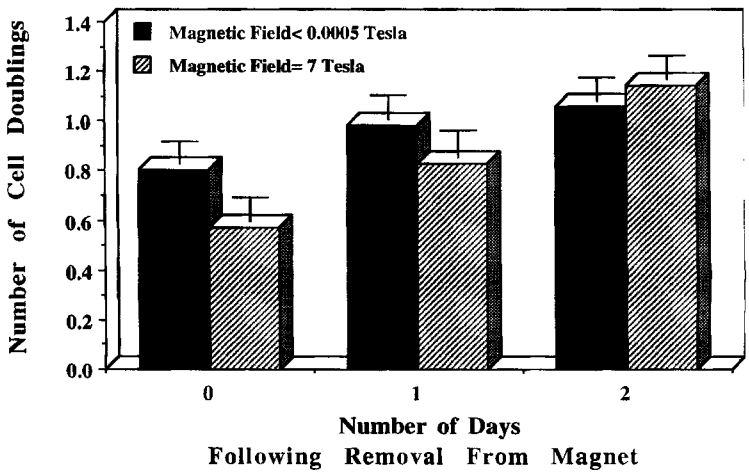

Fig. 3. Growth rate of Raji cells (mean ± S.D.) plotted vs. number of days following removal from the 7 Tesla magnetic field (n = 16).

appropriate control groups. It is interesting to note that the magnetic field did not affect the growth rate of the three cells lines in a uniform manner; the reduction in number of viable cells ranged from 19% for the melanoma cells to 40% for the Raji cells. An obvious difference among cell lines is that the melanoma and carcinoma cells attach to the walls of the culture plates, whereas Raji cells grow in suspension. Lymphomas are generally more radiosensitive than melanomas as well. Thus, the disparity in effect might be related to the individual growth characteristics of cancer cells, but this remains to be proven. This finding is supported by the cell line-specific reactions to a strong magnetic field reported by Tata et al. [1994]. For example, this group found that, whereas an 11.6 T magnetic field produced a very low cell survival fraction (4%) for a mouse sarcoma (S180), a mouse leukemia cell line (L1210) remained virtually unaffected.

Flow cytometry was performed on the cells immediately following termination of magnet exposure to investigate possible mechanisms for the biomagnetic effects. This procedure was performed to test the hypothesis that the magnetic field was slowing growth processes by increasing the length of one (or more) of the cell cycle phases or by arresting them in a particular phase. The results, however, showed that this is not occurring, except possibly in the case of the ovarian carcinoma cells, which demonstrated a shifting of cells from the G1-phase to the S-phase. Furthermore, there is no evidence that magnetically induced apoptosis is present. This confirms the preliminary findings of Tata et al. [1994], who, although reporting significant cell growth retardation, found no evidence of apoptosis in cells exposed to an 11.6 T magnetic field for 1 h. Thus, other causes must be considered. A possible explanation for growth reduction is the effect of a strong static magnetic field on the diffusion of ions such as Na^+, Ca^{2+}, and Fe^{3+}. In a static magnetic field, a force is encountered by moving charged particles (the Lorentz force). Kinouchi et al. [1988] have studied this phenomenon and predicted that, due to the relatively low velocities caused by Brownian motion, significant changes in the diffusion of such ions will occur at magnetic field strengths greater than $\sim 2 \times 10^4$ Tesla. Because the magnetic field studied in this experiment was 7 Tesla, some other effect(s) must be causing the reduction in growth rate.

A more likely explanation for the altered growth rates is that the magnetic field is affecting para- and diamagnetic structures in the cells. For example, the mitochondria and nucleus are among diamagnetic organelles; therefore, they are possible targets of interaction with the magnetic field. Indeed, Cook et al. [1969] measured a decrease in cellular respiration in sarcoma 37 cells when exposed to a magnetic field. In addition, D'Souza et al. [1969] found a decrease of up to 24% in the amount of DNA synthesis in tumor cells exposed to a magnetic field of 7.3 Tesla for from 1 to 3 h. Furthermore, paramagnetic molecules, such as enzymes and free radicals, can be affected by strong magnetic fields. For example, Gross [1961] has shown that magnetic fields can alter the bond angles of large paramagnetic molecules and, thus, possibly change their chemical interactions. The absence of an increased amount of DNA strand breaks, as determined by pulsed-field electrophoresis, reduces the probability that gross DNA damage is responsible for the magnetically-induced slowed growth rate. Mahdi et al. [1994] also found no evidence of DNA fragmentation in *E. coli* bacteria cells exposed to 0.3 and 5.0 T static magnetic fields.

Furthermore, slowed growth continues following removal from the magnetic field, and normal growth rates return only after an approximately 2 day recovery period. This phenomenon indicates that some reversible change is occurring within the cells. These results also show that the growth retardation is not caused by an interaction of the magnetic field with the growth media, because the slowed growth continues after removal from the magnet. At this point, the change that is occurring in the cells is unknown. It is evident, however, that the cells remain viable and that the cell cycle remains largely unchanged, except that it apparently takes longer to complete.

CONCLUSION

In this investigation, we have demonstrated that extended exposure to a strong static magnetic field retards the growth of three human tumor cell lines. Furthermore, the cells regain the ability to reproduce at their normal rate, but only after a several day period of recovery. Flow cytometry performed on the cells immediately following exposure indicated that no consistent change in the normal cell cycle is occurring. Pulsed-field electrophoresis revealed that no increase in DNA strand damage is induced in Raji cells after prolonged exposure to a strong magnetic field. Thus, this experiment has demonstrated that tumor cell growth can be slowed in vitro by the application of a strong magnetic field, but the mechanism for this behavior is as yet unknown. Additional study of this interesting phenomenon, including its mechanism, is warranted. It should be noted that this antiproliferative effect of high static magnetic fields may be augmented by its combination with radionuclide therapy (MERiT). Thus, the cancer is treated by both the magnetic field and the magnetically confined beta particles. It is possible that further study of this effect may lead to other applications in cancer treatments. At the very least, these studies should add to our understanding of tumor cell growth.

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