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Effect of a 60Hz electromagnetic field on the frequency of bleomycin-induced HPRT gene mutation and 1,2,4-benzenetriol-induced sister chromatid exchanges in CHO cell

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Abstract-The interaction of low density extremely low frequency magnetic field (ELF MF) in the frequency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutation induced by bleomycin and on the frequency of sister chromatid exchanges (SCEs) induced by 1,2,4-benzenetriol(BT) was demonstrated. CHO cells pretreated with bleomycin or 1,2,4-benzenetriol were exposed for 24hrs to a sinusoidal 0.8mT magnetic field at 60Hz. Frequency of HPRT mutation and SCEs were determined. ELF MF exposure led to a two-fold increase of the frequency of HPRT mutation induced by bleomycin. No increase of mutation frequency was observed by ELF MF alone. ELF MF also increased the frequency of SCEs induced by BT while no increase of SCE frequencies were observed by ELF MF alone. These results suggest that low density ELF MF field would act as an enhancer rather than as an initiator of mutagenic effects in CHO cell.

Key words : Extremely low frequency magnetic field (ELF MF), Hypoxanthin-guanin phosphoribosyl transferase (HPRT), Sister chromatid exchanges (SCEs)

Introduction

It is widely accepted that the electromagnetic radiation in the extremely low frequency (ELF) range is so weak that it is not mutagenic in itself [1]. McCann et al. (1998) [2] recently reviewed studies on the potential for genotoxicity of electric and magnetic fields and concluded that ELF electric or magnetic field did not have genotoxic potential. In contrast, some studies [3-4] reported that exposure to high density ELF magnetic field could induce sister chromatid exchanges (SCEs) while low density ELF could not.

Yaguchi et al. (1999) [4] reported that the induction of SCE in cultured mouse m5S cell by 400mT ELF was clearly observed but no significant increase of SCE was observed by 5 and 50mT ELF exposure suggesting that there may be a threshold of magnetic density of over 50mT for the elevation of the SCE frequency. Some studies suggested that ELF exposure might enhance tumor development when given together with known carcinogen, but the results were not consistent [5-8].

Recent study by Walleczek et al. (1999) [9] demonstrated that ELF of 0.7mT of 60Hz magnetic filed increased the frequency of point mutation at HPRT gene locus in CHO cells induced by ionizing radiation suggesting that ELF would act as an enhancer of the mutagenicity of a known HPRT mutagen.

Miyakoshi et al. (1996) [10] also reported that 400mT, 50Hz magnetic field increased the frequency of HPRT mutation in malignant melanoma cells induced by 3Gy radiation.

In the present study, we examined whether

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exposure to very low density 0.8mT ELF could enhance HPRT mutation and SCE frequency in CHO cells induced known mutagens. In orderto induce HPRT mutation, we used bleomycin which damages DNA with mechanism to ionizing radiation. SCE frequency was induced by BT, a toxic benzene metabolite, which damages DNA with a mechanism different from ionizing radiation.

Materials and Methods

Cell culture

CHO K1 cells were cultured as monolayer in McCoy's 5A medium supplemented with 10% fetal bovine serum, penicillin(100g/ml) and streptomycin(100g/ml). Cells were cultured in 5% CO^2 in air at 37℃.

Mutation assay at HPRT gene locus

Cells were treated with bleomycin at a concentration of 3.3×10^{-5}M for 3 hours and washed twice with PBS. After resuspending the cell with new medium, cells were exposed to magnetic field of solenoid coils 0.8mT for 24hrs at 37℃. Magnetic field exposure system was set up according to the methods utilized by Walleczek et al. (1999) [9]. Two identical, electrically coupled solenoid coils (length 30㎝; diameter 15㎝) were wound with 350turns/m of bifilar magnet wire No.16 on a cylindrical acrylic support. No significant temperature changes were observed during the experiments (all measurements were 37 ± 0.2℃). The strength of magnetic field was determined with a Gaussmeter (Model 4048, F.W. Bell, Inc. USA). The uniformity of the magnetic field in the active exposure volume was ± 2.5%. After exposure, aliquots of 1×10^6 cells in 75㎠ flask were subcultured at 2 or 3 day intervals for 8 days for the expression of induced mutation. Mutation frequency was determined according to the guidelines described by Li et al. (1987) [11]. Cloning efficiency was determined by plating 200 cells in triplicate in 60㎜ dishes. Mutant cells were selected by plating cells at a density of $1x10^5$ cells in 60㎜ Petridish prefilled with medium containing dialyzed serum and 10㎛M 6-thioguanine. The mutation frequency was calculated after a 14 day incubation period by counting colonies and correcting for cloning efficiency.

Sister Chromatid Exchange

Cells were treated with BT at a concentration of 10 and 20㎛M for 3 hours and washed twice with PBS. After resuspending the cell in new medium containing 10㎛M of BrdU, cells were exposed to magnetic field of solenoid coil(0.8mT) for 24hrs at 37℃. After exposure the cells were further cultured for additional 6hrs. Three hours before harvest colcemid was added to each culture. The chromosome preparation were stained by the fluorescence plus Giemsa technique [12]. The replication index(RI) [13] was calculated according to the formula : RI=(M1+2M2+3M3) /100, where M1, M2 and M3 denote number of cells in the first, second and third division.

Statistical Analysis

Evaluation of statistical significant of results was by student t-test. Intergroup difference was tested by analysis of variance(ANOVA).

Results

The effects of ELF exposure on the frequency bleomycin induced HPRT mutation are shown in Table 1 and fig. 1.

The increased frequencies of HPRT mutation by ELF MF exposure in the bleomycin treated and control group were compared. It was observed that the frequency of HPRT mutation was slightly increased by ELF exposure alone in three (experiment 2, 3, 5) of five independent experiments but it was statistically significant in experiment 3. In order to get net enhancing effect of ELF MF, the increment of mutation frequency by ELF MF exposure in the bleomycin treated group was subtracted with that in the control group. ELF exposure of

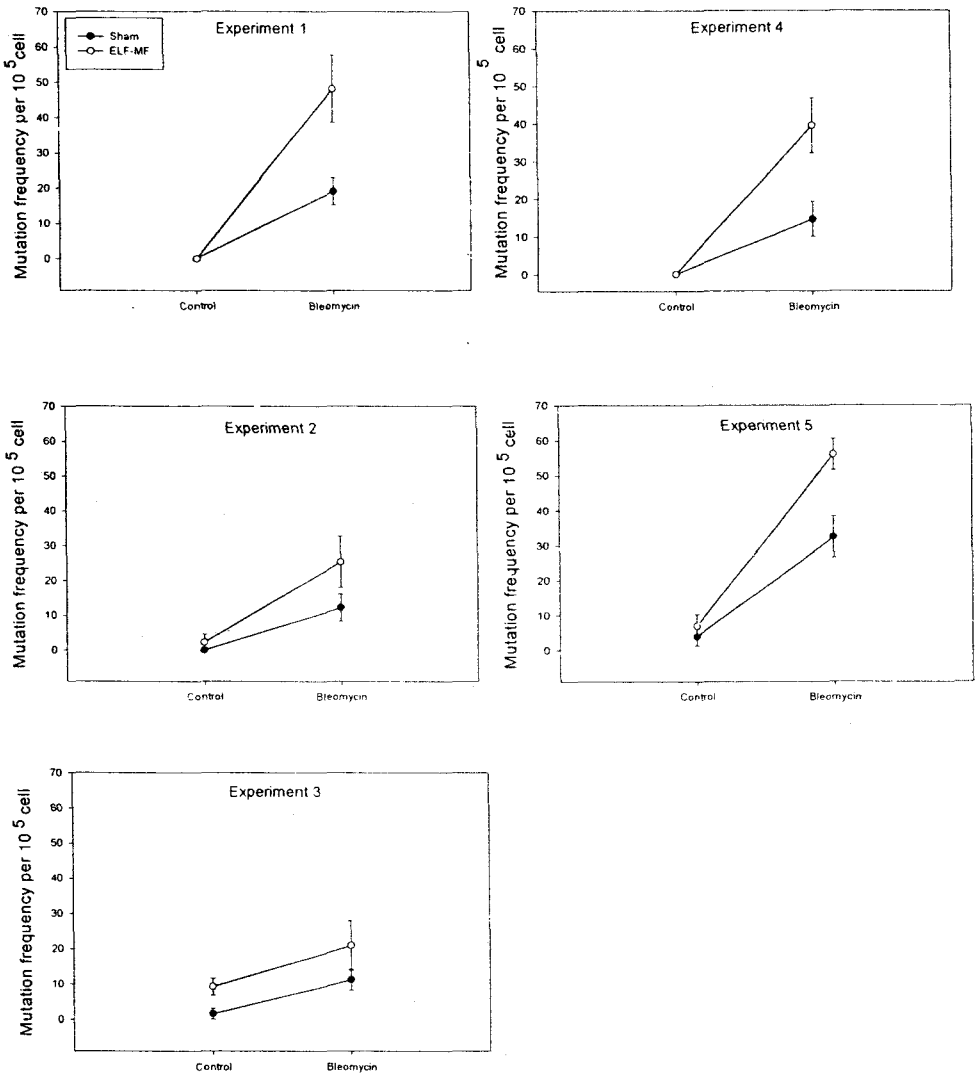

Fig. 1. The increment of the frequency of HPRT mutation by ELF-MF exposure in the bleomycin treated and control group. Five independent experiment was carried out. Error bar indicates standard error of the results from six culture dishes.

CHO cells after bleomycin treatment increased the frequency of HPRT mutation compared to those in bleomycin treated sham exposed cell. In three (experiment 1, 4, 5) of five independent experiments, the increase was statistically significant. Intergroup difference between fiveexperiments was not significant by ANOVA test. When data from five experiments were pooled and analysed, enhancing effect of ELF MF was observed.

As shown in table 2 and fig. 2, the frequency of SCE by BT was increased after exposure to ELF MF.

The frequency of SCE increased in a dose

Table 1. The increment of the frequency of HPRT mutation by ELF-MF exposure in the bleomycin treated and control group

Number of experiment	Sham exposure(S) (0.0mT)	Field exposure(F) (0.8mT)	⊿(F-S)	Significance of increase in HPRT mutation
1 Control (C)	0	0	0	na
BLM (B)	19.05±3.84	48.13±9.46	29.03±7.37	p=0.017
⊿(B-C)	19.05±3.84	48.13±9.46	29.03±7.37	p=0.017
2 Control (C)	0	2.29±2.29	2.29±2.29	p=0.170
BLM (B)	12.26±3.87	25.36±7.35	13.10±10.00	p=0.073
⊿(B-C)	12.26±3.87	23.07±9.08	10.81±6.46	p=0.165
3 Control (C)	1.55±1.55	9.23±2.38	7.68±2.84	p=0.031
BLM (B)	11.28±2.94	20.92±7.00	9.74±6.85	p=0.114
⊿(B-C)	9.63±4.24	11.69±8.82	2.20±3.72	p=0.410
4 Control (C)	0	0	0	na
BLM (B)	14.65±4.63	37.56±6.96	22.91±7.26	p=0.023
⊿(B-C)	14.65±4.63	37.56±6.96	22.91±7.26	p=0.023
5 Control (C)	3.95±2.70	6.94±3.34	3.00±3.45	p=0.251
BLM (B)	32.43±5.92	56.04±4.43	23.61±8.80	p=0.009
⊿(B-C)	28.48±5.43	49.1±6.8	20.64±9.90	p=0.019
Total mean ⊿(B-C)	16.82±2.22	34.30±4.33	17.10±5.79	p=0.000

na : not applicable

Table 2. The increment of SCE frequency by ELF-MF exposure in the 1,2,4-benzenetriol(BT) treated and control group

Treatment		N	SCE mean ± SE	P value	Replication Index(RI)
Control	Sham	60	6.55±0.22	0.095	1.99
	EMF	60	7.03±0.21		2.06
BT 10 μM	Sham	60	6.83±0.22	0.002*	1.99
	EMF	60	7.62±0.18		2.03
BT 20 μM	Sham	60	7.28±0.21	0.073	2.00
	EMF	60	8.00±0.27	-	2.00
BT 30 μM	Sham	60	7.87±0.19	0.031*	2.03
	EMF	60	8.33±0.16		2.02

N = Number of metaphases scored.
SE = Standard error of the mean.
RI = Replication Index.
*= Significantly different from sham exposed group.

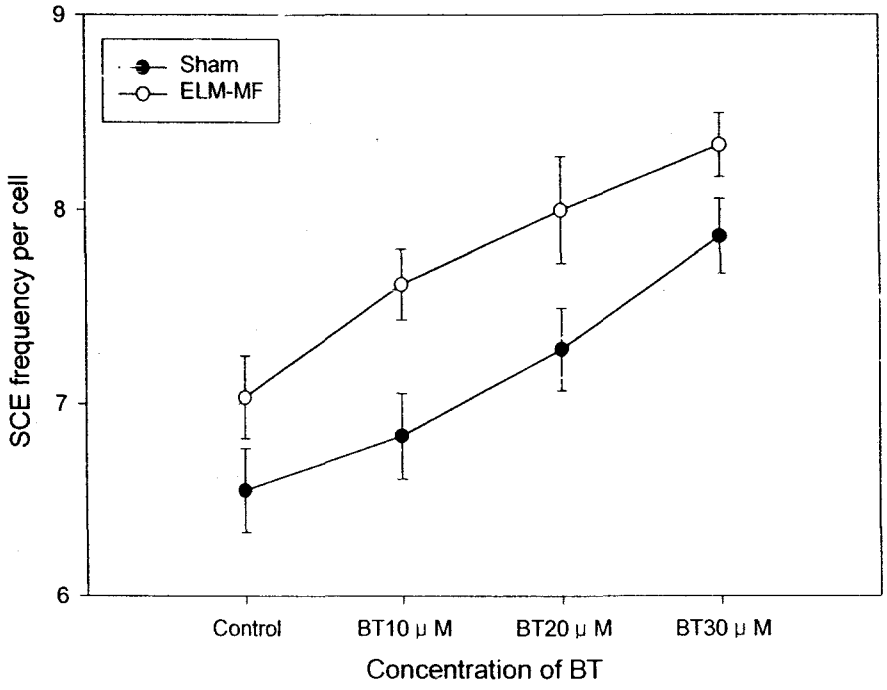

Fig. 2. The increment of SCE frequency by ELF-MF exposure in the 1,2,4-benzenetriol(BT) treated and control group. Error bar indicates error of the mean.

dependent manner in BT treated ELF-MF exposed group as well as sham exposed group. ELF exposure of CHO cells after BT treatment increased the SCE frequency compared to those in BT treated sham exposed cells. No significant difference in Replication index was observed in all exposed group.

Discussion

In the present study, we found no mutagenic effect of 0.8mT ELF MF measured by HPRT mutation frequency assay or sister chromatid exchange induction.

Most in vitro studies using low density (0.1mT to 5mT) ELF MF did not find any elevation of SCE frequency by ELF MF alone [3, 14].

Yaguchi et al. (1999) [4] supposed that there may be a threshold at 50mT magnetic density for the elevation of the SCE frequency through their experiment with mouse m5s cells. They found ELF MF of 400mT could increase the SCE frequencies, but ELF MF of 5 and 50mT could not.

We demonstrated 0.8 mT ELF MF enhanced frequency of sister chromatid exchange induced by BT, which is a toxic metabolite of benzene. BT is mutagenic and known to effectively induce SCE in CHO cells.

Our result also demonstrated that 0.8mT ELF MF could enhance the frequency of HPRT mutation induced by bleomycin.

Walleczek et al. (1999) [9] reported that 0.7mT, 60Hz magnetic field induced an 1.8-fold increase in HPRT mutation frequency but the field alone could not induce the mutation. Since bleomycin utilizes similar DNA damaging mechanism to ionizing radiation (double strand breaking agent and S-phase independent), similar results were expected of the enhancing effect of low density magnetic field.

BT, in contrast to ionizing radiation or bleomycin, can induce only chromatid type aberration like most chemical mutagens.

Since there is not enough available data, we cannot directly compare our results (that 0.8mT ELF MF can enhance frequency of SCE induced by BT) with those using BT or other chemical carcinogens.

Yaguchi et al. (1999) [4] attempted to examine the combined effect of ELF MF (400mT) and mitomycin (MMC) in cultured mouse cells but failed to find any enhancing effect of ELF on SCE frequencies induced by MMC.

Simkó et al.(2001) [16] reported a positive result that 1mT, 50Hz magnetic field induced an 1.6-fold increase in MN frequency compared to BP treatment alone when syrian hamster embryo cells were treated with benzo(a)pyrene during exposure to MF. They suggested that this MF-enhanced co-carcinogenic effect is caused by the genotoxic events via indirect mechanisms, such as the release of reactive intermediates and/or metabolic cell activation processes, leading to genetic instability and cell cycle disturbances.

Some animal studies demonstrated the enhancing effects of low density ELF (100μT-2mT) MF on the carcinogenicity of DMBA [6] or UV radiation [7], although most animal study [5] reported no enhancing effect of ELF MF on the carcinogenicity by known carcinogens. This discrepancy might result from different experimental conditions.

In this study, cells were treated with chemical for 3 hours and followed by a 24-hour exposure period, whereas Yaguchi et al. (1999) [4] treated cells with chemicals and exposed them to ELF MF for entire period of incubation.

Juutilainen et al. (2000) [15] suggested that experiments designed according to the classic initiation-promotion concept may not be sufficient to study the possible role of MF in carcinogenesis. They reviewed several animal studies on cocarcinogenic effect of ELF MF and found that all positive studies have combined chronic MF exposure with known carcinogen exposure during an extended period of tumor development while negative studies have short term exposure to carcinogen followed by a chronic exposure to MF.

In this sense, our experimental design followed the latter occasion while study by Yaguchi et al. (1999) [4] followed the former.

Nevertheless, positive results were obtained in the present study suggesting that results from studies in vitro could be different from those in vivo.

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