Effects of a 50 Hz electric field on plasma lipid peroxide level and antioxidant activity in rats

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ABSTRACT

The effects of exposure to extremely low frequency electric fields (ELF EFs) on plasma lipid peroxide levels and antioxidant activity (AOA) in Sprague-Dawley rats were studied. The test was based on comparisons among rats treated with a combination of the oxidizing agent, 2,2'-azobis(2-aminopropane) dihydrochloride (AAPH), and 50 Hz EF of 17.5 kV/m intensity for 15 minutes per day for seven days, AAPH alone, EF alone or no treatment. EF significantly decreased the plasma peroxide level in rats treated with AAPH, similar to treatment by ascorbic acid or the superoxide dismutase. Ascorbic acid increased AOA, however, EF and superoxide dismutase did not change AOA compared with sham exposure in stressed rat. No influence on the lipid peroxide level and AOA in unstressed rats was observed with EF exposure alone. Although the administration of AAPH decreased AOA, this decrease did not change when EF was added. These data indicate that the ELF EF used in this study influenced the lipid peroxide level in an oxidatively stressed rat.

antioxidant activity; 2,2'-azobis (2-aminopropane) dihydrochloride; ELF electric field; lipid peroxide; tert-butylhydroperoxide

INTRODUCTION

The biological effects of an extremely low frequency electric field (ELF EF) have become a matter of interest and debate as worldwide distribution of electricity, which has markedly increased during the last half century, resulted in increased human exposure to electromagnetic fields (EMFs) [Repacholi et al., 1999]. Clinical techniques using EFs have been developed in disciplines such as complementary and integrative medical therapies [Ghoname et al., 1999; Goldman et al., 1996; Hara 1961; Harakawa et al., 2002; Ito 2000; Lee et al., 1993]. In Japan, EF therapy has been utilized since 1972, when Hara et al. [1961] developed EF exposure equipment (Healthtron, Hakuju Institute for Health Science, Tokyo, Japan), which was approved by the Ministry of Health, Labour and Welfare (Approval number 14700BZZ00904). Recently, we reported the curative effects of EF therapy on several types of pain (headache, stiff shoulders and stomachache) in over a thousand cases [Harakawa et al., 2002]. Reports explaining the mechanism(s) of the response to an exposure to an EF have suggested that either the EF-induced electric current or the perception of the EF through the skin surface act as a trigger on cellular, humoral or behavioral responses [Harakawa et al., 2004a; Harakawa et al., 2004b; Jaffe et al., 1981; Jaffe et al., 1980; Kato et al., 1989; Weigel et al., 1987a; Weigel et al., 1987b].

Some investigators have reported that reactive oxygen species might be active in the mechanisms of ELF EMF effects. Plasma levels of melatonin secreted from the pineal gland and regulated by circadian rhythms may be suppressed by exposure to an EMF, but these results are considered controversial [Levallois et al., 2001; Reiter et al., 1988; Wilson et al., 1981; Wilson et al., 1986]. Additionally, the balance between the generation of reactive oxygen species and the re-uptake of melatonin at the cellular level may modulate

the mechanisms of EMF influences [Reiter et al., 1998]. The increase of phagocytosis activity in mouse bone marrow cells exposed to 50Hz EMF concomitantly upregulates the intracellular level of reactive oxygen species [Simko et al., 2001]. The effects of an EMF potentiate the cellular damage induced in vitro by oxidizing agents in rabbit red blood cells [Fiorani et al., 1997]. A direct current EF pulse induces the production of reactive oxygen species, which in turn mediate Ca+2 release from intracellular stores and activate cell cycle activity in multi-cellular spheroids [Wartenberg et al., 1997]. In the presence of catalase, superoxide dismutase or vitamin E, the enhancement of chick embryo fibroblast cell proliferation from 100 Hz sinusoidal magnetic fields (MF) was reduced and the addition of free radical scavengers during exposure to a MF significantly suppressed the enhancement in cell proliferation caused by the field [Katsir et al., 1998].

However, these reports of the possible harmful effects caused by reactive oxygen species generated by exposure to EMF do not refer to the effects of EF. If the biological effects of various electrical treatments or environments, including MF or EMF, involved free radical metabolism as a common target, it would be very important to confirm these results for EF exposure alone.

The preliminary study presented here was conducted to determine whether the exposure to sine waveform 50 Hz EF, 17.5 kV/m intensity modifies plasma antioxidant activity (AOA) and lipid peroxide levels in oxidatively stressed and/or unstressed rats.

MATERIALS AND METHODS

Experimental procedures using animals in this study were carried out at the Environmental Biological Research Center, Shiga, Japan, and were conducted in

accordance with the guiding principles and requirements for the Environmental Biological Research Center and Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan.

Animals: Male, eight-week-old Sprague-Dawley rats (n = 55), weighing 252-274 grams, were purchased from Japan SLC Inc. (Tokyo, Japan), and were maintained in group housing (five rats per cage) in a conventional, air-conditioned animal room.

EF exposure system: The EF exposure system (Fig. 1) is composed of three major parts, namely, a high voltage transformer (Hakuju Institute for Health Science, Tokyo, Japan), a constant voltage unit (TOKYO SEIDEN, Tokyo, Japan), and EF exposure cages, which have been previously described [Harakawa et al., 2004b]. Briefly, the exposure cage, which is designed for a rat or a smaller animal, is composed of a cylindrical plastic cage (diameter: 400 mm, height: 400 mm) with two electrodes made of stainless steel (1,200 x 1,200 mm) placed over and under the cylindrical cage. In order to form a 50 Hz sine waveform EF 17.5 kV/m RMS intensity in the cage, a stable alternating current (7 kV) was applied to the upper electrode. Experiments were carried out at normal room temperature (25 ± 0.4ºC). In this study, we used four device sets: two sets for exposure to EF and another two for exposure to sham EF. In two device sets for sham exposure, pair of electrodes was connected with wire to generate sham EF (0 V/m) and a switch was turned off during EF session. To estimate an intensity of EF in each set for EF exposure, voltage between electrodes was measured used Fluke 83 (Fluke Co. Ltd., WA) and applied voltage was arranged to 7 kV. Each exposure cage housed one rat during each experimental session in order to avoid an imbalance of EF induced by housing two or more rats at the same time.

Exposure of unstressed rats to EF: To examine the effects of EF on unstressed rats, the animals were exposed to a sine wave 50 Hz, 17.5 kV/m intensity, 15 minutes per day for one week. Rats were randomly divided into five groups. Each rat in five groups (n = 5) was individually treated with an EF or a sham EF for one day or seven days, or was not treated for seven days. Rats exposed to sham EF were equally maintained with EF exposed rats except exposure to sham EF. Exposure sessions for the seven-day exposures always were done in the same sequence. Under pentobarbital (45 mg/kg, i.p.) anesthesia, blood was collected from the abdominal cava vein after treatment on experimental day one or seven and plasma was separated by centrifugation at 1,670 x g for 10 minutes at 4ºC. The plasma samples were stored at -80ºC until tested.

Exposure of EF treated rats to AAPH: To examine the effects of EF during oxidative stress, rats were randomly divided into five groups (n = 8) as follows, Group 1) non treatment; Group 2) rats were treated with the oxidizing agent, AAPH (10 mg/kg, i.p.), on experimental day seven; Group 3) rats were exposed to a sine wave 50 Hz, 17.5 kV/m intensity, 15 minutes per day for one week and treated with AAPH (10 mg/kg, i.p.) at just before EF session on experimental day seven; Group 4) rats were treated with the anti-oxidant agent, ascorbic acid (500 mg/kg, p.o.) 60 minutes before the AAPH (10 mg/kg, i.p.) on experimental day seven; or Group 5) rats were treated with superoxide dismutase (50 mg/kg, s.c., Nacalai Tesque, Tokyo, Japan) just before the AAPH treatment (10 mg/kg, i.p.) on experimental day seven. In addition, Groups 2, 4 and 5 were sham exposed, in which rats were maintained for an equal period of time inside the exposure cage with the system turned off. Exposure sessions for the seven-day exposures always were done in the same sequence. Under pentobarbital (45 mg/kg, i.p.) anesthesia, blood was collected

from the abdominal cava vein 90 minutes after AAPH administration, and the plasma was separated by centrifugation at 1,670 x g for 10 minutes at 4ºC. The plasma samples were stored at -80ºC until tested.

Antioxidant activity (AOA): Plasma AOA was measured by a method using a derivative of methylene blue. Briefly, 0.1 ml plasma or a solution of ascorbic acid (Wako Ltd., Osaka, Japan) and 0.65 ml distilled water was added to 1.5 ml of Good's buffer adjusted to pH 5.8 [Good et al., 1966], including 1 % triton X-100 supplemented with 3,7-bis-demethylamino-10-methyl carbamoyl phenothiazine (MCDP, 40 µM, KYOWA MEDEX Co., Ltd., Tokyo, Japan) and hemoglobin (67.5 µg/ml). After incubation for one minute at 37ºC, 0.25 ml of tert-butylhydroperoxide (84 µM, BHP, Aldrich) as an alkoxyl radical initiator was added and reacted for 10 minutes at 37ºC. In addition, AOA measurements in a reaction using AAPH (50 mM) as a peroxy radical initiator were conducted in Good's buffer without hemoglobin for 20 minutes at 37ºC. A radical initiator not trapped by an antioxidant in each plasma sample will oxidize MCDP. The absorbance of the resultant blue color was subsequently measured at 675 nm using a spectrophotometer (150-20, Hitachi). The equation to calculate AOA was the following: AOA (%) = (1-Abs1/Abs2) x 100, where Abs1 is the absorbance of the plasma sample and Abs2 is a value of the blank. All samples were tested in one day.

Lipid peroxide: Thiobarbituric acid reactive substance (TBARS), as an indicator of the plasma concentration of lipid peroxide, was measured as level of malondialdehyde using a commercial kit (Lipid peroxide-test Wako, Wako Ltd., Osaka, Japan). All samples were tested in one day.

Statistical analyses: The results are expressed as the mean ± standard error of

the mean (SEM). The statistical significance of the differences among all groups was calculated by a one-way ANOVA and that between two groups was calculated by Tukey's multiple comparison test. The level of significance was defined as $P<0.05$. All computations for the statistical analyses were carried out using Prism Version 4.0b (GraphPad Software Inc., San Diego, CA).

RESULTS AND DISCUSSION

There were no significant differences in the plasma lipid peroxide levels between EF and sham groups in unstressed rats (Table 1). Table 2 summarizes the plasma lipid peroxide levels in rats administered AAPH just before exposure to EF on day seven. There were no significant differences between the non-treatment and sham group. However, the plasma levels of lipid peroxide in the group exposed to EF showed a remarkable decrease compared to those two groups ($P<0.01$). AAPH is well known as a generator of peroxy radical, which induces lipid peroxide. Ascorbic acid is able to trap free radical species. The plasma levels of lipid peroxide in rats treated with ascorbic acid or superoxide dismutase were lower when compared to the sham group ($P<0.01$ and $P<0.001$, respectively). Present finding might indicate the involvement of exposure to ELF EF in lipid peroxide metabolism. The data provided by an earlier report [Kimura et al., 1988] were consistent in suggesting that exposure to ELF EF would suppress plasma levels of lipid peroxide.

The AOA of plasma, which had been added to AAPH or BHP in a test tube, are summarized in Table 3. The AOA of plasma against AAPH-induced peroxy radicals in rats exposed to EF days did not show any differences when compared to the sham group.

Similarly, the AOA against BHP-induced alkoxyl radicals did not show any differences among the three groups. However, the addition of ascorbic acid to the plasma of the non-treatment group significantly elevated the AOA against both oxidizing agents (P<0.001). In unstressed rats, plasma AOA was neither enhanced nor inhibited by exposure to EF. These results indicate that exposure to the 50 Hz EF used in this study does not have a significant effect on the plasma AOA of an unstressed rat.

The AOA against BHP or AAPH in the plasma from rats co-treated with AAPH and EF are listed in Table 4. The plasma of the rats treated with AAPH did not show any changes in AOA against BHP, but did show a suppression in AOA against AAPH. Plasma AOA in the sham group was significantly suppressed compared to those of the non-treatment group (P<0.05). By contrast, plasma AOA in rats administrated ascorbic acid was significantly higher than that of the other four groups (P<0.01). Plasma AOA in EF-exposed or superoxide dismutase-treated groups was not different from that of the sham exposed group. The administration of ascorbic acid resulted in an increase of AOA against BHP (P<0.01) when compared to the other four groups.

Studies focused on the safety of EMF exposure in humans have reported the involvement of oxidative stress, such as that induced by reactive oxygen species, in the mechanism(s) of EMF exposure to organisms [Fiorani et al., 1997; Katsir et al., 1998; Moustafa et al., 2001; Reiter et al., 1998; Simko et al., 2001; Wartenberg et al., 1997]. However, this linkage is controversial and the discussion of a possible influence of exposure to a pure EF constructed without a MF has been largely ignored. Whether exposure to ELF EF alone has any impact on the biological response to oxidative stress is not known. This study specifically addressed whether exposure to ELF EF modifies

plasma AOA and lipid peroxide levels in unstressed and oxidatively stressed rats. Lipid peroxidation products have been accepted as biomarkers for oxidative stress in biological systems [Laval 1996]. If cellular or plasma reactive oxygen species levels were influenced by exposure to ELF EF, plasma levels of lipid peroxide would be altered as well. Although these results indicates that exposure to 50 Hz EF used in this study, which were in the ELF range and were "pure" (i.e., not magnetized) EF, did not affect plasma AOA against AAPH-induced peroxy radical or BHP-induced alkoxyl radical in rats administered AAPH, concomitantly suggests that the EF used in this study might has some influence on lipid peroxide metabolism. Because plasma AOA might not be influenced by presence of EF, EF would not significant effect in the mechanism against reactive oxygen species, which are already generated. In future, we need to clarify whether EF has some effects in lipid peroxide metabolism including glutathione peroxidase.

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LEGENDS

FIGURE 1. The electric field (EF) exposure system [Harakawa et al., 2004b]

The EF exposure cage (A). A cylindrical plastic cage (2) is placed between one upper and three lower stainless steel electrodes. The cylindrical cage has slits (length: 100 mm; width: 5 mm) at 5 mm intervals. The slits prevent contamination due to feces and saliva from disturbing the formation of a stable EF. Overview of the system (B).

REFERENCES

Fiorani M, Biagiarelli B, Vetrano F, Guidi G, Dacha M, Stocchi V. 1997. In vitro effects of 50 Hz magnetic fields on oxidatively damaged rabbit red blood cells. Bioelectromagnetics 18(2):125-31.

Ghoname EA, William FC. 1999. The effect of stimulus frequency on the analgesic response to percutaneous electrical nerve stimulation in patients with chronic low back pain. Anesth Analg 88:841-846.

Goldman R, Pollack S. 1996. Electric fields and proliferation in a chronic wound model. Bioelectromagnetics 17:450-457.

Good NE, Winget GD, Winter W, Connolly TN, Izawa S, Singh RM. 1966. Hydrogen ion buffers for biological research. Biochemistry 5(2):467-77.

Hara H. 1961. On the effect of AC electrostatic high potential load upon the blood-electrolytes. Niigata Igakkai Zasshi 75:1-8.

Harakawa S, Doge F, Saito A. 2002. Exposure to electric field (EF): Its palliative effect on some clinical symptoms in human patients. Research Bulletin of Obihiro University 22:193-199.

Harakawa S, Inoue N, Saito A, Doge F, Nagasawa H, Suzuki N, Martin DE. 2004a. 60 Hz electric field upregulates cytosolic Ca2+ level in mouse splenocytes stimulated by lectin. Bioelectromagnetics 25(3):204-10.

Harakawa S, Takahashi I, Doge F, Martin DE. 2004b. Effect of a 50 Hz electric field on plasma ACTH, glucose, lactate, and pyruvate levels in stressed rats. Bioelectromagnetics 25(5):346-51.

Ito F. 2000. The role of EF therapeutic device in the therapy of acute low back pain. Nihon

Onsen Kikou Igakkai Zasshi 63:127-137.

Jaffe RA, Laszewski BL, Carr DB. 1981. Chronic exposure to a 60-Hz electric field: effects on neuromuscular function in the rat. Bioelectromagnetics 2(3):227-39.

Jaffe RA, Laszewski CA, Carr DB, Philips RD. 1980. Chronic exposure to a 60-Hz electric field: Effects on synaptic transmission and peripheral nerve function in rat. Bioelectromagnetics 1:131-148.

Kato M, Ohta S, Shimizu K, Tsuchida Y, Matsumoto G. 1989. Detection-threshold of 50-Hz electric fields by human subjects. Bioelectromagnetics 10:319-327.

Katsir G, Parola AH. 1998. Enhanced proliferation caused by a low frequency weak magnetic field in chick embryo fibroblasts is suppressed by radical scavengers. Biochem Biophys Res Commun 252(3):753-6.

Kimura R, Sato M, Kato Y, Aimoto T, Hara A. 1988. The effect of high-intensity electric-field exposure on lipid peroxidation in blood and organs in rats. J Bioelectricity 7(1):77-88.

Laval J. 1996. Role of DNA repair enzymes in the cellular resistance to oxidative stress. Pathol Biol (Paris) 44(1):14-24.

Lee RC, Canaday DJ, Doong H. 1993. A review of the biophysical basis for the clinical application of electric fields in soft-tissue repair. J Burn Care Rehabil 14(3):319-35.

Levallois P, Dumont M, Touitou Y, Gingras S, Masse B, Gauvin D, Kroger E, Bourdages M, Douville P. 2001. Effects of electric and magnetic fields from high-power lines on female urinary excretion of 6-sulfatoxymelatonin. Am J Epidemiol 154(7):601-9.

Moustafa YM, Moustafa RM, Belacy A, Abou-El-Ela SH, Ali FM. 2001. Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide

and antioxidase activities in human erythrocytes. J Pharm Biomed Anal 26(4):605-8.

Reiter RJ, Anderson LE, Buschbom RL, Wilson BW. 1988. Reduction of the nocturnal rise in pineal melatonin levels in rats exposed to 60-Hz electric fields in utero and for 23 days after birth. Life Sci 42(22):2203-6.

Reiter RJ, Tan DX, Poeggeler B, Kavet R. 1998. Inconsistent suppression of nocturnal pineal melatonin synthesis and serum melatonin levels in rats exposed to pulsed DC magnetic fields. Bioelectromagnetics 19(5):318-29.

Repacholi MH, Greenebaum B. 1999. Interaction of static and extremely low frequency electric and magnetic fields with living systems: health effects and research needs. Bioelectromagnetics 20(3):133-60.

Simko M, Droste S, Kriehuber R, Weiss DG. 2001. Stimulation of phagocytosis and free radical production in murine macrophages by 50 Hz electromagnetic fields. Eur J Cell Biol 80(8):562-6.

Wartenberg M, Hescheler J, Sauer H. 1997. Electrical fields enhance growth of cancer spheroids by reactive oxygen species and intracellular Ca2+. Am J Physiol 272(5 Pt 2):R1677-83.

Weigel RJ, Jaffe RA, Lundstrom DL, Forsythe WC, Anderson LE. 1987a. Stimulation of cutaneous mechanoreceptors by 60-Hz electric fields. Bioelectromagnetics 8:337-350.

Weigel RJ, Lundstrom DL. 1987b. Effect of relative humidity on the movement of rat vibrissae in a 60-Hz electric field. Bioelectromagnetics 8(1):107-10.

Wilson BW, Anderson LE, Hilton DI, Phillips RD. 1981. Chronic exposure to 60-Hz

electric fields: effects on pineal function in the rat. Bioelectromagnetics 2(4):371-80.

Wilson BW, Chess EK, Anderson LE. 1986. 60-Hz electric-field effects on pineal melatonin rhythms: time course for onset and recovery. Bioelectromagnetics 7(2):239-42.

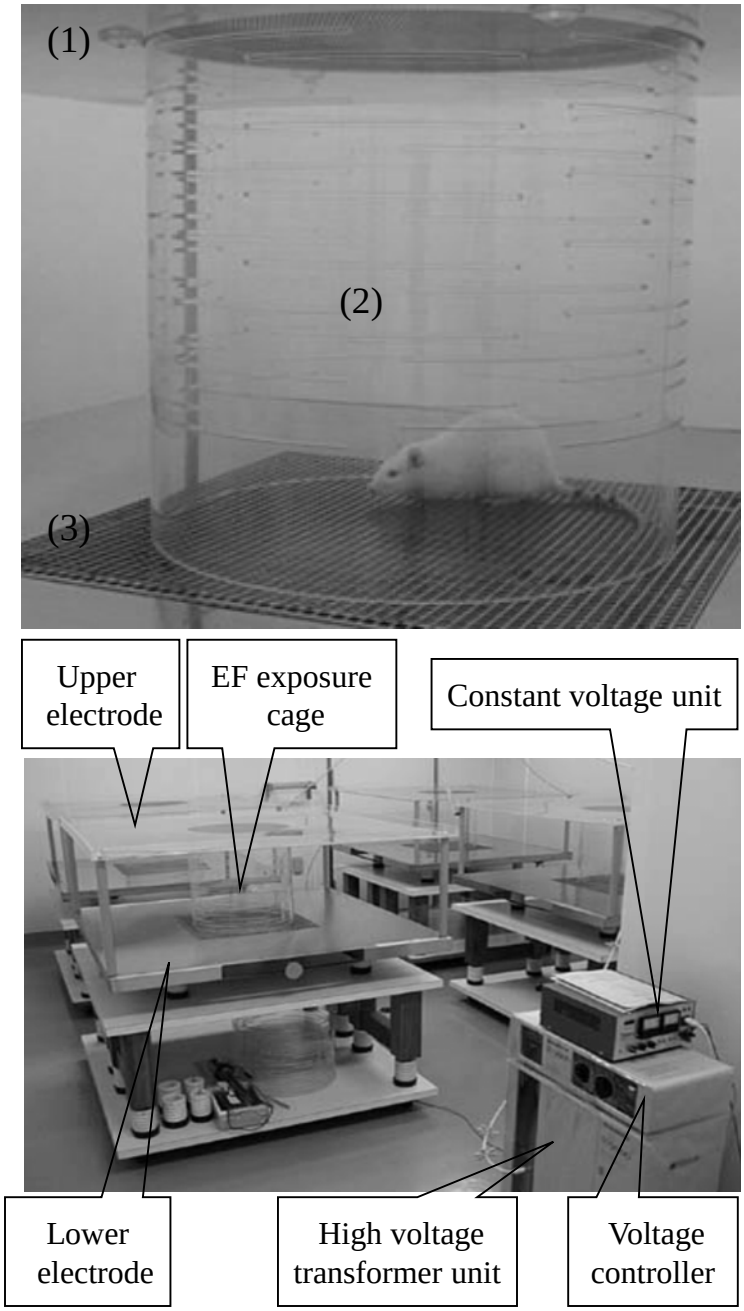

Figure 1

From Harakawa, S. et al. (2004b)
Effects of a 50 Hz electric field on plasma ACTH, glucose, lactate and pyruvate levels in stressed rats

Treatment	Malondialdehyde concentration nmol/ml±SEM
Non-treatment	2.60 ± 0.06
Sham-exposure (1 day)	2.22 ± 0.12
EF exposure (1 day)	2.28 ± 0.11
Sham-exposure (7 days)	1.90 ± 0.05
EF exposure (7 days)	2.12 ± 0.09

TABLE 1. Effects of electric field (EF) exposure on plasma lipid peroxide levels without AAPH oxidative stress in rats.

Each value represents the mean±SEM (n = 5). No significant differences between EF exposure and sham-exposures were found.

Treatment	Dose (mg/kg)	Route	AAPH on day 7	EF status days 1-7	Malondialdehyde concentration nmol/ml ±SEM
Non-treatment	-	-	-	-	2.014 ± 0.13
Sham-exposure	-	-	10 mg/kg, i.p.	Sham EF	1.957 ± 0.09
EF exposure	-	-	10 mg/kg, i.p.	50 Hz 17.5 kV	1.503 ± 0.03 * #
Ascorbic acid on day 7[a)]	500	p.o.	10 mg/kg, i.p.	Sham EF	1.509 ± 0.05 * #
SOD on day 7[b)]	50	s.c.	10 mg/kg, i.p.	Sham EF	1.139 ± 0.10 ** ## † ‡

TABLE 2.
Effects of electric field (EF) exposure on plasma lipid peroxide levels in AAPH-treated rats.

a) Water was used to prepare the ascorbic acid solution, 10 ml/kg; b) Saline was used to prepare the SOD solution, 2 ml/kg
AAPH: 2,2'-azobis(2-amidinopropane)dihydrochloride; SOD: superoxide dismutase
Each value represents the mean±SEM (n = 8). * and **: significant difference from non-treatment at $P<0.01$ and 0.001. # and ##: significant difference from sham-exposure at $P<0.01$ and 0.001. †: significant difference from EF exposure at $P<0.05$. ‡: significant difference from ascorbic acid at $P< 0.05$.

Treatment	Dose (ng/ml)	AOA_{AAPH} %±SEM	AOA_{BHP} %±SEM
Non-treatment	-	36.7 ± 4.7	75.7 ± 0.9
Sham-exposure (1 day)	-	41.5 ± 2.6	75.5 ± 0.7
EF exposure (1 day)	-	31.4 ± 6.7	75.0 ± 0.7
Sham-exposure (7 days)	-	25.0 ± 2.6	75.4 ± 2.1
EF exposure (7 days)	-	18.0 ± 3.0 * ##	73.7 ± 0.7
Ascorbic acid (a)	440	62.0 ± 3.8 ** # † ‡ æ	87.7 ± 0.3 *** ### † ‡ æ

TABLE 3. Effects of electric field (EF) exposure on the antioxidant activity (AOA) of plasma without AAPH oxidative stress in rats.

AAPH: 2,2'-azobis(2-amidinopropane)dihydrochloride; BHP: tert-butylhydroperoxide
Each value represents the mean±SEM (n = 5). No significant difference between EF exposure and sham-exposure. (a): the plasma in non-treatment group was partly separated to the other test tube to measure AOA in addition of ascorbic acid (440 ng/ml). *, ** and ***: significant difference from non treatment at $P<0.05$, 0.01 and 0.001. #, ## and ###: significant difference from sham-exposure (1 day) at $P<0.05$, 0.01 and 0.001. †: significant difference from EF exposure (1 day) at $P<0.001$. ‡: significant difference from sham-exposure (7 days) at $P< 0.001$. æ: significant difference from EF exposure (7 days) at $P< 0.001$.

Treatment	Dose (mg/kg)	Route	AAPH on day 7	EF status days 1-7	AOA(AAPH) %±SEM	AOA(BHP) %±SEM
Non-treatment	-	-	-	-	32.4 ± 4.0	76.8 ± 1.1
Sham-exposure	-	-	10 mg/kg, i.p.	Sham EF	18.4 ± 0.8 *	76.2 ± 1.5
EF exposure	-	-	10 mg/kg, i.p.	50 Hz 17.5 kV	19.6 ± 4.4	72.8 ± 1.4
Ascorbic acid on day 7	500	p.o.	10 mg/kg, i.p.	Sham EF	46.3 ± 3.2 * # †	87.5 ± 0.3 ** ## †
SOD on day 7	50	s.c.	10 mg/kg, i.p.	Sham EF	22.1 ± 2.5 ‡	78.0 ± 2.1 ‡

TABLE 4. Effects of electric field (EF) exposure on the antioxidant activity (AOA) of plasma in AAPH-treated rats.

AAPH: 2,2'-azobis(2-amidinopropane)dihydrochloride; BHP: tert-butylhydroperoxide; SOD: superoxide dismutase
Each value represents the mean±SEM (n = 8). No significant difference between EF exposure and sham-exposure. * and **: significant difference from non-treatment at $P<0.05$ and 0.001. # and ##: significant difference from sham-exposure at $P<0.01$ and 0.001. †: significant difference from EF exposure at $P<0.001$. ‡: significant difference from ascorbic acid at $P< 0.001$.