J. Radiat. Res., 45, 393–397 (2004)

Regular Paper

Genotoxic Effects of Strong Static Magnetic Fields in DNA-Repair Defective Mutants of *Drosophila melanogaster*

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Static magnetic field/Mutagenesis/Dose-response relationship/Wing spot test/*Drosophila melanogaster*

To assess the possibility that strong static magnetic fields cause DNA damage and mutation, we examined the genotoxic effects of magnetic field exposure by using the somatic mutation and recombination test system in DNA repair-proficient and -deficient strains of *Drosophila melanogaster*. A postreplication repair-defective mutation *mei-41*^{D5} and/or a nucleotide excision repair-defective mutation *mei-9*^{a} was introduced into the conventional loss of the heterozygosity assay system by the use of *mwh* +/ + *flr* transheterozygotes, and were exposed to static magnetic fields of up to 14 Tesla (T). We found that exposure to 2, 5, or 14 T fields for 24 h caused a statistically significant enhancement in somatic recombination frequency in the postreplication repair-deficient flies, whereas the frequency remained unchanged in the nucleotide excision repair-deficient flies and in the DNA repair-proficient flies after exposure. An increase linearly dependent on the flux density was observed between 0.5 T and 2 T, but it was saturated at exposure levels over 2 T. These findings suggest that exposure to high-density magnetic fields induce somatic recombination in *Drosophila* and that the dose-response relationship is not linear.

INTRODUCTION

Biological effects of static magnetic fields have been well documented. Positive findings have been reported for the effects of static magnetic fields on several end-points, including effects on blood flow[1)], elution pattern of red blood cells[2)], embryonal development[3,4)], orientation of macromolecules[5)], and oxygen dissolution in tissues[6)]. However, there are only a few reports on the genotoxic effects of static magnetic fields[7)]. Ikehata *et al*.[8)] showed that exposure to a static magnetic field (2 or 5 T) increased the point mutation rates induced by DNA-reactive chemicals on bacterial mutation assay. Zhang *et al*.[9)] reported that a 9 T static magnetic field was sufficient to induce mutation in *E. coli*, which lacked the superoxide stress response gene. Nakahara *et al*.[10)] showed that exposure to a 10 T static magnetic field enhanced the micronucleus formation induced by X-ray irradiation on Chinese hamster ovary cells. Recently, the use of strong static magnetic fields is increasing in various fields. Medical appliances such as magnetic resonance imaging (MRI) and transportation systems using superconducting magnets are routinely used in civilized countries. Energy storage rings are also coming into practical use in the near future. Therefore it is important to estimate the possible genetic effects, especially the carcinogenic effects, of strong static magnetic fields.

We have indicated previously that the somatic mutation and recombination test (SMART)[11)] was useful to detect the mutagenic activity of static[12)] or extremely low-frequency (50Hz)[13)] magnetic fields. In these reports, the use of a mutation *mei-41*, which is defective in postreplication repair function, made sensitivity of the test higher. We have found that exposure to a 5 T static magnetic field increases the frequency of somatic recombination in a heterozygous of *mei-41* defective mutant, and that the increase was suppressed to a control level by supplementation with vitamin E, a lipid-soluble antioxidant[12)]. This suggests a possibility that somatic recombination increases the following exposure to a static

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magnetic field, by affecting the lifetime of free radicals spontaneously produced in living cells.

Here, we examined the dose-response relationship of strong static magnetic fields up to 14 T by using the *Drosophila* somatic mutation and recombination test. Besides the postreplication defective *mei-41*, a nucleotide excision repair defective mutation *mei-9* was also used to obtain information on the mechanisms of mutagenicity of magnetic fields.

MATERIALS AND METHODS

Fly strains and culture conditions

In a conventional wing spot test, mating is performed to produce transheterozygotes of *mwh* (3-0.3) and *flr* (3-38.8). The frequency of mitotic recombination between the homologous chromosomes is determined by the examination of wing hairs with a mutant morphology. A nucleotide excision repair-defective mutation, *mei-9*^{a}[14], and a postreplication repair-defective *mei-41*^{D5} [15] were introduced into the conventional wing spot test system[11]. Virgin females of *y mei-9*^{a} *v f* · *y*$^{+}$ / *FM6; flr* / *TM6* were crossed with *y mei-9*^{a} *v f* · *y*$^{+}$ / *Y; mwh jv; spa*^{pol} males. Among eight kinds of F_1 offspring, homozygous *mei-9*^{a} females (*y mei-9*^{a} *v f* · *y*$^{+}$ / *y mei-9*^{a} *v f* · *y*$^{+}$*; mwh jv* / *flr*^{3}*; spa*^{pol}/+) were used as tester individuals. For the construction of *mei-41*^{D5} homozygote, *w mei-41*^{D5} / *FM6; flr* / *TM6* females were crossed with *w mei-41*^{D5} / *Y; mwh jv; spa*^{pol} males. For details about genes and balancers, see Lindsley and Zimm[16].

The flies were maintained on standard cornmeal-sugar-agar medium at 24 ± 0.5°C, 55 ± 5% relative humidity. Thirty mated females four days after eclosion were put in a culture vial with fresh medium and allowed to lay eggs for 24 h. The parental females were then removed, and the eggs were incubated at 24 ± 0.5°C for another 48 h. Thereafter, hatched (a mixture of second and third instars) larvae were exposed to a magnetic field or sham exposed for 24 h. After this exposure period, they were returned to the culture room and reared under normal conditions until molting. Background magnetic flux density in the culture room was less than 30 μT for the static field and less than 0.25 μT for the 50 Hz field. The lighting condition was L:D=12:12 in all experiments.

Magnetic field exposure and X-ray irradiation

Two superconducting magnets were used, producing static fields of up to 5 T (Toshiba JS-500) and 14 T (Oxford Instruments SCM type 14/70/1), respectively. Culture vials were placed in the center of the exposure bore of one of the two superconducting magnets where the field was homogenous. Temperature in the exposure bores was maintained at 25 ± 0.5°C. Larvae were exposed to a static magnetic field with a horizontal flux for 24 h. X-ray irradiation was performed with a Hitachi MBR-1505R operated at 130 kVp, 5 mA with 0.5 mm Al, and 0.1 mm Cu filters with a dose rate of 0.27 Gy/min. Third-instar larvae (84 ± 12 h after egg laying) were irradiated with 1 Gy. Thereafter they were returned to the culture room and reared again under normal culture conditions. Background magnetic flux density in the X-ray irradiation space was almost the same as that in the culture room.

Wing spot analysis

Microscopic observation was performed similarly to the previous reports[12,13]. When the tester flies were molted from pupal cases, their wings were excised, mounted on a glass slide and examined under a microscope for the presence of mutant hair spots. According to the original method of Graf *et al.*[11], large mutant spots with 3 or more mutant hairs and small spots with 1 or 2 mutant hairs were analyzed separately. Chromosomal deletion is considered to cause small spots because these phenomena decrease cell viability. Large *flr* spots and large *mwh* spots were also distinguished because only the latter resulted from somatic recombination. Large *flr* spots are inferred to result from cellular events other than recombination, most probably from point mutation. By contrast, *mwh* spots result from either point mutation or somatic recombination between the *flr* and *mwh* loci. Twin spots result solely from a somatic recombination between the *flr* locus and centromere. Somatic recombination or point mutation at a later developmental stage might result in small spots that are not distinguishable from spots caused by deletion.

The statistical significance of spot frequency differences was determined by applying Welch's *t*-test for equality of means between groups with unequal variances.

RESULTS

Three types of mutant hair spots appeared in the wings of the exposed flies in common with the sham-exposed ones in DNA repair proficient flies (Table 1). They were small single spots with 1 or 2 *mwh* hairs, large *mwh* spots with 3 or more hairs, and twin spots (*flr* and *mwh* spots appearing side by side). There were no spot types that were specific to the exposed or the sham-exposed group. Small *flr* spots and large *flr* spots were not observed in this experiment. The incidence of small spots per wing was approximately 0.2 in sham-exposed flies, and the incidence of large *mwh* spots per wing was much less frequent and approximately 0.03. A static magnetic field of 5 or 14 T did not significantly alter the spontaneous frequencies in all kinds of spots. Irradiation of the flies to X-ray (1 Gy) enhanced small and large *mwh* spot frequencies.

When DNA repair-defective mutations were introduced in tester flies, four types of the mutant hair spots appeared (Table 1). The incidence of small spots per wing increased drastically. It was approximately 15 spots per wing in sham-exposed *mei-41* flies and 8 in sham-exposed *mei-9* flies. The

Table 1. Wing spot frequency induced by exposure to static magnetic fields.

	No. of wings	small spot	large *flr* spot	large *mwh* spot	twin spot
DNA repair-proficient (*mwh jv/flr^3 ; spa^{pol}/+*)					
14 T	97	20 (0.21)	0 (0)	2 (0.02)	0 (0)
5T	203	39 (0.19)	0 (0)	4 (0.02)	2 (0.01)
X-ray 1Gy	87	63 (0.72)**	0 (0)	9 (0.18)**	2 (0.04)
Sham-exposed	281	54 (0.19)	0 (0)	8 (0.03)	2 (0.01)
mei-41-deficient (*w mei-41^{D5} / w mei-41^{D5} : mwh jv/flr^3 ; spa^{pol}/+*)					
14 T	100	2,050 (20.5)	1 (0.01)	81 (0.81)**	11 (0.11)
5T	76	1,361 (17.9)	1 (0.01)	76 (1.00)**	8 (0.11)
X-ray 1Gy	70	1,786 (25.4)*	5 (0.07)	96 (1.37)**	5 (0.07)
Sham-exposed	528	8,071 (15.3)	8 (0.02)	297 (0.56)	54 (0.10)
mei-9-deficient (*y mei-9^a v f · y$^+$ /y mei-9^a v f · y$^+$; mwh jv/flr^3 ; spa^{pol}/+*)					
14 T	100	740 (7.40)	0 (0.02)	51 (0.51)	14 (0.14)
5T	168	1,398 (8.32)	5 (0.03)	64 (0.38)	27 (0.16)
X-ray 1Gy	48	384 (8.0)	0 (0)	54 (1.1)**	6 (0.1)
Sham-exposed	269	2,187 (8.13)	2 (0.01)	101 (0.38)	33 (0.12)

Numbers in parentheses represent spot frequency per wing. X-ray irradiation is shown as a positive control. *significantly higher than the corresponding sham-exposed group at $P<0.05$. **significantly higher than the corresponding sham-exposed group at $P<0.01$.

incidence of large *mwh* spots per wing also increased to approximately 0.6 and 0.4, respectively. X-ray irradiation of 1 Gy enhanced the spot frequency in both *mei-41*-deficient and *mei-9*-defecient flies. The frequency of the large *flr* spot, the twin spot, or the sum of the large *flr* spot and the twin spot was not increased in all strains. This shows that defects in DNA repair function, either postreplication repair or nucleotide excision repair, resulted in an increase in somatic mutations.

When *mei-41*-deficient flies were exposed to a static magnetic field of 5 or 14 T for 24 h, large *mwh* spots appeared at a significantly higher frequency than in sham-exposed flies. Twin spots also increased, but they were not statistically significant. The frequency of small spots and large *flr* spots, however, remained unchanged. When *mei-9* flies were exposed to a 5 T or a 14 T field, no significant differences were observed in all kinds of spots.

The dose-response relationship of large *mwh* spot frequency to the magnetic flux density up to 14 T is shown in Fig. 1. The incidence of large *mwh* per wing increased significantly when the cells were exposed to 2, 5, or 14 T field for 24 h.

The increases of mutation frequencies in these exposed groups were quite similar to one an other; thus the dose-response relationship was not detected from 2 to 14 T. The exposure to 1 T field also increased, but this was not statistically significant. No increase was observed in flies exposed to a 0.5 T field. Instead, the mutant spot frequency in the 0.5 T group was lower than in 0 T group; therefore it appears that the dose-response relationship has a threshold of about 0.5 T.

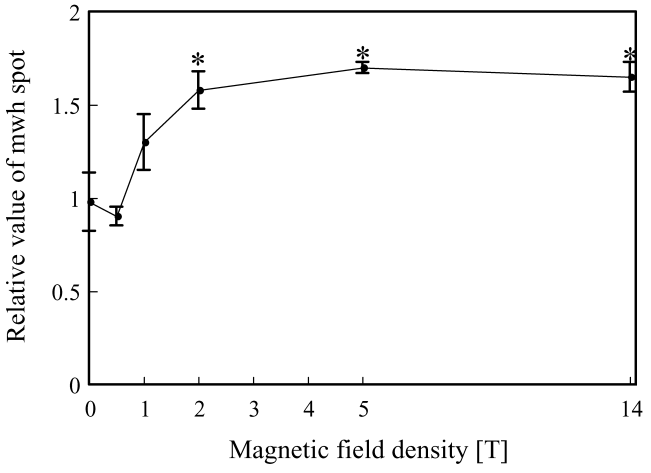

Fig. 1. Dose-dependence of the somatic mutation spot in the repair-deficient *mei-41^{D5}* flies. The ordinate is the ratio between large *mwh* spot frequency in the magnetic field exposed groups and that in the sham-exposed group. Results are expressed as means ± SD for four independent experiments. * Significantly higher than the corresponding sham-exposed group ($P < 0.01$).

DISCUSSION

We found that an exposure of *mei-41* flies to strong static magnetic fields caused an increase in large *mwh* spot frequency, but the incidence of other spots did not change (Table 1). In the previous study, Zimmering *et al.*[17] showed that the late third instar larvae were more sensitive to form the large *flr* and twin spots by an exposure of ethyl methanesulfonate; it is then possible that the second or early third instar larvae were insensitive to form these spots by an exposure to the static magnetic field. However, the large *mwh* spot frequency was extremely high. It is suggested that point mutation and chromosomal deletion were not affected by the exposure and that most of the large *mwh* spots resulted, as in the sham-exposed group, from somatic recombination. Actually, Koana *et al.*[12] indicated that in *TM6*-carrying flies, in which homologous recombination on the third chromosome is totally suppressed, large *mwh* spots scarcely appeared even when exposed to a 5 T field. In contrast, the spot frequency did not increase on exposure of the DNA repair-proficient strain or the nucleotide excision repair-defective mutant strain. These results indicate that the increase in somatic mutation frequency was caused by a defect of *mei-41* functions. Recently, we have also reported that *mei-41*-proficient and -deficient flies showed different shapes of the dose-response curve in X-ray irradiation. This suggests that the DNA repair function is involved in the cellular response to ionizing radiation[18]. The *mei-41* gene is a structural and functional homologue of the human Ataxia telangiectasia gene[19], and homozygous *mei-41* flies are defective in phosphatidylinositol-3 kinase activity and postreplication repair function[20]. It was previously shown that the introduction of *mei-41*^{D5} mutation increased the sensitivity of the wing spot test[12]. The *mei-41* deficient cells display high levels of mitotic chromosome instability and fail to show DNA damage-induced cell cycle arrest[19]. It is then possible that the exposure to static magnetic fields affects damage-induced the cell cycle checkpoint or DNA replication.

The effect of magnetic fields on the rate of radical pair recombination is the best-understood mechanism by which magnetic fields interact with biological systems[21]. Free radicals or reactive oxygen species (ROS) are major sources of damage to DNA and proteins. Several reports have suggested that ROS induced by a static magnetic field may cause mutation or apoptosis. Zhang *et al.*[9] reported that a 9 T static magnetic field was sufficient to induce mutation in *E. coli,* which lacked the superoxide stress-response gene. This study suggests that exposure to a static magnetic field induces mutations through an elevated production of intracellular superoxide radicals. Jajte *et al.*[22] reported that the exposure of rat lymphocytes to a 7 mT static magnetic field and iron ions caused an increase in apoptosis and necrosis. Although this study showed the positive effects of static magnetic field exposure at lower flux density, this is consistent with our results because highly reactive hydroxyl radical is produced in response to an exposure to a static magnetic field in the presence of transition metals (principally iron ions). We have also reported previously that exposure to a 5 T static magnetic field increases the frequency of mutation in *mei-41* heterozygotes, and that the increase was suppressed to control levels by supplementation with vitamin E, a lipid-soluble antioxidant[12]. In the present study, we demonstrated a dose-response relationship between magnetic flux density and somatic recombination rate. A linear increase was observed up to exposure levels of 2 T, and the increase was saturated above 2 T (Fig. 1). In the field of organic chemistry, Sakaguchi *et al.*[23] found that in some radical reactions, the decay rate of free radicals was affected dose-dependently by exposure to an external static magnetic field and that the effect was saturated at about 1 T. The shape of the dose-response curve in the present study is similar to the dose-dependence of free radicalsí lifetimes found by Sakaguchi *et al.*[23] Although the existence of the threshold is unknown in chemical reactions, the threshold of biological effects may exist. Therefore the present data offer other circumstantial evidence that is supportive of the free radical hypothesis for the mutational effects of magnetic fields.

ACKNOWLEDGMENTS

We thank Ms. Mikie O. Okada (Railway Technical Research Institute) for technical suggestions. This work was supported in part by a Grant-in-aid from Research for the Future Program of Japan Society for the Promotion of Science.

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Received on December 16, 2003
1st Revision on April 7, 2004
2nd Revision on July 7, 2004
Accepted on July 7, 2004