STATIC MAGNETIC FIELDS INHIBIT RADIATION-INDUCED APOPTOSIS IN BONE MARROW STEM CELLS

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Abstract
There are various sources of magnetic fields (MF) around us; natural earth’s field as well as man-made. To investigate the effects of these fields on living organisms, bone marrow stem cells (BMSC) were exposed to 15 milli Tesla (mT) spatially uniform static magnetic field (SMF) in the presence and the absence of 0.5 Gray X-ray as a radical producer. The results didn’t show a significant difference when the cells were treated with SMF alone. The percentages of apoptotic cells in the cells encountered MF didn’t show a significant difference from 43.40% to 47.58% in treated cells. On the other hand, the percentage of apoptosis induced by 0.5 Gy X-ray showed a significant increase compared to the controls. SMF significantly decreased the extent of apoptosis from 72.65% to 58.88% in treated cells, respectively. Therefore in the presence of X-ray, SMF increased the percentage of survived cells significantly and rescued approximately 20% of the X-ray induced apoptotic cells. These data show that the exposure of SMF suppresses apoptosis and promotes cell survival in BMSC. Thus MF might act as a co-mutagenic and co-carcinogenic agent with increasing the risk of tumor development by inhibiting apoptosis.

Introduction
We encounter Magnetic fields (MFs) every day, both naturally occurring and manmade fields such as power lines, electric appliances at homes and offices, electrified transportation systems including urban railway systems and diagnostic devices such as Magnetic Resonance Imaging [1]. This leads to exposure both in our homes as well as in our various workplaces. Interest in the interaction of MFs with living organisms has been triggered primarily from epidemiology studies, which have reported associations between MF exposure and tumorigenicity in several biological systems [2-5]. Specifically, increased tumorigenesis in chemically induced tumors after exposure to MFs has been reported [6]. These data have indicated that not all types of tumors are sensitive to MFs, with the incidence of some specific types of leukemia and nerve cell tumors (especially from glial cells) most significantly increased [7-9].

There have been a series of reports describing the effects of electromagnetic fields, but there are only a few reports on the effects of Static Magnetic Fields (SMFs). Previous studies at cellular level have been investigated from different endpoints [10]; but because of the variety in cell lines, magnetic fields intensity and the duration of exposure, findings are often contradictory.

In the present study, the effects of SMF treatment after a cute exposure to X-ray on the rate of apoptosis were investigated. The goal was to determine a potential relationship between MFs and the risk of tumor development in Bone Marrow Stem Cells.

Material and Methods

Chemicals.
Cell culture reagents, Fetal Bovine Serum (FBS) and Trypsin were obtained from Gibco-BRL (Invitrogen LT, Merelbeke, Belgium). Propidium iodide (PI), Triton X-100 and Ficoll-hypaque were obtained from Sigma-Aldrich Chemicals, ribonuclease A (RNase A) from Fermentas-Life sciences (EU) and dimethylsulphoxide (DMSO) from Merck (Darmstadt, Germany). All chemicals were stored as stock solutions at -20°C.
Isolation and culture of MSCs.
The primary source for Bone Marrow Stromal Stem Cells (BMSCs) is the Bone Marrow, although recent reports indicate that BMSCs can be isolated from other sources, such as peripheral blood [11], fat [12], skin [13, 14], vasculature [15], and muscle [16]. BMSCs are isolated as described by Woodbury [17]. In order to isolate BMSCs, Rat Bone Marrow aspirates were subjected to fractionation on density gradient solution such as ficoll-hypaque. The mononuclear cell fraction obtained at the interface was then explanted in αMEM with 20% FBS and incubated at 37°C in an atmosphere having 5% CO2. After 24 hours, nonadherent cells were discarded and adherent cells were thoroughly washed twice with phosphate buffered saline (PBS). BMSCs were allowed to grow until 70-80% confluence of the surface area of flask. After three or four passages when the density of cultured BMSCs was approximately 5×104cells/cm2, BMSCs were ready to treat with MF.

Magnetic field application.
Exposure to MF was performed by a locally designed MF generator. The electrical power was provided using a 220 V AC power supply equipped with variable transformer as well as a single-phase full-wave rectifier. The maximum power and passing current were 1 kW and 50 A DC, respectively. This system was designed to generate MF in range of 0.5 mT–30 mT. It was consisted of two coils (each with 3000 turns of 3 mm copper wire) on a U-shaped laminated iron core (to prevent eddy current losses). Using two vertical connectors, the arms of the U-shaped iron core were terminated in four circular iron plates covered with thin layer of nickel (each 23mm thickness, 260 cm in diameter). A water circulation system around the coils was employed to avoid the increase of the temperature. Without this system, the temperature of the coils increased from 25 to 40°C, after the apparatus was working for 10 minutes. Applying the water circulation system helped was useful for long exposures (5 h) as well as to keep the maintenance of the apparatus. The temperature between the circular iron plates, where the samples were located, was measured and was almost the same as the other parts of room ±1°C. Presence of any pulsation in the current from rectifier into the MF generating apparatus, was tested by an oscilloscope (40 MHz, model 8040, Leader, Japan) and a pulsation frequency of 50 Hz with a range of voltage variation about ±1 V, was shown. The presence of this pulsation frequency may be related to the shortcoming of the single-phase full-wave rectifier, which provides a ripple voltage around 5%. This small ripple voltage suggests that the generated MF can be considered highly homogeneous.

Calibration of the system as well as tests for the accuracy and uniformity of the MFs were performed by a teslameter (13610.93, PHYWE, Germany) with a probe type of Hall Sound. The accuracy of the system was ±0.1% for MF and the range of measurements was 3 mT–30 mT [18].

Apoptosis Induction and treatment of cells with Static Magnetic Field.
In order to perform the experience 25ml flasks containing at least 10^6 BMSCs were used. Apoptosis was induced by acute exposure to X ray. Irradiation was performed with X rays at 140 kVp and 40 mA equipped with a filter of 2.5 mm Aluminum. All irradiation was performed at 37°C. Absorbed Dose was approximately 0.5 Gy in a period of 1 second. The flasks were placed in the gap between the two plates of the SMF application to encounter SMF. The treatment group was exposed to static magnetic field of 15mT for 5 hours continuously, while the rest served as controls. The control samples were kept far enough from the coils and only were exposed to the extremely low SMF of the earth (60±5 μT), as the treatment groups were too.

Cell fixation, staining and data collection.
Cultured cells were harvested using Trypsin-EDTA, and re-suspended in αMEM at a concentration of 1.0×10^6 cells/flask. The cells were suspended in PBS and centrifuged at 200g at 4°C for 6 minutes, the supernatant was decanted and the cells gently re-suspended in 0.5 ml PBS. Cells were fixed by adding 4.5 ml cold ethanol (70%). Fixed cells were left at 4°C for 24 hours before further analysis. Then they were again centrifuged as above, washed once and re-centrifuged with cold PBS. Centrifuged cells were re-suspended in 0.5 ml PBS containing 10 μl of 10 mg/ml Ribonuclease A (RNase A) and 1 μl Triton-X100 and incubated at 37°C for 10 minutes. Incubated cells were stained by adding 10 μl of 1 mg/ml propidium iodide. Flow cytometric measurements were performed using a LSR II flow cytometer (Becton Dickinson). PI fluorescence was collected with a 575/25 nm band pass filter, orange-red fluorescence (FL2), after linear amplification. Flow cytometric data was stored according to a standard format. According to the flow cytometry standard (FCS), a data storage file includes a description of the sample acquired, on which the data was collected, the data set, and the results of data analysis.

Detection of Apoptosis.
Flow cytometry is one of the methods that are utilized to detect apoptosis. The way by which the apoptotic cells were distinguished from nonapoptotic cells is that apoptotic cells have fragmented DNA and their attachment to specific fluorocromes such as propidium iodide (PI) was diminished [19].
Histogram plots were created using the Cell Quest program. For each measurement, data from 10,000 single cell events were collected. Cells have traditionally been gated according to physical characteristics. For instance, sub cellular debris and clumps can be distinguished from single cells by size, estimated by forward scatter. Also, dead cells have lower forward scatter and higher side scatter than living cells [20, 21]. Cell doublets and aggregates were gated out using a two parameter dot plot of FL2-Area versus FL2-Width shown in figure 1.

Fig. 1. Application of the two parameter Dot plot of FL2-Area versus FL2-Width to gate out Cell doublets and aggregates

Statistical analysis.
All of the experiments were carried out with at least three independent repetitions and all data were expressed as the mean values ± Standard Deviation (SD). Statistical analysis was performed using Student’s t-test and the differences were expressed as significant at level of P < 0.05.

Results
The effect of SMF on the extent of damage-induced apoptosis in BMSCs was investigated. The percentages of apoptotic cells in the cells encountered MF didn’t show a significant difference from 43.40% to 47.58% in treated cells. In the other hand, the percentage of apoptosis induced by ionizing radiation after flow cytometric analysis of cells showed a significant decrease compared to the controls. SMF decreased the extent of apoptosis from 72.65% to 58.88% in treated cells, respectively (P < 0.05). Therefore MFs rescued 18.95% of the X ray induced apoptotic cells. As showed in table 1, the percentage of Apoptotic cells was increased by SMF alone; but in the presence of 0.5 Gy X-ray, SMF modulated the effect of ionizing radiation significantly. Table 1 summarized the obtained results showing the rate of apoptosis ± SD in four groups. For better illustration, the percentage of apoptotic cells has been demonstrated in figure 2.

Table 1: Effects of 15 mT Static Magnetic Field for 5 hours on the percentage of apoptotic cells in BMSCs

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Apoptotic cells</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>43.40 ± 15.15 ac*</td>
</tr>
<tr>
<td>SMF</td>
<td>47.58 ± 8.33 bc</td>
</tr>
<tr>
<td>0.5 Gy X Ray</td>
<td>72.65 ± 5.91 b</td>
</tr>
<tr>
<td>0.5 Gy X Ray + SMF</td>
<td>58.88 ± 10.6 c</td>
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*Different letters show the significant differences in Dead cells
Figure 2. Percentage of dead cells after treating with 0.5 Gy X ray 15 mT Static Magnetic Field for 5 hours

Discussion
Stem Cells are responsible for generating many different types of cells, including the various kinds of blood cells and the cell types that make up the immune system. Since stem cells can be damaged in certain cancer treatments, studying the effects of ionizing radiation have become considerable public concern. Bone Marrow Stromal Stem Cells (BMSCs) provide a physical support for maturing hematopoietic stem cells. These undifferentiated cells are very sensitive to genotoxic agents and are one of the most accessible adult Stem Cells have been used in therapies at the cellular level [22]. When BMSCs were grown in the absence and the presence of a 15 mT Static Magnetic Field for 5 hours, the number of cells showed a significant difference in cell survival in comparison with the controls (Tab. 1).

The presumptive mechanism that can explain the alternations in the rate of apoptotic cells is the hypothesis in which MF influences the kinetics of two major processes: (1) kinetics of chemical reactions with radical pair intermediates and (2) kinetics of Ca^{2+} influx from the extracellular environment. MFs enhance radical’s life span, which can initiate chain reactions to form new free radicals [23,24,25]. It can also affect the Ca^{2+} influx with an immediate and reversible effect, and without affecting Ca^{2+} mobilization from intracellular stores [26]. So, as a hypothesis, the alternation of dead cells is result of conflict with these two major processes.

In this study, we demonstrated that MFs as weak as 15 mT can modulate apoptosis in BMSCs, allowing the survival of possibly mutated cells when there is a high content of potential apoptotic cells. Our data suggest that MFs might act as a co-mutagenic and co-carcinogenic agent, thus behaving as an epigenetic mechanism inducing tumor development. This mechanism preferentially would be directed to some type of tissue or organ, as observed in one epidemiologic report [27]. Thus, MFs might increase the risk of tumor development by inhibiting apoptosis without being a tumor initiator.

References
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