

Influence of constant, alternating and cyclotron low-intensity electromagnetic fields on fibroblast proliferative activity in vitro

Einfluss schwacher elektromagnetischer Felder (konstant, wechselnd bzw. durch Zyklotron erzeugt) auf die Fibroblastenproliferation in vitro

Abstract

Available data allow assuming the presence of stimulation of reparative processes under influence of low-intensity electromagnetic field, commensurable with a magnetic field of the Earth. Research of effects of low-intensity electromagnetic fields on fibroblast proliferative activity in human lungs in cell culture was performed.

The influence of a constant electromagnetic field, an alternating electromagnetic field by frequency of 50 Hz and cyclotron electromagnetic field with identical intensity for all kinds of fields – 80 mcTI – on value of cellular mass and a correlation of live and dead cells in culture is investigated in three series of experiments. We used the universal electromagnetic radiator generating all three kinds of fields and supplied by a magnetometer which allows measuring the intensity of accurate within 0.1 mcTI including taking into account the Earth's magnetic field intensity.

The peak value for stimulation cellular proliferation in the present experiences was two-hour influence by any of the specified kinds of electromagnetic fields. The irradiation by cyclotron electromagnetic field conducts positive dynamics in growth of live cells (up to $206 \pm 22\%$) and decreases the number of dead cells (down to $31 \pm 6\%$). Application of cyclotron magnetic fields promoted creation of optimum conditions for proliferation. As a result of researches we observed the reliable 30% increase of nitro-tetrazolium index (in nitro-tetrazolium blue test) after irradiation by cyclotron electromagnetic field in experience that testifies to strengthening of the cell breathing of living cells.

In our opinion, it is necessary to pay attention not only to a pure gain of cells, but also to reduction of number dead cells that can be criterion of creation of optimum conditions for their specific development and valuable functioning.

Keywords: electromagnetic field, human lung fibroblasts, cell culture, stimulation of growth, decrease of number of dead cells

Zusammenfassung

Vorhandene Daten erlauben die Schlussfolgerung, dass reparative Prozesse unter dem Einfluss eines schwachen elektromagnetischen Felds vergleichbar der Stärke des Erdmagnetfelds stimuliert werden. Deshalb wurden weitere Untersuchungen zum Einfluss schwacher elektromagnetischer Felder auf die Proliferation von Fibroblasten humanen Lungengewebes in der Zellkultur durchgeführt.

Geprüft wurde der Einfluss eines konstanten elektromagnetischen Felds, eines wechselnden elektromagnetischen Feldes mit einer Frequenz von 50 Hz und eines elektromagnetischen Feldes eines Zyklotrons mit identischer Intensität der drei Felder von mcTI 80 auf den Massezuwachs und die Korrelation zwischen lebenden und abgestorbenen Zellen

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in der Zellkultur. Die drei Felder wurden mit einem Universalgenerator erzeugt, dessen Intensität mit einem Magnetometer einer Messgenauigkeit von 0.1 mCTI ermittelt wurde.

Das Maximum der Zellproliferation wurde bei allen drei Feldarten nach 2 h erreicht. Durch das Zyklotron wurde die Vermehrung um bis zu $206 \pm 22\%$ gesteigert und die Anzahl abgestorbener Zellen bis auf $31 \pm 6\%$ reduziert, d.h. durch das elektromagnetische Feld des Zyklotrons wurden optimale Bedingungen für die Zellproliferation erreicht. Nach Einwirkung des Zyklotrons konnte mit der Nitrotetrazolium Blau-Methode ein 30%iger Anstieg des Nitrotetrazolium-Index zuverlässig nachgewiesen werden, der die Zunahme der Zellenatmung bestätigt.

Unserer Meinung nach ist es wichtig, als Kriterium für optimale Bedingungen nicht nur den Zellzuwachs, sondern auch die Anzahl abgestorbener Zellen zu bewerten.

Schlüsselwörter: elektromagnetisches Feld, humane Lungenfibroblasten, Zellkultur, Wachstumsstimulierung, Reduktion der Zahl abgestorbener Zellen

Introduction

Today, various kinds of electromagnetic effects are widely used in theory and practice of medicine, and according to specific requirements a wide range of electrotherapy equipment was established. Their use is accompanied by both positive and negative effects, mainly having metabolic nature [1], [2], [3], [4], [5]. In particular, there is both stimulating and inhibiting effect of electromagnetic fields on the growth of tissue culture [6], [7], [8], [9]. Mechanisms of interaction of magnetic and electromagnetic fields with different cellular structures of the organism are inadequately studied, as evidenced by the many hypotheses attempting to give their theoretical interpretation [8], [10], [11], [12], [13]. At the same time, available data allows assuming the presence of stimulation of reparative processes under the influence of low-intensity electromagnetic radiation that is commensurate by intensity with Earth's magnetic field [4]. In the present work the effects of low-intensity electromagnetic fields on the proliferative activity of human lung fibroblasts in cell culture are given.

Methods

In three series of experiments there was studied the effect of constant electromagnetic field (CoEF), alternating electromagnetic field (AEF) with frequency of 50 Hz, and cyclotron electromagnetic field (CyEF) with the same intensity for all types of fields – 80 mCTI – on the magnitude of increase of cell mass and the ratio of live and dead cells in culture. To create a pilot model the suspension of fibroblasts in Eagle growing medium (supplemented with 10% calf serum) was poured by 15 ml in Carrel vessels (seeding concentration of 80–120 cells/ml) and thermostatically controlled at 37 °C for 16–18 hours to complete attachment of viable cells.

After the change of growing medium vessels with the experimental samples were exposed to CoEF, AEF or CyEF

for 1, 2, 4, 6 hours and remained at 37 °C for 24 hours to form a confluent monolayer. To determine growth and ratio between dead and living cells the monolayer was resuspended in supportive Eagle environment with 0.02% of chemopsin solution in phosphate buffer, after which the cells were stained with 0.2% trypan blue. Counting of cells was produced in cell of Goryaev with 20x lens and 10x eyepiece. Total number of cells in the vessel (X) was calculated using the formula:

$$X = A \times B \times C \times 10,000,$$

where A is the number of cells in 25 large squares of the cell of Goryaev; B is the volume of growth medium where the cells were resuspended; N – dilution of suspension as a result of staining with trypan blue solution, 10,000 is a constant value.

From each vessel produced 5–6 independent cell counts formed a monolayer. The data are processed statistically using Student's criterion and the confidence interval for the probability levels ($p < 0.05$). There were made 5–6 independent cell counts of formed monolayer from each vessel. The data was processed statistically using Student criterion and confidence interval for the probability levels ($p < 0.05$). A universal electromagnetic transducer was used in the experiment that generates all three types of fields, equipped with a magnetometer, which allowed measuring the intensity of fields with an accuracy of 0.1 mCTI, including the field intensity of the Earth. To exclude the interaction of magnetic field of the Earth and radiators in the experiments, the direction of the emitter magnetic field vector has was strictly perpendicular to the direction of magnetic field vector of the Earth.

Results and discussion

In the experiment with the CoEF statistically significant increase in the number of living cells was noted after irradiation for one and two hours (Table 1). The parallel counting of dead cells showed a sharp jump in their quantity after two hours of irradiation regarding control (Table 2). Longer exposure did not lead to reliable experienced significant changes in the cultures compared with control.

Table 1: Quantity of living cells with respect to control (taken as 100%)

Exposure time (hours)	CoEF %	AEF %	CyEF %
1	130±17	85±11	154±12
2	152±22	98±14	206±22
4	117±5	81±25	160±16
6	62±6	80±22	133±25

Table 2: Quantity of dead cells with respect to control (taken as 100%)

Exposure time (hours)	CoEF %	AEF %	CyEF %
1	115±27	67±4	68±3
2	600±26	54±5	79±5
4	80±32	29±3	31±6
6	142±28	85±3	61±6

Irradiation with AEF for one, four and six hours showed reliable decrease in the number of living cells compared to control (Table 1). During exposure within one, two and four hours a significant decrease in the number of dead cells is defined (Table 2). The ratio of dead cells to living in culture after one, two and four hours tended to be reduced by 22–64% compared to control due to more pronounced decrease of the former. Six-hour exposure led to the increase in ratio of dead cells to living by 6% higher than at control.

In the experiment with the CyEF there was a significant increase in the number of living cells compared to control after exposure within one, two, four and six hours (Table 1). There was significant decrease in the number of dead cells after exposure within one hour (Table 2). A stable reduction dynamics of the ratio of dead to living cells in culture was revealed.

From the experiments with the use of CoEF and AEF it follows that changes in the culture are caused not by the increase of cell mass but by the decrease or increase in the number of dead cells in culture. Exposure to CoEF for more than two hours, from our point of view, leads to the adaptation to external factor and the lack of reaction to

it. Exposure to AEF evokes a response of cells during four-hour exposure, but during six-hour exposure the adaptation occurs.

In the known works on reparative osteogenesis there is primarily considered the absolute growth in cell mass under the influence of some physical factor [14]. Certainly this argument is very important in terms of stimulation per se. But, in our opinion, one should focus not only on the absolute growth in cells but also on the reduction in number of dead cells, what may be a criterion of establishing optimal conditions for their specific development and full operation. Impact, based on the effect of the cyclotron magnetic resonance has led to more pronounced changes in the process of fibroblast proliferation. There has been a reliable growth of living cells in all the expositions with reduction of number of dead cells, which apparently indicates a lack of development of adaptation processes during CyEF exposure.

Summarizing the preliminary results of work with the culture of human fibroblasts, we want to highlight a few points obtained in the analysis of results. Firstly, the peak value for the stimulation of cell proliferation in these experiments was a two-hour exposure of any of these types of electromagnetic fields. Secondly, the exposure with CyEF resulted in positive dynamics of growth of living (up to 206±22%) and reduce in number of dead cells (down to 31±6%). Application of cyclotron magnetic fields promoted the creation of optimal conditions for cell proliferation.

In our opinion, it is necessary to pay attention not only to a pure gain of cells, but also to reduction of number dead cells that can be criterion of creation of optimum conditions for their specific development and valuable functioning. As a result of researches we observed the reliable 30% increase of nitro-tetrazolium index (in nitro-tetrazolium blue test) after irradiation by cyclotron electromagnetic field in experience that testifies to strengthening of the cell breathing of living cells.

However, these data still require further verification, and methodological issues require working out in experiments on animals.

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