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AN EVALUATION OF THE BIOLOGICAL EFFECTS OF THREE DIFFERENT MODES OF MAGNETIC FIELDS ON CULTURED MAMMALIAN CELLS

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ABSTRACT

The biological effects of static magnetic fields, and their combined effects with ionizing radiation, were studied using a cultured mammalian cell line (FM3A). The three different modes of magnetic fields evaluated in this report were the 0.3 Tesla (T) field with a gradient of 0.3T/m, the 0.7T field with a gradient of 0.7T/m and the 6.34T field with no gradient. Exposure to the 0.3T and 0.7T fields had no effect on cell survival. Exposure to the 6.34T field decreased cell survival. Survival curves showing the combined effect of the 0.3T and 0.7T fields with radiation had a smaller mean lethal dose (D_{37}) value. The survival curve of the 6.34T field was influenced by the interval between magnetic exposure and ionizing irradiation. When the interval was 6 or 12 h, the survival curve showing the combined effect of the 6.34T field had smaller D_{37} and quasithreshold dose (D_q) values, indicating the potentiation of the radiation effect. Flow cytometric analysis indicated that exposure to the 0.3T and 0.7T fields showed no change and that exposure to the 6.34T field showed an increase in the percentage of G1 phase cells. Our conclusions were as follows: 1) magnetic fields decreased the colony-forming abilities of cultured mammalian cells; 2) magnetic fields can affect the cell cycle; 3) a stronger magnetic field strength does not always have stronger biological effects and 4) the gradient of a magnetic field may be an important factor when combined with ionizing radiation. Despite the foregoing analysis, the biological effects of magnetic fields on mammalian cells remains a complex phenomena.

Key Words: FM3A, Magnetic field, Biological effect, Combined effect, Ionizing radiation

INTRODUCTION

The biological effects of magnetic fields have been reported,[1)] and the combined effects of magnetic fields and ionizing radiation have also been investigated.[2-6)] However, there have been no conclusive results. One of the reasons is that there have been no reports on objective phenomena. Previously, we reported the biological effects of a static magnetic field on intracellular DNA amounts, using a peak strength of 5.8×10^{-2}T with a mean gradient of 0.6T/m.[4)] In order to further understand the combined effects of magnetic field exposure and ionizing radiation, we studied the biological effects of static magnetic fields, and their combined effect with ionizing radiation *in vitro* using three different magnetic field modes. These results are discussed in comparison with previously reported results.[4-6)]

MATERIALS AND METHODS

The FM3A cell line originally established from a spontaneous mammary carcinoma in a C3H

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mouse and maintained continuously as a suspension culture,[7,8] was cultured at 37 ± 0.5°C in Minimum Essential Medium (MEM) supplemented with 10% calf serum, 0.3 mg/L of glutamine and 0.12% $NaHCO_3$ as a buffer. Under this condition, the average cell population doubling time (PDT) was 12.5 h.[8] The cells used in this study were in the same exponential phase of growth. Cell survival was equated with colony-forming ability.[6] In brief, the cells were plated into 0.4% agar with MEM containing 20% fetal bovine serum (GIBCO) and were incubated at 37 ± 0.5°C for 10 days. The colonies containing more than 50 cells were counted. Every examination was repeated three times, and each set of data was compared with the control using Student's t-test. When the P value was less than 0.1, we concluded that there was a significant difference.

A dose rate of 0.3 or 0.75 Gy/min of telecobalt gamma ray unit (Toshiba Corporation, Japan) was used for ionizing radiation. Three kinds of magnetic fields were evaluated in this study. The first one had a peak strength of 0.3T with a mean gradient of the 0.3T/m, and the second had a peak strength of 0.7T with a mean gradient of 0.7T/m. These two magnetic fields were generated from the same conductive magnet machine. The third magnetic field had a peak strength of 6.34T with no gradient. The amounts of intracellular DNA and the cell cycle were analyzed with two kinds of flow cytometer. One was a cytofluorograft ICP22A (Orto., USA), which measured intracellular DNA after exposure to the 0.3T and 0.7T magnetic fields. The other was the JNM-GSX270 (Electronics Co. Ltd., Japan), which measured intracellular DNA after exposure to the 6.34T magnetic field. FM3A cells were fixed by 70% ethanol, treated with 1mg/ml RNase, stained with 50 μg/ml propidium iodide (Sigma Chemicals, St. Louis, MO, USA) then counted using a flow cytometer. The data was analyzed by the Multicycle Software cell cycle analysis program.[9]

1) Effect of magnetic field on cell survival

The cells were exposed to a given magnetic field for between 10 to 60 min. Thereafter, colony-forming ability was investigated, as described above.

2) Combined effects of magnetic field exposure and ionizing radiation

FM3A cells in test tubes were exposed to a given magnetic field at room temperature for 1 h. Immediately after this exposure, the cells were irradiated with telecobalt gamma rays at a dose rate of 0.3 or 0.7 Gy/min. Immediately after irradiation, cell survival was evaluated using the colony-forming method.

3) Influence of the time interval between magnetic field exposure and ionizing radiation

After exposure to the 6.34T magnetic field, FM3A cells were placed in an incubator for 0, 3, 6, 12 and 24 h. After each interval, the cells were irradiated and then their colony-forming abilities were measured.

4) Influence of magnetic field on cell cycle kinetics

After exposure to the magnetic field for 1 h, the cells were incubated from 0 to 24 h. Amounts of intracellular DNA were measured using a flow cytometer.

RESULTS

1) Effect of magnetic field on cell survival

The survival curves for cells after exposure to a magnetic field for up to 60 min are shown in Figure 1. The survival curve for the 0.3T and 0.7T fields indicate no effect on cell survival. On the other hand, cell survival decreased after exposure to the 6.34T field. After exposure for 60 min, the survival rate decreased to 93.7 ± 2.03%. As reported previously, after exposure to the 5.8×10^{-2}T field, cell survival rate decreased to 0.80 compared to the control after exposure as short as 10 min.

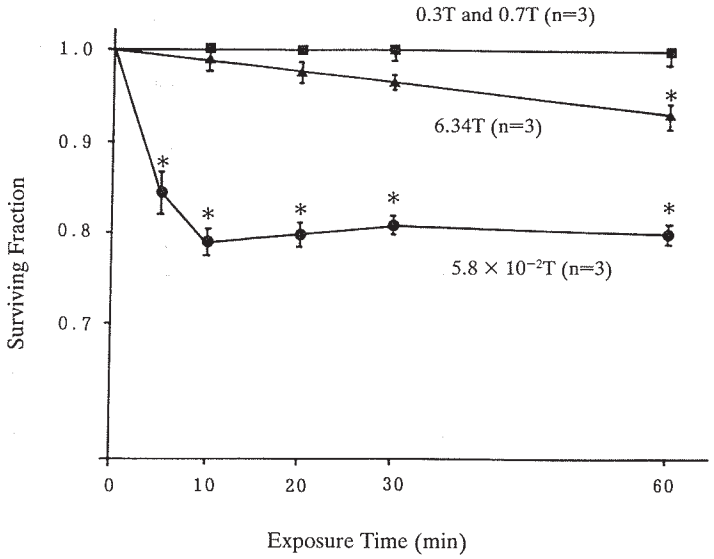

Fig. 1. Survival curve after exposure to a given magnetic field for 1 h.
— ● —: Exposure to 5.8×10^{-2}T. — ■ —: Exposure to the 0.3T and 0.7T. — ▲ —: Exposure to 6.34T.
*: Significant difference ($p < 0.1$). n: Number of examinations.

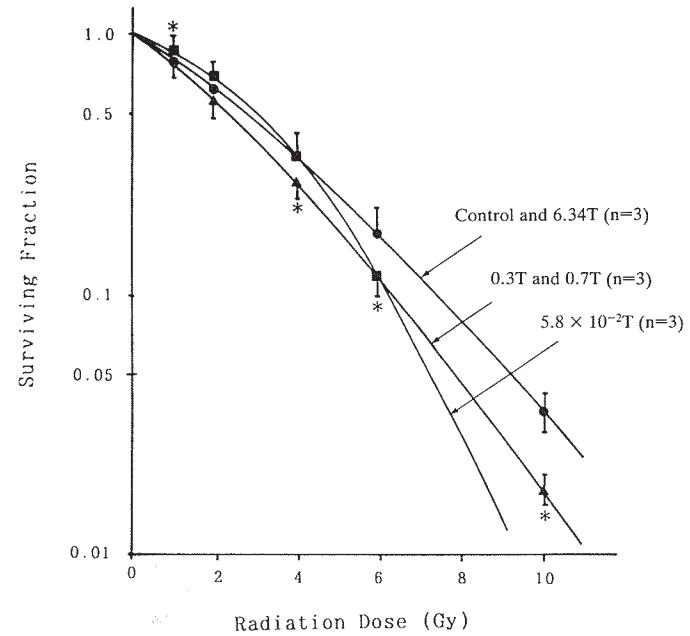

Fig. 2. Survival curve after combined exposure to a given magnetic field and ionizing radiation.
— ■ —: Exposure to 5.8×10^{-2}T. D_{37}: 2.3Gy; D_q: 2.6Gy. — ▲ —: Exposure to the 0.3T and 0.7T. D_{37}: 2.6Gy; D_q: 1.2Gy. — ● —: Control and Exposure to 6.34T. D_{37}: 3.1Gy; D_q: 1.3Gy.
*: Significant difference ($p < 0.1$). n: Number of examinations.

2) Combined effects of magnetic field exposure and ionizing radiation

Survival curves combining magnetic field exposure and ionizing radiation were corrected by each decrease of survival rate after exposure to each magnetic field alone (Fig. 2). The survival curve combining 6.34T magnetic exposure for 1 h and irradiation immediately thereafter coincided with the control (without magnetic field exposure). The D_{37} value (the dose required to reduce the cell survival fraction to 37% in the terminal exponential phase, thereby indicating the cell's radiosensitivity) and the D_q value (the intercept of the extraplotted curve with the 100% survival level, which indicates the cell's ability to repair itself following radiation damage) were obtained from each survival curve. In the control and with 6.34T exposure, the D_{37} and D_q values were the same; 3.1Gy and 1.3Gy, respectively. Survival curves for 0.3T and 0.7T exposures showed a smaller D_{37} value (2.6Gy) with a D_q value of 1.2Gy, which was the same as the control. However, the combined effect survival curve of the 5.8×10^{-2}T field showed a smaller D_{37} value (2.3Gy) and a larger D_q value (2.6Gy).

3) Influence of time interval between magnetic field exposure at 6.34T and ionizing radiation

The survival curves at intervals of 0, 3 and 24 h coincided well with the control. In contrast, the survival curves after intervals of both 6 and 12 h showed smaller D_{37} values (2.6Gy) and D_q values (1.2Gy) (Fig. 3), respectively.

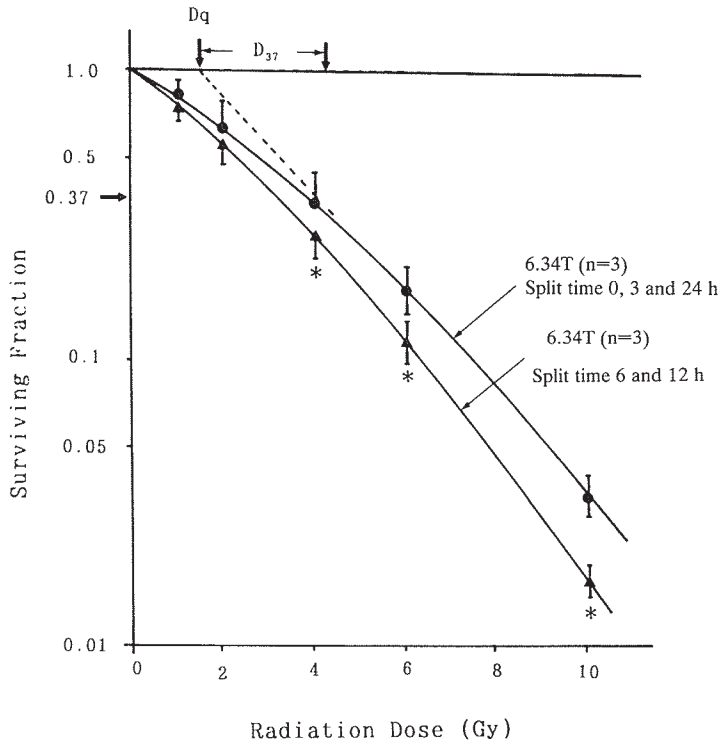

Fig. 3. Survival curve after combined exposure to a given magnetic field and ionizing radiation.
— ● —: Irradiation at intervals of 0,3 and 24 h after exposure to 6.34T. D_{37}: 3.1Gy; D_q: 1.3Gy
— ▲ —: Irradiation at intervals of 6 and 12 h after exposure to 6.34T. D_{37}: 2.6Gy; D_q: 1.2Gy
— — —: Extrapolated curve with the 100% survival level
*: Significant difference ($p < 0.1$)
n: Number of examinations

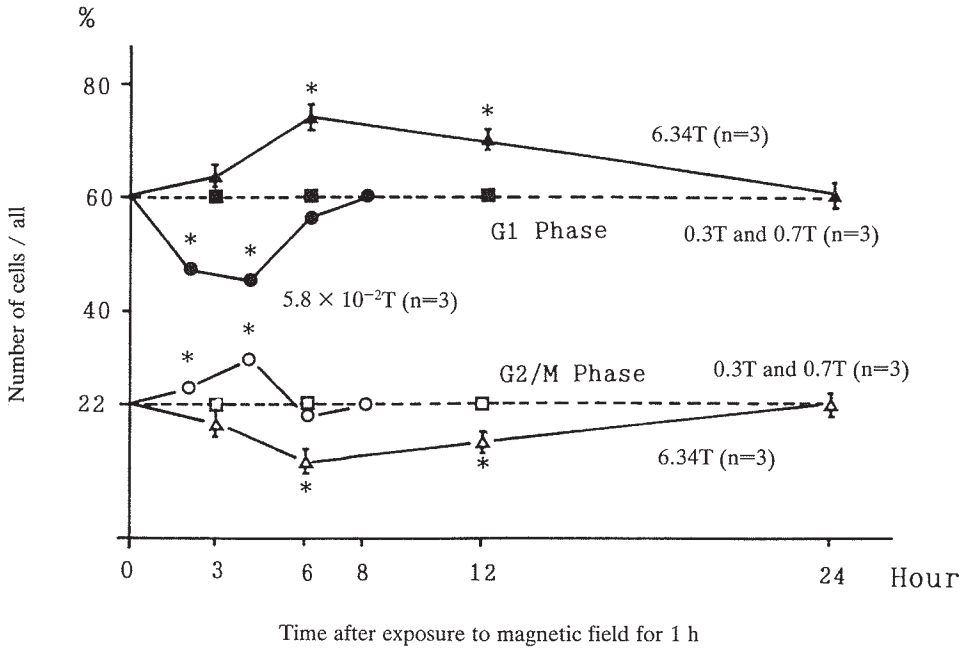

Fig. 4. Influence of magnetic fields on cell cycle kinetics. The percentage of S phase cells was constant in all four magnetic fields.
— ●, ○ —: Exposure to 5.8×10^{-2}T, — G1, G2/M —
— ■, □ —: Exposure to the 0.3T and 0.7T, — G1, G2/M —
— ▲, △ —: Exposure to 6.34T, — G1, G2/M —
*: Significant difference ($p < 0.1$)
n: Number of examinations

4) Influence of magnetic field on cell cycle kinetics

The influence of a given magnetic field on cell cycle kinetics showed complex results. In the case of the 0.3T and 0.7T fields, there seemed to be no influence. With the 6.34T field, the percentage of G1 phase cells increased and this effect continued until 24 h after exposure. As for the 5.8×10^{-2}T field, a decrease of G1 phase cells we shown, and this influence disappeared after 8 h, as observed previously. The percentage of S phase cells were constant in all four magnetic fields (Fig. 4).

DISCUSSION

There are many reports about the biological effects of magnetic field on normal cells,[10-14] cancer cell lines[4,15,16] and animals.[17,18] Assays were made measuring cell survival,[16] cell growth,[16] turnover rate,[3,14] RNA synthesis,[14] radiosensitivity,[3,14] thymidine uptake,[19] membrane depolarization,[20] membrane electrical parameters[21] and amounts of mRNA.[16] Magnetic fields were thought to have a little effect on cultured mammalian cells and planted animal tumor cells.[5,10,11,15,18] There are some hypotheses about the mechanism causing the effects which accompany mammalian cell exposure to magnetic fields. The locus of activity is suspected to be the cell membrane, the cell nucleus or the macromoleculus.[1] Until Kobayashi reported that

exposure to magnetic fields might affect intracellular DNA,[4] these hypotheses remained speculative. However, other papers insisted that no biological effects ensued following exposure to magnetic fields.[22,23] For example, mammalian visual functions have been found to be unaffected by static magnetic fields up to 1.5T.[24] Many researchers increased the strength of the magnetic fields[1] in order to obtain more obvious effects, but the results have remained obscure. We have suspected that another factor was influencing the biological effects of magnetic fields; not only the strength of the magnetic field, but also its gradient may be an important factor. In this report, we examined the biological effects of a 0.3T magnetic field with a gradient of 0.3/m, a 0.7T field with a gradient of 0.7T/m and a 6.34T field (with no gradient) and compared the results with a previous experimental report using a 5.8×10^{-2}T field with a gradient of 0.6T/m. The most effective magnetic field was the 5.8×10^{-2}T field with a gradient of 0.6T/m in terms of evident biological effects. As for cell cycle arrest and cell killing, the 6.34T field with no gradient was more effective than the 0.3T and 0.7T fields with gradients, and was less effective than the 5.8×10^{-2}T field with gradient.

When combined with ionizing radiation, the 6.34T magnetic field with no gradient was less effective than either the 0.3T and 0.7T fields with gradients. Thus, the gradient of a magnetic field may be an important factor in potentiating ionizing radiation. But examination of magnetic fields with gradients showed different results. The 0.3T and 0.7T fields with gradients had smaller D_{37} and D_q values, and the 5.8×10^{-2}T magnetic field had the smallest D_{37} value and the

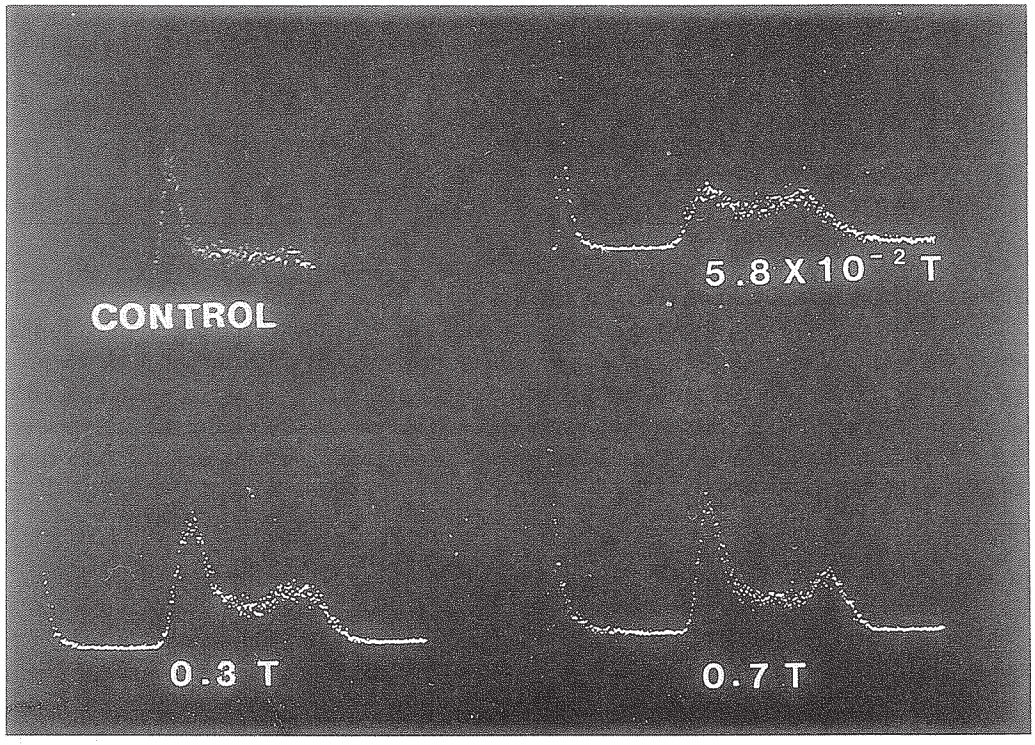

Fig. 5. DNA histograms 4 h after exposure to magnetic fields for 1 h. This data was obtained by a cytofluorograft ICP22A (Orto., USA).
After exposure to 5.8×10^{-2}T, the 0.3T or 0.7T field for 1 h, FM3A cells were incubated at 37°C for 4 h before analysis. Control: No exposure to magnetic field.

largest D_q value. The reason for this inconsistency is still unknown. It has been reported that the growth of T lymphocytes were influenced by the exposure strength of a magnetic field.[2] On the other hand, the 6.34T magnetic field with no gradient showed potentiation of radiation when the time interval was 6 or 12 h.[4]

In the case of the 5.8×10^{-2}T field, combined treatment at the same time caused the most potentiation. The time interval between magnetic field exposure and ionizing radiation made the FM3A less effective at forming colonies, and the FM3A became radioprotective after 4 h. After exposure to the 5.8×10^{-2}T field, the percentage of G1 phase cells decreased and the percentage of G2/M phase cells increased. In contrast, after exposure to the 6.34T field, the percentage of G1 phase cells increased at the expense of G2/M phase cells. Therefore, the differences between the combined effects of the 5.8×10^{-2}T and 6.34T fields might be due to the different populations of cells in the cell cycle. In the case of the 0.3T and 0.7T fields, magnetic exposure had no effect on either the cell cycle or cell survival for unknown reasons.

In conclusion, the biological effects of magnetic fields were influenced by many factors; for example, the magnetic field's strength, gradient, and the duration of exposure. Effects on the cell cycle became obvious after some hours, and the cycle returned to normal 24 h after exposure in all cases. This biological effect on the cell cycle was transient and variable with each magnetic field.

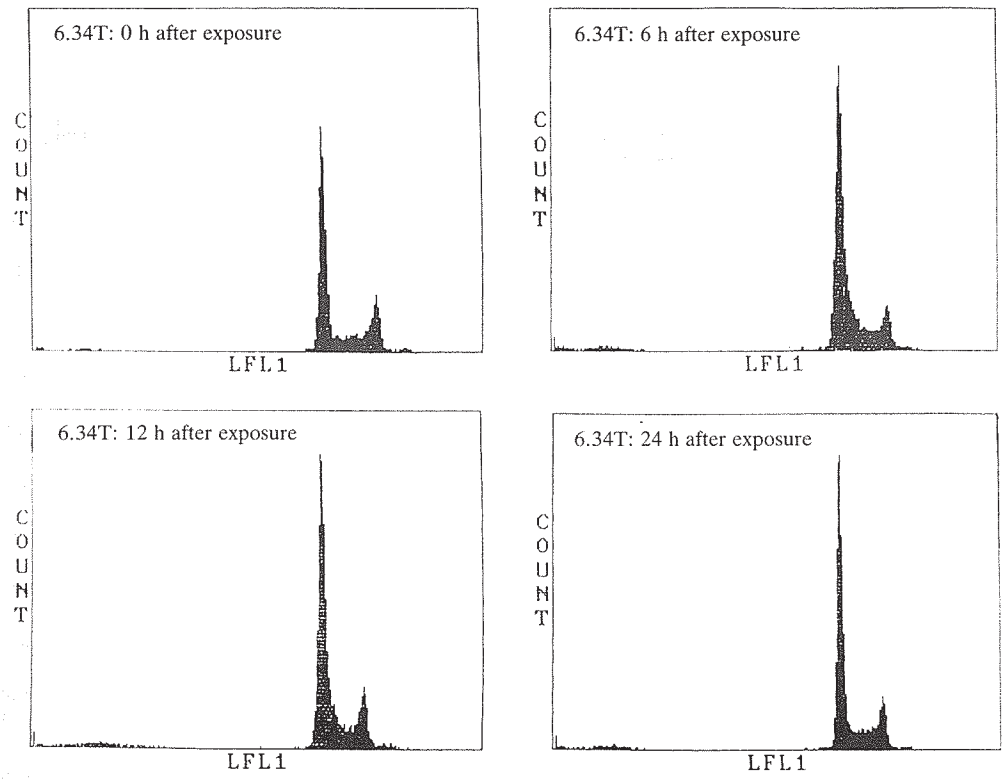

Fig. 6. DNA histograms after exposure to a 6.34T magnetic field. This data was obtained by a JNM-GSX270 (Electronics Co., Ltd., Japan).
After exposure to the 6.34T field, FM3A cells were incubated at 37°C from 0 to 24 h before analysis.

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