

## The Effect of Time-Varying Low Intensity ELF Magnetic Field on Growth Rate of Invasive Ductal Carcinoma on Balb/C Mice

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### Abstract

**Background:** Extremely low frequency (ELF) magnetic fields have been considered by researchers as a noninvasive treatment approach. Citing documented biological effects of ELF magnetic fields in recent studies, we investigate the effect of low intensity ELF magnetic field on the treatment of invasive ductal carcinoma tumor.

**Materials and Methods :** Female Balb/c mice were transplanted with invasive ductal carcinoma tumors. Extremely low frequency magnetic field (100 pulse per second EMF (150  $\mu$ T)) with static magnetic field (400  $\mu$ T) as a background exposure was applied as a combined protocol for the treatment of the tumors. Other treatment groups in this study were control group (with no treatment), static magnetic field exposure group at 400  $\mu$ T (sham group), chemotherapy by bleomycin intratumoral injection group and finally a group with the combined protocol after intratumoral injection of bleomycin. All groups were treated in the magnetic field for 10 minutes per day for 12 days.

**Results:** The results of this study showed that static and ELF magnetic field had no significant effect on the invasive ductal carcinoma tumor growth process. But static magnetic field indicated a significant decrease in tumor growth in the last days of the follow up. Also combined protocol significantly increased toxic effect of bleomycin as a chemotherapy agent.

**Conclusion:** Low intensity static magnetic field can be considered as an antitumor factor. Also combined magnetic fields can be used for increasing the effects of chemotherapy in cancerous patients.

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### Introduction

Given the importance of examination of therapeutic effects of magnetic fields as a noninvasive treatment, various tests have been conducted in the past two decades, such as magnetic field effects on the broken bones healing; treatment of headaches, ulcers, pain reduction and etc [1-4]. In addition, carcinogenesis effects of low-frequency magnetic fields have been studied for years and in this regard, conflicting results have been reported indicating the carcinogenesis nature of magnetic fields. Some reports indicate that exposure to 50 Hz magnetic field with intensity of 1m Tesla (T), as well as the intensity of 20-1000  $\mu$ T, for 1-24 hours (5 minutes on and 10 minutes off), creates DNA strand breaks and chromosomal damages [5-7]. Also some studies have been conducted to review the effect of field on tumor progression in which the field either has not shown any significant effect or has led to more growth of tumor than the control group.

During the studies conducted on the effects of combined, static and time varying sinusoidal 50-Hz magnetic fields on the treatment of tumor and cancer cells, according to the parameters of the magnetic fields, different results have been

reported that suggest the magnetic field can effect on the tumor growth rate or in vitro tests on the apoptosis induction and decrease of cell proliferation [8, 9]. The main intended parameters are the intensity of total applied field, the duration of radiation per day and the number of treatment days. Exposure by 50 Hz magnetic field with flux density of 100  $\mu$ T continued for 24 hours per day, 7 days a week and over 13-27 weeks consequently, no significant effect on the tumors was observed [10]. Thus, according to the more activity of chemotherapy drugs such as bleomycin at presence of free radicals, the importance of simultaneous examination of the effect of field and drug on the tumor is evident.

Considering the importance of chemotherapy in the treatment of cancer and the side effects of chemotherapy drugs in high doses used, the researchers have intended to determine some methods to reduce the dose of drug as well as increase its toxic effects. For this purpose, some tests have been conducted to examine the effects of time varying, combined static and time varying magnetic field on the effect of different chemotherapy drugs to treat various human and

animal tumors [11, 12]. For further evaluation of the effects of the magnetic field in this study, the effect of time-varying magnetic field with a frequency of 100 pulses per second and 150  $\mu\text{T}$  on treatment of breast adenocarcinoma tumors of Balb/c mice has been studied.

### Materials and Methods

In this experimental study, using the results of similar articles, 40 adult female Balb/c mice of 6 to 8-week old were prepared from the Pasteur Institute in Tehran, and 10 days after purchase and maintenance in the animal house environment, they were induced to tumor through transplantation.

The tumor used in this study was invasive ductal carcinoma of murine breast which was prepared from the Pasteur Institute in Tehran. The tumor was placed in the animal side after surgery. Ketamine 10%, Xylazine 5% and injection saline 85% were used to anesthetize the animals. Finally, the animal was transferred to the animal house and required care was taken.

After about 2 weeks of tumor transplantation when the diameter of tumors was 8 mm, they were divided into 5 groups each containing 8 animals. In this study, we reviewed low-intensity fields in 12 consecutive days as chronic radiation to reduce the damage to healthy tissues which are caused by the exposure to magnetic field. Also, we used rectified magnetic field to increase the effect of field on polar bleomycin molecule.

Treatment groups in this research are: control group (no radiation), radiation group of 400  $\mu\text{T}$  static magnetic field as sham magnetic field, radiation group of time-varying magnetic field with a frequency of 100 pulses per second and intensity of 150  $\mu\text{T}$  and static background exposure of 400  $\mu\text{T}$ , chemotherapy alone group with intratumoral injection of bleomycin and radiation group of combined magnetic field (static background and time varying) with intratumoral injection of bleomycin. One reason to use bleomycin in this study is the increased activity of this drug at presence of free radicals and active ions; because magnetic fields affect the production of free radicals [8], and thus the effectiveness can be assessed better.

Bleomycin (the chemotherapy drug used) supplied by NIPPON KAYAKU Co, Tokyo, and after dissolving the drug in normal saline (1.5 mg/ml), 0/016mg/ml of the drug was injected into the tumor. Intratumoral injection is recommended to reduce drug side effects on healthy tissues and increase the drug concentration in the tumor area. Two minutes after injection, the animals were exposed by combined magnetic field.

First, magnetic field generator was designed and built (Fig. 1). These systems was a C-shaped iron core and a coil mounted on a core side with 1000

round wires with opening dimensions of 10x12cm and width of 6 cm, which was connected to the power rheostat of the city through full-wave rectifier (diode bridge). Static magnetic field was measured for sham group of 400  $\mu\text{T}$ .

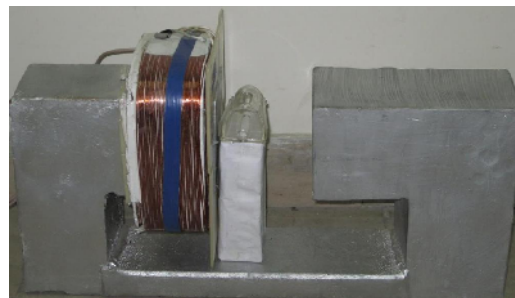


Figure 1. Iron core and coil and mouse placement chamber

In this study, combined static magnetic field of 400  $\mu\text{T}$  was considered as background and uniform half-sinusoidal magnetic field with frequency of 100 pulses per second and peak intensity of 150  $\mu\text{T}$  was considered for 10 minutes per day for 12 consecutive days [13]. The static magnetic field of 400  $\mu\text{T}$  was produced due to the ferromagnetic core get magnet, by passing rectified magnetic field lines through the core, which is considered as background exposure in total tests.

Tumor diameter was measured for 30 days, Tertian, by a digital caliper with an accuracy of 0/02mm and the volume was obtained from the relation of  $V = ab^2\pi/6$  where a and b are respectively large and small diameters of tumor. The results were reported as normalized volume obtained from the following relation.

$$\text{Normal volume in the nth day} \\ = \frac{\text{volume in the nth day after the treatment}}{\text{volume in the day of treatment}} \times 100$$

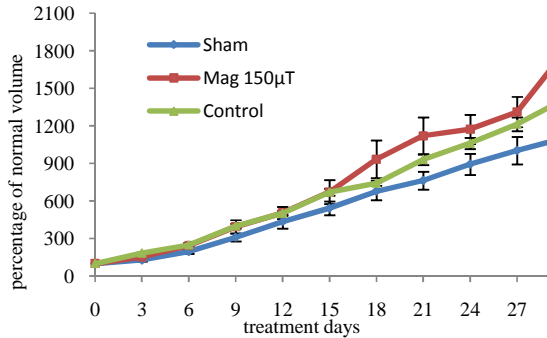
In this study, statistical analysis was performed using SPSS-16 software. Gaussian distribution of data were evaluated through Kolmogorov-Smirnov test and it was shown that the data in the experimental groups with accuracy of  $p < 0/05$  have a normal distribution Thus, ANOVA was used in the intergroup statistical investigation and complementary LSD test was used to review the significant difference between both groups.

### Results

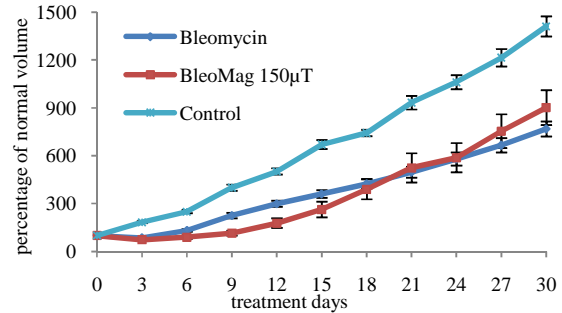
The research results were analyzed in two stages, the examination of effect of combined static and time varying half-sinusoidal magnetic field with 100 pulses per second on tumor growth, the examination of effect of static magnetic field (background exposure) on tumor growth and the examination of effect of combined magnetic field

on the toxicity of bleomycin. The results of which were presented in (Fig 1 & 2) as follows.

According to the graphs shown for the five treatment groups, there was no significant difference between the normalized volume of the control groups and combined magnetic field during the 30 days of study. There was a significant difference between the normalized volume for sham and control group on the days 3, 9, 15, 21, 24, 27 and 30 with  $p < 0/05$ , but no significant difference was observed on the other days.



**Figure 1.** Normalized volume by percentage and the day of treatment in the 3 groups. : sham (static magnetic field with intensity of 400µT) : the control group and : the combined static magnetic field of 400µT and time-varying magnetic field of 150 µT with frequency of 100 pulses per second. Data are shown as Mean ± SEM.



**Figure 2.** Normalized volume by percentage and the day of treatment in the 3 groups. : control, : Bleomagnetics (magnetic field combined with bleomycin) and : Bleomycin. Data are shown as Mean ± SEM

In addition, there was a significant difference between the normalized volume for sham and combined magnetic field on the days 21, 24, 27 and 30. A significant difference was observed between the normalized volume for treatment groups of Bleomycin and Bleomycin with combined magnetic field on the days 6, 9, 12 and 15. According to the results, it was observed that combination of two alternating magnetic field of 150 µT with a frequency of 100 pulses per second and static magnetic field of 400 µT, has no effect on tumor growth. The data on sham group showed the effect of static magnetic field of 400 µT on tumor growth, which reduced tumor growth.

**Table 1:** Changes in normalized volume of treatment groups by percentage compared to the zero day.

Groups	Sham (magnetic field) Mean±SEM	Bleomycin Mean±SEM	Bleomagnetic Mean±SEM	Control Mean±SEM	Combined magnetic field Mean±SEM
0	100	100	100	100	100
3	132.39 ± 6.8	82.56 ± 4.73	71.7 ± 5.76	175.74 ± 4.14	158.17 ± 13.57
6	209.85 ± 17.86	125.93 ± 8.43	90.19 ± 13.79	245.28 ± 8.25	250.49 ± 27.56
9	315.28 ± 33.51	230.68 ± 20.03	110.26 ± 17.27	399.95 ± 12.88	423.17 ± 61.98
12	415.11 ± 56.79	299.28 ± 22.75	157.95 ± 27.73	504.23 ± 21.19	529.14 ± 52.83
15	539.35 ± 63.37	363.75 ± 29.9	220.47 ± 37.02	698.13 ± 11.89	714.93 ± 108.18
18	639.99 ± 75.38	422.3 ± 37.06	357.32 ± 61.66	771.79 ± 7.29	1005.3 ± 168.98
21	699.16 ± 66.88	489.85 ± 42.99	478.8 ± 85.34	981.89 ± 30.45	1168.2 ± 171.88
24	796.49 ± 62.27	568.64 ± 51.22	519.34 ± 69.4	1117.09 ± 29.25	1213.53 ± 124.79
27	865.68 ± 65.08	640.41 ± 51.39	683.1 ± 87.75	1278.83 ± 39.09	1327.7 ± 118.44
30	965.05 ± 63.66	730.86 ± 49.78	844.04 ± 98.12	1491.92 ± 37.61	1860.76 ± 240.03

**Discussion**

The results obtained in this study showed that combined half-sinusoidal 100 Hz magnetic field by intensity of 150 µT and static magnetic field of 400µT has no significant effect on tumor. This result is consistent with the research on the human adenocarcinoma cell lines conducted by Tofani et al in 2001. In this study, combined static and time varying sinusoidal 50 Hz magnetic field with a total intensity of 3.59mT were used and no significant

effect was observed [8]. However, another study conducted by the same group, has reported combined static and time varying sinusoidal 50 Hz magnetic field with an average intensity of 5.5 mT have had a significant effect on reduction of the growth rate of breast cancer [9] and this is inconsistent with the results presented in this study. Lack of field effect in this study may be due to the low intensity of the applied field or because of large tumor size on the

treatment day; because the tumor cells will get resistant to the treatment with tumor progression. Ruggier et al. performed a test in 2004 to study the effect of static magnetic field on angiogenesis in chick embryo membranes, in which the static magnetic field with 3 hours chronic radiation at intensity of 200mT was used, which could significantly reduce the membrane angiogenesis [14]. The results of this research showed that static magnetic field of 400 $\mu$ T alone could significantly reduce the tumor growth rate compared to the control group. Thus, the reduction of tumor growth rate can be attributed to tumor angiogenesis reduction by the static field. The results obtained from the effect of static magnetic field with an intensity of 1mT which was topically applied to rabbits for 10 minutes [15], increased blood flow after the exposure, which leads to a result inconsistent with the results obtained in this study.

The applied combined magnetic field enhances the therapeutic effect of bleomycin during the days of magnetic field exposure. This observation has been performed consistent with the results reported in the previous works. In a study conducted by Tofani et al in 2003 to investigate the effect of combined sinusoidal and static magnetic field with intensity of 5.5 mT on the toxicity effect of cisplatin on the tumors of rats. The magnetic field leads to longer survival of rats compared to the cisplatin group alone [9].

The test conducted by Charles et al in 1994 using pulsed magnetic field with the average intensity between 0.525 and 0.276mT to investigate the field effect on treatment power of cisplatin, showed that the group of combined drug and magnetic field has had the lowest growth among the other treatment groups [12]. Thus, in this test, it was estimated that the field along with chemotherapy drugs may have a synergic effect.

Given that bleomycin is converted to its active form at presence of bivalent iron ions and oxygen, and thus

has the capability to attack the DNA strand and breaks its strands by creating free radicals [16].

Therefore, the magnetic field may increase the toxicity of bleomycin in tumor treatment on the exposure days by affecting on the production of free radicals of oxygen [17]. According to the results, we also notice that combined magnetic field alone has had no effect on tumor, but has increased the toxicity of chemotherapy drugs on the radiation days. This effect can be attributed to the increased possibility of bleomycin molecule binding to cellular DNA caused by the magnet exposure [18].

The results and the discussion presented reveal that combined magnetic field with maximum intensity of 150  $\mu$ T for the alternating field with a frequency of 100 pulses per second and 400  $\mu$ T for static field alone has no effect on the tumor, but it increases the impact of bleomycin on the tumor on the exposure days when combined with anti-tumor bleomycin. Thus, it is hoped that the effective field parameters would be determined for use in the clinic through further evaluation of the effect of magnetic fields with different frequencies and intensities on the effect of chemotherapy drugs. Also, according to what is obtained from the results, the static magnetic field alone reduces tumor growth rate. Therefore, the static magnetic field alone can be considered as a factor influencing tumor growth rate.

Given that in this study, it was not possible to review the effect of time varying magnetic field alone with the desired specifications, the observed effect on drug toxicity is dependent on both static and time varying field and the effect cannot be separated. Therefore, it is recommended to evaluate the effect of static field on the toxicity of bleomycin.

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