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EFFECT OF MAGNETIC FIELD ON L-STRAIN CELLS*

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INTRODUCTION

Magnetic field's bioeffects started with the research done by GALVANI (1737-1798) in the middle of the 18th century. The data obtained from many research works show that magnetic fields have many kinds of effects on both cells and organisms. The evaluation of the effects formed on the organisms exposed to electromagnetic or magnetic fields is difficult. The main basic cause lies in the very complex structure of the biological systems (1). Since most of the biological structures are not homogeneous, they show significant differences when exposed to magnetic or electromagnetic field. These differences were found to be effective at the molecular level, ionic level and in the structure and physiology of the membrane (1).

It has been shown that electromagnetic and magnetic fields affect also DNA in the cells. In the investigations it was found that magnetic field, sometimes either stimulated or inhibited DNA synthesis according to its strength (1).

In our research work we aimed to examine the nature of the effect of a magnetic field of 20-26 mT strength on L-Strain cells, which are tumor cells.

MATERIAL AND METHOD

The L-Strain cells used in our experiments were first defined by EARLE in 1943 (2). These cells were originally sent to our tissue culture laboratory from the Radiotherapy Department of Cambridge University in England in 1973. Continually cell lines have been prepared from this original stock in our laboratory since then.

Propagation and preparation of the cells for the experiments

The cells were propagated in Medium 199 (Gibco), supplemented with 10% Fetal Bovine Serum (Gibco), 100 mg/ml Streptomycine and 100 UI/ml Penicilline, Medium pH was adjusted to 7.2 by 4.4% $NaHCO_3$ (3,4).

Passages of the L-Strain cells were made when they formed a monolayer culture in the culture bottle, generally twice a week. For this procedure, the cells are first washed with HBSS (Hank's Balanced Salt Solution), 2,5% Trypsin is added to the culture bottle and after the cells are lifted from the bottom they are transferred to the centrifuge tube. Trypsin is inactivated by the addition of a certain amount of medium. The cells in suspension are centrifuged at 1500 rpm/min for 3 minutes and supernatant is decanted. Amount of medium is added to the pellet and a cell suspension is again obtained. These cells in suspension are counted at the hematocytometre and they are diluted according to the requested concentration.

Characteristics of magnetic field

The cells were exposed to a magnetic flow density of 20-26 mT under exactly 3 cm magnet in the form of a rectangular prisma with dimensions of 0.45 m – 0.065 m – 0.022 m in the magnetic system prepared by JINR laboratories, Magnetic field group.

The periods of the cells kept in this field were changed in order to find magnetic field's optimum value in causing the decrease in cell number.

In our experiments the effect of the magnetic field was investigated in two steps:

a) The effect of magnetic field on cell number:

For this experiment 500.000 cells, as 100.000 cells/ml L-Strain cells, were seeded into each petri dish. They were kept in a desicator under a mixture 5% CO_2 and 95% air at 37°C and incubated at pH 7.2. After 24 hours the petri dishes were divided into groups. The experimental groups were exposed to the magnetic field for 1, 2, 3 and 4 minutes. After 24 and 48 hours, these cells and those of the control group were treated with 2.5% Trypsin and then they were centrifuged. After the resulting cell pellet was suspended in a specific amount of medium, the suspension of cells were treated with Trypan blue and the number of cells in each petri dish were determined by counting them on the hemacytometer. The results of the countings were evaluated statistically by Duncan Single Direction Variance Analysis test.

b) The effect of magnetic field on DNA synthesis:

For this experiment L-Strain cells were seeded in equal amounts on coverslips in petri dishes 5 ml of medium was added to the petri dishes and they were incubated in a desicator under a mixture of 5% CO_2 and 95% air at 37°C and 7.2 pH for 24 hours. At the end of this period the petri-dishes were separated into groups again. The experimental groups were exposed to the magnetic field for 1, 2, 3 and 4 minutes. To the petri dishes of L-Strain cells of the control group and the ones exposed to the magnetic field, 3 μCi/ml ^{3}H-Thymidine (methyl-H-Thymidine, TRA 120, 1μCi Amersham, England) was added after 24 and 48 hours. After 1/2 hour ^{3}H-Thymidine was removed from the petri dishes and fixative (1:3 v/v glacial acetic acid and ethyl alcohol) was added to them. After a fixation period of 15 minutes, the cells were washed with 70 % of ethyl alcohol and then dried. The cover slips with cells seeded on them were attached on jelatinized microscope slides and their autoradiograms were prepared. For this process the slides were covered with K2 jel emülsion(Ilford) and kept at 4°C in an icebox for an exposure period of 5 days. At the end of this period, the slides were immersed in D19 Developer (Kodak) and Fixative (Kodak) and then they were stained with Giemsa for 5 minutes. The stained cells were examined under the microscope and the amount of labelled and unlabelled cells were counted. The data obtained were evaluated by Single Direction Variance Analysis and Duncan test.

RESULTS AND DISCUSSION

Any literature about an experiment made with L-Strain cells and magnetic field is not available. The effects of the magnetic field with a dose of 20-26 mT, used in the experiments on fibrosarcoma type L-Strain cells were investigated in two steps. 1) The effect on cell number 2) The effect on DNA synthesis.

1- The effect of the magnetic field on cell number

In the experiments made to determine the optimum value of the decrease in cell number caused by the magnetic field, in the groups exposed for 1 and 4 minutes in comparison to the controls, statistically ($P<0.05$) significant decrease per ml in cell number was observed. In the ones exposed to the magnetic field for 2 and 3 minutes a significant difference from the controls was not observed (Tab. 1, Fig. 1).

Table 1: Effect of magnetic field on cell number after 24 hours.

Exposure time of magnetic field (minute)	Control	1	2	3	4
Cell number/ml	218033.3 a*	122360.7 b	165000.0 b	159444.0 b	139722.0 b

*Means with the same letter are not significantly different by Duncan multiple range test ($P<0.05$).

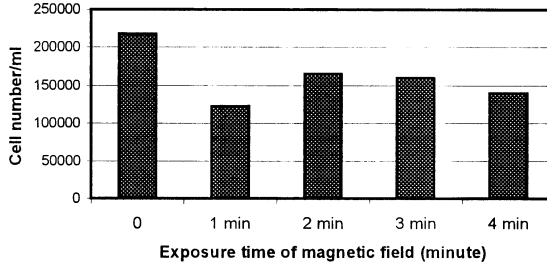

Fig 1: Effect of magnetic field on cell number after 24 hours.

In order to determine the magnetic field's effect after 24 hours, another experimental series was performed. When the number of the cells exposed to the magnetic field of 20-26 mT for 1, 2, 3 and 4 minutes were counted after 24 and 48 hours, the decrease observed 1 and 4 minutes was statistically significant ($P<0.05$) and the value for 2 and 3 minutes were statistically insignificant (Tab. 2, Fig. 2).

Table 2: Effect of magnetic field on cell number after 24 and 48 hours.

Exposure time of magnetic field (minute)	Cell number/ml	
	24 hour	48 hour
Control	236666.7	341133.3
1	138333.3	196666.7
2	177777.3	381666.7
3	143333.3	283333.3
4	106666.7	263333.3

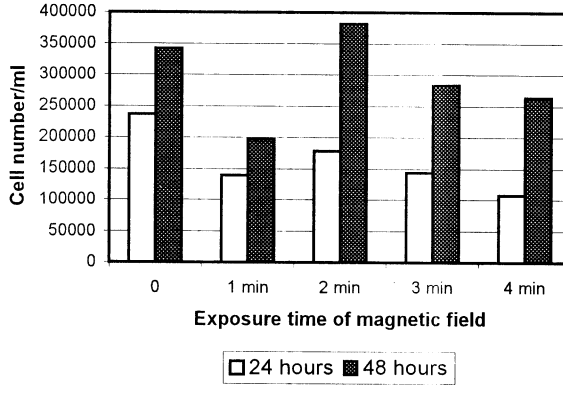

Fig 2: Effect of magnetic field on cell number after 24 and 48 hours.

According to these results, the multiplications of the L-Strain cells exposed to the magnetic field of 20-26 mT for 1 and 4 minutes are inhibited during a period of 24 and 48 hours.

2- The effect of magnetic field on DNA synthesis

The decrease in DNA synthesis of the cells, in comparison to controls exposed to a magnetic field at a dose of 20-26 mT for 1 and 4 minutes showed a parallelism with the decrease in cell number. On the other hand, in the cells exposed to the magnetic field for 2 minutes, an increase in DNA synthesis close to that of controls was especially observed after 48 hours (Tab. 3, Fig. 3).

Table 3 : Effect of magnetic field on labelling index after 24 and 48 hours.

Exposure time of magnetic field (minute)	Labelling index %	
	24 hour	48 hour
Control	100	100
1	57.33	76.47
2	61.33	98.53
3	45.33	75.00
4	53.33	67.64

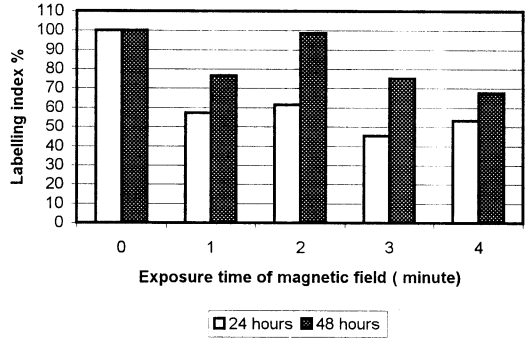

Fig 3 : Effect of magnetic field on labelling index after 24 and 48 hours.

Many research works are present on the effect of magnetic and electromagnetic fields on DNA and RNA synthesis, enzyme activities and Ca^{+2} ions of different cell types. Ovary cells of Chinese hamster cells were exposed to 10 Hz, 20 μT pulse 25 μ sec. wide magnetic field in a studies done in 1986. In this work, it was observed that ^{3}H-Thymidine incorporation increased 13% in these cells. When the dose was raised to 100 Hz, the increased in ^{3}H-Thymidine incorporation increased to 30%. When the magnetic field's strength was raised to 200 μT or more 80 % inhibition of ^{3}H-Thymidine incorporation was observed (1). In research done in 1993 where 6 hours after the mouse fibroblasts were exposed to 50 Hz electric field or 2 mT magnetic field for an hour while a small but a significant decrease in DNA amount occurred, an increase in those exposed to electric field was observed. When the cell cycle analysis of these cells was analyzed, it was observed that the magnetic field increased the number of the cells in G_1, whereas the electric field induced the collection of cells in S, G_2 and M (1). In another experiment done in 1994, both bidirectional and unidirectional waves were given to chicken tendon fibroblasts in primary culture for a period of 24 hours. 2-3 days after this treatment, it was observed that both waves stimulated DNA synthesis and growth. On the other hand, after 3-4 days, they observed that the growth of the cells exposed to magnetic field, was slowed down (1).

By the data obtained from this research, we can conclude that the increase or decrease in DNA synthesis changes according to the magnetic field's strength, period of exposure and cell type. This conclusion verifies the results obtained from our experiments.

It was shown that electromagnetic field at low frequency (7-8 mT, 20 Hz) always inhibited the proliferation and speeded the differentiation of human fibroblasts (5). At the same time electromagnetic field at low density causes the fluctuation of Ca^{+2} ions (6). It was observed that RNA synthesis of CCRF-CEM cells, obtained from T-lymphoblastoid cell type was twice as increased as of the controls when exposed to 72

Hz magnetic field 30 minutes in vitro. This synthesis increased 3.2 times more than that of the control ones, when the were exposed to the same for 2 hours (7). Research work is present showing the change in enzymatic activity by the magnetic field in vitro. Magnetic field above 7-8 T decreases glutamic dehydrogenase enzyme, and 6 T magnetic field increases catalase activity (8). It was shown that when mouse fibroblasts were exposed to 0.61 T magnetic field statistically a significant increase of 0.5-1.0 times more increase occuried in both DNA and protein synthesis (8).

Many hypothesises are put forth about the interaction mechanism of cells and electromagnetic or magnetic field. Work is present where it is asserted that for the realization of the interaction, an electric field or flow should be present in a part or the whole of the cell.

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Улакоглу Г. и др.
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Воздействие магнитного поля на клетки L-штамма

Эффекты магнитного поля в настоящее время широко используются во многих областях науки, особенно в медицине. В настоящем исследовании клетки L-штамма типа фибросаркомы подвергались воздействию магнитного потока силой 20–26 мТл в течение одной, двух, трех и четырех минут. Клетки L-штамма, подвергавшиеся воздействию магнитного поля, через 24 и 48 часов были сосчитаны и сравнены с контрольными образцами. При этом в группах клеток, подвергавшихся экспонированию в магнитном поле в течение одной и четырех минут, было обнаружено существенное уменьшение ($P < 0,05$) числа клеток по сравнению с контрольной группой.

Процент синтеза клеток L-штамма, экспонировавшихся одну и четыре минуты, также существенно уменьшался по сравнению с контрольными образцами.

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Effect of Magnetic Field on L-Strain Cells

The effects of electromagnetic and magnetic fields are currently being made useful in many fields, especially in medicine. In this research work, L-Strain cells which are a type of fibrosarcoma cells were exposed to a magnetic flow of 2–26 mT in periods of 1, 2, 3 and 4 minutes. The L-Strain cells, which were exposed to the magnetic field for these periods, were counted after 24 and 48 hours, when compared with the controls, it was observed that in groups of 1 and 4 minutes exposure a significant decrease ($P < 0.05$) in the number of cells occurred.

The per cent of labelling index of L-Strain cells exposed to the magnetic field for 1 and 4 minutes decreased significantly also in comparison to the controls.

The investigation has been performed at the Dzhelepov Laboratory of Nuclear Problems, JINR.

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