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Effects of 13 T Static Magnetic Fields (SMF) in the Cell Cycle Distribution and Cell Viability in Immortalized Hamster Cells and Human Primary Fibroblasts Cells*

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Abstract Magnetic resonance image (MRI) systems with a much higher magnetic flux density were developed and applied for potential use in medical diagnostic. Recently, much attention has been paid to the biological effects of static, strong magnetic fields (SMF). With the 13 T SMF facility in the Institute of Plasma Physics, Chinese Academy of Sciences, the present study focused on the cellular effects of the SMF with 13 T on the cell viability and the cell cycle distribution in immortalized hamster cells, such as human-hamster hybrid (A_L) cells, Chinese hamster ovary (CHO) cells, DNA double-strand break repair deficient mutant (XRS-5) cells, and human primary skin fibroblasts (AG1522) cells. It was found that the exposure of 13 T SMF had less effect on the colony formation in either nonsynchronized or synchronized A_L cells. Moreover, as compared to non-exposed groups, there were slight differences in the cell cycle distribution no matter in either synchronized or nonsynchronized immortalized hamster cells after exposure to 13 T SMF. However, it should be noted that the percentage of exposed AG1522 cells at G0/G1 phase was decreased by 10% as compared to the controls. Our data indicated that although 13 T SMF had minimal effects in immortalized hamster cells, the cell cycle distribution was slightly modified by SMF in human primary fibroblasts.

Keywords: static magnetic fields, immortalized hamster cells, human primary fibroblasts cells

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1 Introduction

Strong, static magnetic fields, such as those in magnetic resonance imaging (MRI) for medical diagnosis and transportation systems, is increasingly applied in recent years [1]. One of the most significant advances in the development of diagnostic equipments, such as magnetic resonance imaging and nuclear magnetic resonance (NMR), is the enhancement of the magnetic field strength to realize both higher resolution and sensitivity. Although most MRI systems are made up of a magnet with a magnetic flux density of 1.5 T, systems with a magnetic flux density of 3 T have recently been used for diagnosis. Recently MRI images were obtained in humans using a magnetic field of 8 T. It should be noted that MRI systems with a much higher magnetic flux density have already been developed and applied for potential medical diagnostic use. Moreover, several groups in chemical and biochemical studies work with NMR units with magnetic strengths ranging from 4.7 T to 11.4 T [2]. Despite the fact that the safety guidelines of magnetic field have been established by the U.S. Food and Drug Administration (FDA) and International Electrotechnical Commission, there are still important safety issues regarding exposure to strong SMF. It is extremely important to understand the mechanisms of the magnetic fields on living organisms, which will be needed to protect human health in consideration of the strong magnetic field brought by the imminent introduction of new technologies such as magnetically levitated trains and the therapeutical use of magnetic fields.

Various in vivo and in vitro models have been widely used to assess the potential biological effects of the strong SMF in the past several decades [3,4]. There is some evidence that magnetic field dose not affect the aggregation of melanophores [5], proliferation of human breast cancer cells [6], axonal outgrowth and proliferation of moto-neurons in chick embryos [7,8], alignment of cortical neurons in mouse embryos [9], regeneration in rat sciatic nerve [10], early embryonic development of frogs eggs [11], behavior of normal lymphocytes and monocytes [12] or growth of T cells under normal cell-culture conditions [13]. Cell cycle analysis of synchronized and nonsynchronized human fetal lung fibroblasts (HFLFs) cells does not reveal statistically significant differences between the cells exposed to magnetic field of 0.2 T, 1.0 T, or 1.5 T for 1 hr/day in 5 consec-

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utive days and control cells [14]. Exposure to a SMF of 7 T has no significant effect on the growth rate of P-388 and V-79 cells [15]. On the other hand, considerable evidence has indicated that strong SMF could induce some effects such as the reorientation of the mitotic apparatus in frog eggs [16], the modification of the electroencephalograms of monkeys [17], the changes in the intracellular concentration and membrane flux of Ca^{2+} [18~20], the induction of c-fos gene expression [21], and the inhibition of poly-adenosine 5'-diphosphate ribosylation of proteins [22]. Different influences of SMF on apoptosis have been reported depending on the cell type [23~25], and prolonged exposure to a 7 T field appeared to inhibit growth of three human tumor cell lines in vitro [26]. These reports suggest that the effect of the strong, static magnetic fields differs depending on the cell types and exposure intensity or time. The effect of SMF on the cell growth or viability has also had contradictory results. Previous studies showed that a static magnetic field alone did not have a lethal effect on the cell growth and viability, with different magnetic field strength (from 0.2 T to 10 T) or exposure time (from 3 hr to 7 days) [2,14,19]. Recently it was reported that 1~5 weeks exposure to 0.5 T SMF inhibited the growth of the GH3 cells [27].

The cellular effects of SMF were mostly tested below 5 T in various biosystems, and with controversial results [4]. With the development of the strong magnetic fields in medical applications, it is critical to assess the biological safety of SMF with a high magnetic flux density. In this study, a SMF biological exposure system was established and the cellular effects of 13 T SMF was determined either in immortalized hamster cell lines or human primary cell lines.

2 Materials and methods

2.1 Cell culture

Human-hamster hybrid (A_L) cells, Chinese hamster ovary (CHO) cells, DNA double-strand break repair deficient mutant (XRS-5) cells, and Human primary skin fibroblasts (AG1522) cells were used in this study. CHO cell line was derived from a Chinese hamster ovary. XRS-5 cells was a CHO-derived cell line and deficient in Ku80 (XRCC5) protein, which is an important element of the non-homologous end-joining (NHEJ) pathway of rejoining DNA DSBs [28]. The A_L hybrid cells contain a standard set of CHO-K1 chromosomes and a single copy of human chromosome 11 which also derived from CHO cells [29]. AG1522 cells were human primary skin fibroblasts. CHO and XRS-5 cells were cultured in F12/DMEM (1:1) medium supplemented with 9% heat-inactivated FBS, 2×10^{-4} M glycine, 100 units/mL penicillin and 0.1 mg/mL streptomycin. A_L cells were cultured in Ham's F-12 medium supplemented with 8% heat-inactivated fetal bovine serum (FBS, Hyclone), 25 μg/mL gentamicin, and 2×10^{-4} M glycine. AG1522 cells were cultured in α-MEM (Invitrogen) supplemented with 2.0 mM L-glutamine and 20% FBS (Hyclone) plus 100 unit/mL penicillin and 0.1 mg/mL streptomycin. All those cells were cultured at 37°C in a humidified 95% air/5% CO_2 incubator.

2.2 SMF exposure system

In this study, the SMF exposure system was composed of a superconducting magnet, cell culture holders, an air supply, and a temperature control system. The superconducting magnet (Oxford Company, England) generated a homogeneous static magnetic field held at 13 T. The cell culture holders were made of copper with a perpendicular bore having an inner diameter of 40 mm, and an altitude diameter of 115 mm. The mixed air (5% CO_2 and 95% air) detector and the temperature detector were located on the top of the cell culture holders. The temperature of the holders was maintained at 37.0±0.2°C during the whole experiment process. A shammed exposure unit without a superconducting magnet was located nearby the SMF exposure unit, as shown in Fig. 1.

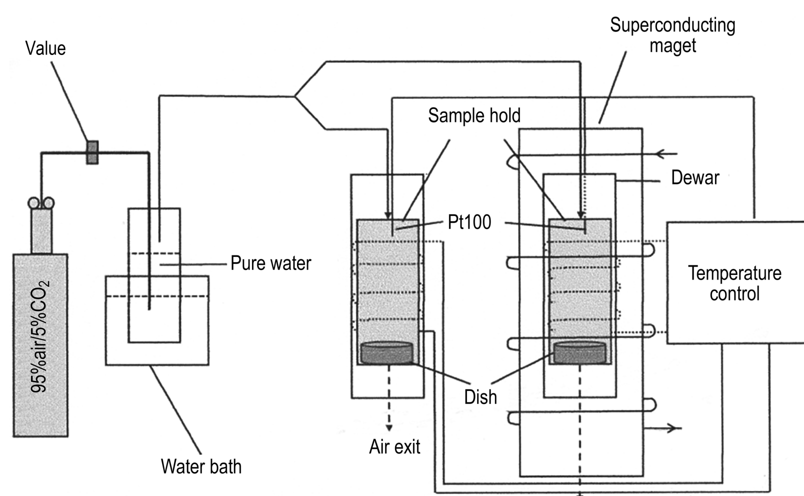

Fig.1 Schematic diagram of the built superconducting magnet biosystem

2.3 Synchronization of cells

To synchronize A_L cells, the exponentially growing A_L cells at a density of 8×10^5 cells/dish were inoculated into 100 mm culture dishes. 72 hours later, more than 85% of suspending cells were determined at G0/G1 phase by flow cytometry [30]. With a further incubation of 2.5 hours in 35 mm dishes, the cells were exposed to 13 T SMF.

The synchronizing process for CHO and XRS-5 cells was described in Ref. [31]. Briefly, to harvest the cells at the G1/S phase, the cells were plated into 35 mm dishes with medium containing 1 μg/mL of aphidicolin (Sigma, USA). After incubation for 16 hr, the medium containing aphidicolin was removed and the cells went into G2 phases with further incubation for 6 hr in fresh medium. The cultures in the G1/S phase were exposed to SMF.

To synchronize AG1522 cells, the exponentially growing AG1522 cells (1×10^6 cells/dish) were inoculated into 35 mm cell culture dishes. After 48 hr, the media were replaced by α-MEM media supplemented with 1% FBS. With further incubation for 24 hr, more than 90% cells were in G0/G1 phase as determined by flow cytometry [32]. Then the cells were trypsinized and inoculated into 35 mm cell culture dishes with the density of 8×10^5 cells/dish. With a further incubation of 25.5 hr, the cells were exposed to 13 T SMF.

2.4 Exposure to SMF

In a preliminary study we found that the cell cycle distribution had the most significant change from 6 hr to 9 hr after the A_L cells were synchronized to (G0/G1) phase. So synchronized (G0/G1) A_L cells were cultured for 6 hr before exposed to the SMF and then treated for 3 hr to evaluate the effect of the 13 T SMF on A_L cells. Similarly, the cell cycle distribution had the most significant change from 1 hr to 6 hr after the CHO and XRS-5 cells were synchronized to the (G0/G1) phase. So synchronized (G0/G1) A_L cells were cultured for 1 hr before exposed to the SMF and then treated for 5 hr to evaluate the effect of Ku protein deficiency on the 13 T SMF-treated cells. To evaluate the effect of the 13 T SMF on primary cells, AG 1522 cells were treated with 13 T SMF for 3 hr. Also, the cell cycle distribution of the synchronized AG1522 cells had the greatest change in the 3 hr period.

2.5 Colongenic assay

After exposure to SMF, the cultures were replated into 60 mm diameter petri dishes for colony formation as described previously [33]. The cultures were incubated for 7 days, and then they were fixed with formaldehyde. The colonies were defined as consisting of 50 more cells and counted by staining with Gimsa.

2.6 Cell cycle analysis

Propidium iodide (PI) is a typical cell cycle fluorescent probe, which passes through a permeabilized membrane and intercalates into cellular DNA. Since the intensity of the signal is directly proportional to DNA contents, the cell cycle distribution can be determined by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA) [34]. After exposure to SMF, the cells were harvested by trypsinization and centrifugation. After being washed with PBS, the cells were fixed with ice-cold 70% ethanol at -20°C for 30 min, then washed with PBS, followed by RNase A (25 μg/mL in citric phosphate buffer) treatment at 37°C for 30 min. Cells were harvested by centrifugation and further stained with 50 μg/mL PI (Becton - Dickinson, San Jose, CA) at room temperature for 60 min in darkness. The DNA contents of 1×10^6 cells were collected by flow cytometry and the cell cycle profile was analyzed with the software ModFit.

2.7 Statistical analysis

All data were expressed as the mean values, standard deviation (SD). Statistical analysis was performed using the Student's t-test.

3 Results

3.1 Colongenic effect of 13 T SMF

The normal plating efficiencies used in the present study was 86.2% and 59.5% in nonsynchronized and synchronized A_L cells (G0/G1), respectively. To detect the colongenic effects of strong SMF, the nonsynchronized A_L were exposed to 13 T SMF for either 3 hr or 5 hr; while the synchronized A_L cells in the G0/G1 phase were exposed to SMF for 1 hr. As shown in Fig. 2(a) and (b), the different durations of exposure to SMF had no significant effect on the plating efficiencies in either the nonsynchronized or synchronized A_L cells, as compared to the shammed cells.

3.2 Effects of 13 T SMF on cell cycle distribution

The cell cycle distribution was determinate after exposure to 13 T SMF. As shown in Fig. 3(a),(b) and Fig. 4, there was no significant difference in the cell cycle distribution between SMF-treated groups and the controls in either synchronized or unsynochronized A_L cells. Similarly, exposure to 13 T SMF had a minor effect on the cell cycle distribution in both CHO cells and XRS-5 cells. However, the cell cycle distribution was modified by 13 T exposure in AG1522 cells. The cell percentage at the G0/G1 phase decreased from 52.39% to 42.34% ($P=0.029$) as compared to the controls (Fig. 5). These results suggested that primary cells might be more sensitive to 13 T SMF than immortalized cells.

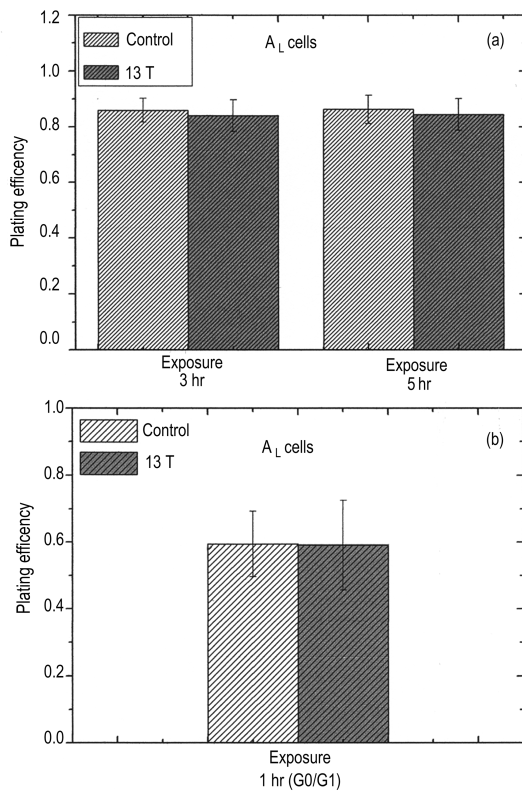

Fig.2 The colongenic assay of the synchronized and non-synchronized A_L cells exposed to the 13 T SMF for different periods of time. No differences between the exposed and corresponding groups were found

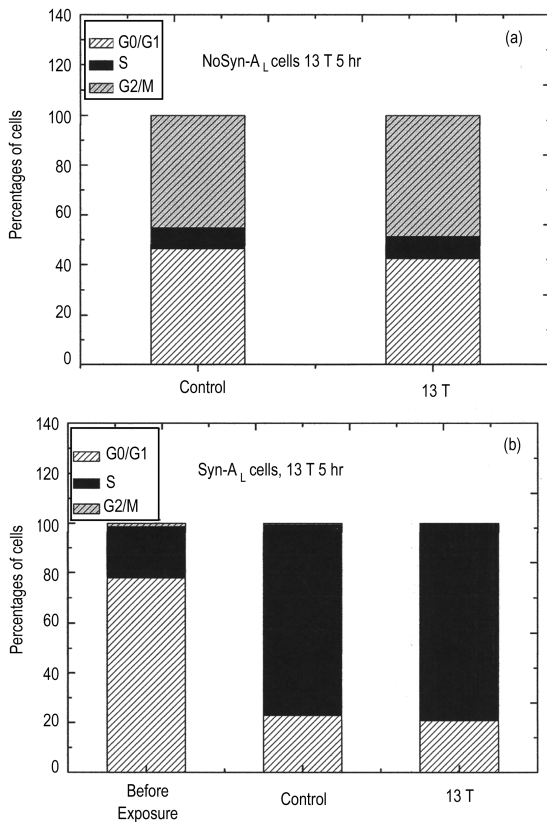

Fig.3 Cell cycle distribution of nonsynchronized and synchronized A_L cells after exposure to magnetic fields of 13 T for 3 hr. No differences between the exposed and corresponding control groups were found

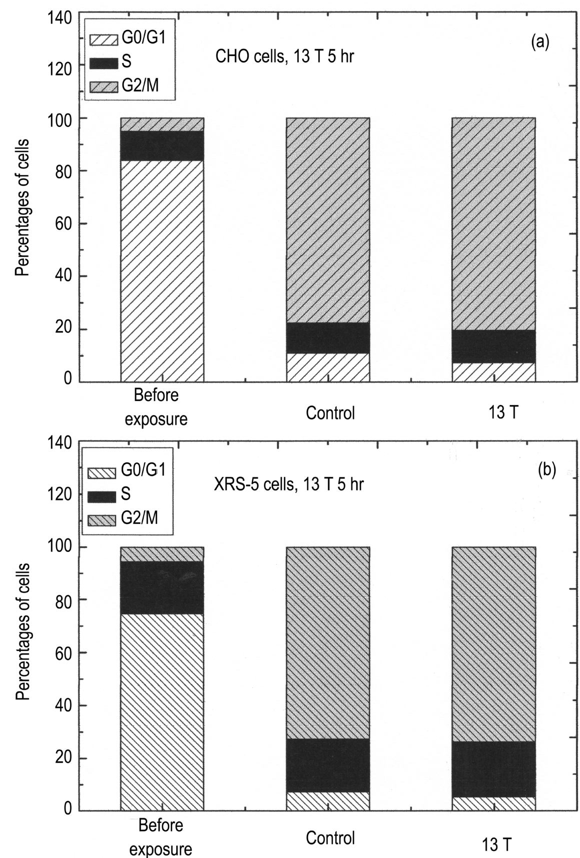

Fig.4 Cell cycle distribution of synchronized CHO and XRS-5 cells after exposure to magnetic fields of 13 T for 5 hr. No significant differences between the exposed and corresponding control groups were found

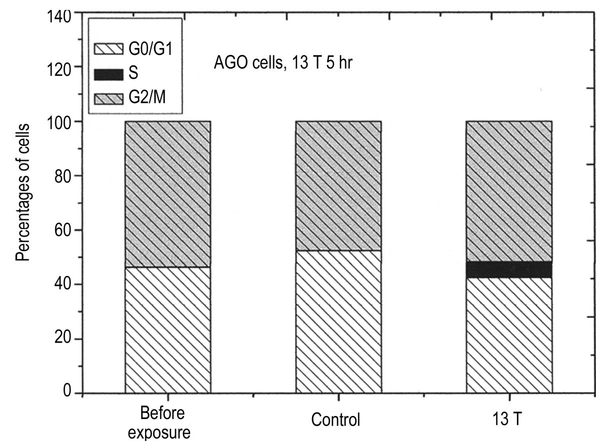

Fig.5 Cell cycle distribution of synchronized primary AG1522 cells after exposure to magnetic fields of 13 T for 3 hr. A slight change between the exposed and corresponding control groups was found

4 Discussion

The rapid development and the wide application of high intensity magnetic facilities lead to increasing exposure levels of static magnetic fields in general populations [1]. The data generated from in vivo and in vitro

studies have been controversial for decades [4]. Clonogenic activity, DNA synthesis, cell cycle, and proliferation kinetics were not altered by exposure to the magnetic field, and repetitive exposure to a static magnetic field up to 10 T exerted no effects on the proliferation in human fetal lung fibroblast cells [35,36]. In the contrary, there is the evidence that the prolonged exposure to 7 T field appeared to inhibit the growth of three human tumor cell lines in vitro [26], which was consistent with the observation in human WI-38 fibroblasts and murine L-929 cells exposed to a 0.5 T magnetic field [37]. NORIMURA [13] et al. reported the growth inhibition of strong magnetic field exposure in phytohemagglutinin-stimulated T lymphocytes due to the higher field strength (up to 6.3 T) and the exposure time (up to 60 hours).

In present study, we observed that a 13 T static magnetic field did not affect the cell cycle distribution in either synchronized or nonsynchronized A_{L}, CHO and XRS-5 cells, respectively. As the cell cycle distribution of nonsynchronized cells could not be well controlled while the change in synchronized cells might be easy to detect, we subsequently examined the synchronized cells. Similarly, no change was found in synchronized cells. Among the above three cell lines, XRS-5 cells are DNA-double strand break (DSB) repair-deficient ones. DSB was usually regarded as the most deleterious type of DNA damage, induced either by environmental stress such as irradiation or oxidative stress by the stalling of DNA replication forks [38]. Since there was no significant difference in the cell cycle distribution between exposed XRS-5 cells and the shammed controls, our results indicated that DSB repair system might not be involved in the cell cycle process with SMF exposure. However, it should be noted that AG1522 cells at the G0/G1 phase were decreased by 10% with 13 T SMF exposure. This observation was not consistent with the findings that the cell cycle analysis in human fetal lung fibroblasts (HFLFs) cells did not reveal differences between the cells exposed to 0.2 T, 1.0 T, or 1.5 T for 1 hr/day for 5 consecutive days and the control cells [14]. AG1522 cells were primary fibroblasts with intact cell cycle check point gene. This might be the reason why we only observed the SMF exposure changed cell cycle in AG1522 cells. Therefore, the effect of intensity of SMF together with that of the exposure time needs to be further studied in various primary cell lines.

Previous reports indicated that long-term exposure to a 10 T SMF for up to 4 days did not affect the growth rate CHO-K1 cells, and repetitive exposure to the static magnetic field (1.5 T) of a diagnostic MR scanner did not affects the proliferation of human fetal lung fibroblasts on a mid- or long-term basis [35,36]. In this study, we observed that the exposure to a 13 T static magnetic field for either 1 hr or 5 hr did not affect the colongenic assay of the synchronized or nonsynchronized A_{L} cells, a finding consistent with the report in CHO-K1 cells and human fetal lung fibroblasts cells mentioned above. These data suggested that the exposure to strong SMF of up to 13 T at least—may not affect on cell viability.

A possible mechanism involved in the interaction of strong SMF with living cells is the generation of free radicals that are spontaneously produced during metabolization [39]. The radical pairs are modified by strong SMF, leading to an increased number of free radicals [40], such as reactive oxygen/nitrogen species (ROS/RNS) generated in the presence of metal ions. The ROS/RNS may further act directly or indirectly to damage neighboring biomolecules, such as DNA, protein, and membrane lipid [41].

The contradictory results from SMF study may be caused by several factors [42]. For example, the parameters of SMF (field strength, exposure) duration used for cell exposure may play a crucial role in the cellular effects of SMF. The cell type should be taken into account as well for different types of cells which may react in different ways to magnetic field exposure. There is an urgent need for comprehensive investigation of the cellular and molecular mechanisms of the strong SMF induced biological effects both in vitro and in vivo, which will allow us to evaluate the safety of strong SMF and its medical application.

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