Influence of pulsing electromagnetic field on the frequency of sister-chromatid exchanges in cultured mammalian cells

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Summary. Exposure of Chinese hamster cells to pulsing electromagnetic field (PEMF) with 0.18–2.5 mT did not influence the baseline frequency of sister-chromatid exchanges (SCE). The results suggest that PEMF with the magnetic intensity examined does not interfere with DNA replication nor produce DNA lesions, thereby leading to an increased frequency of SCE.
Key words. Chinese hamster V79 cells; electromagnetic field; sister-chromatid exchange.

A pulsing electromagnetic field (PEMF) has been shown to influence a variety of biological processes, including cell proliferation[2], DNA synthesis[3,4] and DNA transcription[5], and has been clinically applied to stimulate bone healing[6–8]. The increasing use of PEMF for clinical applications has led to the need to assess possible risks. Some biological processes have been shown to be perturbed by magnetic fields under specific experimental conditions; there were vacuolar degenerations in rat liver and kidney[9], abnormal development of chick embryos[10], and inhibition of the DNA synthesis in cultured mammalian cells[4]. While the mechanism involved in the modification of these biological processes by the magnetic fields is still largely unexplained, several physical as well as chemical factors which interfere with cellular DNA synthesis are known to influence the formation of sister-chromatid exchanges (SCE)[11–16]. These events serve as indicators of genetic damage, in both laboratory experiments and in humans. In the present study we examined the possible influence of the magnetic field on the frequency of SCE in Chinese hamster V79 cells exposed to PEMF, with different magnetic intensities. In a previous study we noted that the direction of the influence of the magnetic fields; i.e. stimulation or inhibition of cellular DNA synthesis, is largely dependent upon the magnetic intensity rather than on the pulse frequency or width[4].

Materials and methods. Chinese hamster V79 cells were maintained as previously described[4]. Two independent series of experiments were carried out as follows. Experiment (I): cells at 4×10^4 cells/5 ml were incubated at 37 °C for 24 h and then exposed to PEMF for a successive 24 h. After exposure to the magnetic field, 2.4 μg/ml 5-bromodeoxyuridine (BrdU) was added to the cells and they were cultured for another 24 h in the dark, in the absence of PEMF. The cultures were placed between a pair of 10 by 10 cm Helmholtz coils placed horizontally in a CO_2 incubator, and were kept in the magnetic field at various intensities of a 25-μs pulse that repeated at 100 Hz. The control cultures were in a separate incubator and were not perturbed by the magnetic fields. Mitotic delay in cells in this series of experiment was negligible. In experiment (II), cells inoculated at 1.2×10^5 cells/5 ml were cultured for 24 h and then exposed to PEMF for another 24 h in the dark together with BrdU. PEMF resulted in a slight reduction in mitotic frequency, without causing any significant mitotic delay in the experimental cultures. To each culture 0.06 μg/ml colcemid was added 3 h before harvesting. Chromosome preparations were made according to a standard protocol and the slides were air-dried and stained using the fluorescence plus Giemsa technique[17]. The frequency of induced SCEs was determined by scoring 100 metaphases for each culture. Statistical analysis was performed using Student's t-test.

Results and discussion. Two independent experiments were carried out in cultured V79 cells. In the first experiment, exponentially growing cells were exposed to PEMF for 24 h and then cultured for two additional cell cycles (24 h) with added BrdU, not in the magnetic field. The magnetic waveform and its first time-derivative are shown in the figure. The PEMF was generated by a 25-μs pulse, repeating at 100 Hz. In control cultures not perturbed by the magnetic field, the frequency of SCE was 7.0 ± 2.2 (mean ± SD). When cells were exposed to PEMF with a magnetic intensity in the range from 0.18 to 2.5 mT, the lowest level of SCE induced was 6.5 ± 2.6 in the case where exposure was at 0.66 mT, and the highest level of SCE was 7.1 ± 2.1 at 0.44 mT (table 1). However, these values were not statistically different from values for cells not exposed to the fields (t-test; $p > 0.1$). These results showed that exposure of cells to the magnetic field with 0.18 to 2.5 mT, prior to the addition of BrdU, did not increase nor decrease the baseline frequency of SCE. SCEs have been shown to occur during DNA replication[13,18,19] and agents that interfere with DNA replication could increase the frequency of SCE[15,16]. The magnetic field at 0.4 mT represses the DNA synthesis to 80% of that in the control not exposed to the fields[4]; however, the present results observed in experiment (I) suggest that exposure of cells to the magnetic fields at 0.18–2.5 mT may interfere with the DNA synthesis but without causing significant changes in the frequency of SCE.

In experiment (II) the frequency of SCE induced in cells which were exposed to PEMF with various magnetic intensities together with BrdU was examined. Mean number of SCEs per metaphase plus or minus standard deviation induced in cells which were exposed to PEMF in the range from 0.18 to 2.5 mT was 6.5 ± 2.3 at 0.66 mT to 7.3 ± 2.3 at 2.1 mT (table 2). These values were not significantly different ($p > 0.05$) from that observed in the control cultures. This indicates that exposure of cells to the magnetic fields with 0.18–2.5 mT, when BrdU is present, induces no additional SCEs in the cells.

Metaphase cells observed in experiment (II) seem to be exposed to the magnetic fields at the G2 or late S phase of the cell cycle.

Table 1. Influence of PEMF on the frequency of SCE (Experiment I)

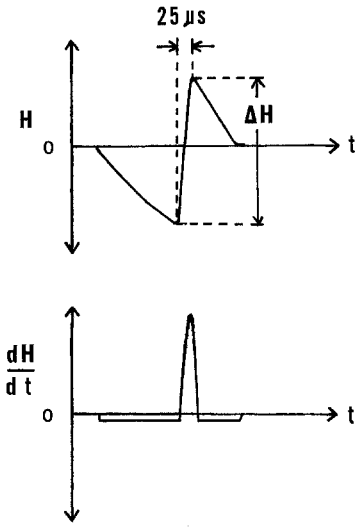
Magnetic intensity (mT)	SCEs per metaphase (mean ± SD)	Range
0	7.0 ± 2.2	2–14
0.18	7.0 ± 2.1	3–12
0.44	7.1 ± 2.2	3–12
0.66	6.5 ± 2.6	2–14
1.2	6.9 ± 2.1	3–12
1.7	6.6 ± 2.4	2–13
2.1	6.6 ± 2.4	2–14
2.5	6.6 ± 2.4	2–13

100 metaphases were scored for each treatment.

Table 2. Influence of PEMF on the frequency of SCE (Experiment II)

Magnetic intensity (mT)	SCEs per metaphase (mean ± SD)	Range
0	7.0 ± 2.2	2–14
0.18	7.1 ± 2.3	3–12
0.44	6.8 ± 2.5	2–12
0.66	6.5 ± 2.3	2–13
1.2	7.2 ± 2.2	3–12
1.7	7.1 ± 2.4	3–14
2.1	7.3 ± 2.3	3–14
2.5	7.1 ± 2.6	2–14

100 metaphases were scored for each treatment.



The electromagnetic field waveform of the pulse signal and its first time-derivative (below) measured using a calibrated search coil. The magnetic fields with various intensities used in the present work were generated by a 25-µs pulse, repeating at 100 Hz.

Physical agents, such as UV or ionizing radiations, increase the SCE frequency[11-13,15,16] and drugs, such as bleomycin or adriamycin, which are known to intercalate into or break the DNA molecules, have also been shown to increase the SCE frequency[14,20,21]. Contrary to these agents, which can modify the DNA molecules themselves, magnetic fields with an intensity in the range from 0.18 to 2.5 mT do not seem to damage DNA in cells which are entering the M phase of the cell cycle, thereby leading to an increased frequency of SCE.

The possible mechanisms involved in the modification of a variety of biological processes by magnetic fields are still largely unexplained at present. The present results show that magnetic fields with 0.18–2.5 mT seem to lack the ability to induce SCEs in cultured Chinese hamster cells. The intensities used here amount to 3.6–50-fold of the geomagnetic field; however, the magnetic fields produced by modern generators, such as those used for clinical diagnoses, have magnetic intensities far larger than those used here. Further investigations with the use of other cell types as well as other experimental conditions would be required to assess possible risk effects of magnetic fields.

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2 Rodan, G.A., Bourett, L.A., and Norton, L.A., Science *199* (1978) 690.
3 Liboff, A.R., Williams, T. Jr, Strong, D.M., and Wister, R. Jr, Science *223* (1984) 818.
4 Takahashi, K., Kaneko, I., Date, M., and Fukada, E., Experientia *42* (1986) 185.
5 Goodman, R., Bassett, C.A.L., and Henderson, A.S., Science *220* (1983) 1283.
6 Bassett, C.A.L., Pawluk, R.J., and Pilla, A.A., Science *184* (1974) 575.
7 Bassett, C.A.L., Mitchell, S.N., and Gaston, S.R., J. Am. med. Ass. *247* (1982) 623.
8 Sutcliffe, M.L., and Goldberg, A.A.J., Clin. Orthop. *166* (1982) 45.
9 Ohashi, T., Inoue, S., Sasaki, H., Ibaragi, K., Tada, M., and Ashihara, T., Trans. 3rd Ann. Meeting of the Bioelectr. Repair and Growth Soc., San Francisco *3* (1983) 50.
10 Trillo, M.A., Jimenez, M.A., Leal, J., Ubeda, A., and Delgado, J.M.R., Trans. 3rd Ann. Meeting of the Bioelectr. Repair and Growth Soc., San Francisco *3* (1983) 49.
11 Kato, H., Exp. Cell Res. *82* (1973) 383.
12 Gatti, M., and Oliveli, G., Mutat. Res. *17* (1973) 101.
13 Wolff, S., Bodydote, J., and Painter, R.B., Mutat. Res. *25* (1974) 73.
14 Perry, P., and Evans, H.J., Nature *258* (1975) 121.
15 Ishii, Y., and Bender, M.A., Mutat. Res. *79* (1980) 19.
16 Takahashi, K., Kaneko, I., Nishiyama, C., and Nakano, K., Mutat. Res. *144* (1985) 265.
17 Perry, P., and Wolff, S., Nature *251* (1974) 156.
18 Kato, H., Nature *252* (1974) 739.
19 Kato, H., Cancer Genet. Cytogenet. *2* (1980) 69.
20 Popescu, N.C., Turnbull, D., and DiPaolo, J.A., J. natl Cancer Inst. *59* (1977) 289.
21 Sono, A., and Sakaguchi, K., Cell Struct. Funct. *3* (1978) 341.

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Extra-nuclear inheritance in a sexually produced aphid: the ability to overcome host plant resistance by biotype hybrids of the greenbug, *Schizaphis graminum*

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Summary. Greenbugs of biotype E that grow and reproduce well on a biotype C-resistant sorghum (IS 809) were crossed with biotype C greenbugs. The resulting hybrids were tested on IS 809 to study how the ability to overcome the cultivar's resistance is inherited. Only those hybrids stemming from biotype E mothers were able to overcome the resistance of IS 809 plants, indicating that this trait is inherited by the sexually-produced fundatrices in an extra-nuclear manner from the mother. A plausible explanation for this phenomenon is presented.

Key words. Extra-nuclear inheritance; aphids; greenbugs; *Schizaphis graminum;* biotypes; hybrids; fundatrix; plant resistance; symbiotes.

The rapid evolution of aphid biotypes able to overcome resistance bred into graminaceous plants is well documented for the greenbug, *Schizaphis graminum* (Rondani)[1,2], the most destructive insect attacking small grains in the Great Plains region of the USA[3]. Most of the seasonal generations of the aphid are produced by asexual parthenogenetic reproduction. Each aphid thereby gives rise to genetically identical progeny and it is difficult therefore to elucidate the mechanism of inheritance of biotype traits. Under fall conditions, however, the aphid can reproduce sexually, and, from fertilized overwintering eggs, spring generations are produced by fundatrices which differ genetically from each other. This paper reports on the inher-