Zinc supplementation ameliorates static magnetic field-induced oxidative stress in rat tissues

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Abstract

The present study was undertaken to find out the effect of zinc supplementation on the antioxidant enzymatic system, lipid peroxidation and DNA oxidation in liver and kidney of static magnetic field (SMF) exposed rats. The exposure of rats to SMF (128 mT, 1 h/day during 30 consecutive days) decreased the activities of glutathione peroxidase (GPx), catalase (CAT) and the superoxide dismutase (SOD) in liver and kidney. By contrast, sub-chronic exposure to SMF increased the malondialdehyde (MDA) concentration in liver and kidney. Our results revealed an increase of the 8-oxo-7,8-dihydro-2′-desoxyguanosine (8-oxodGuo) in kidney of SMF-exposed rats. However, this biomarker of DNA oxidation remained unchanged in liver. Zinc supplementation (ZnCl$_2$, 40 mg/l, *per os*) in SMF-exposed rats restored the activities of GPx, CAT and SOD in liver to those of control group. However, only CAT activity was restored in kidney. Moreover, zinc administration was able to bring down the elevated levels of MDA in the liver but not in the kidney. Interestingly, zinc supplementation attenuated DNA oxidation induced by SMF in kidney to the control level.

Our investigations suggested that zinc supplementation minimizes oxidative damage induced by SMF in rat tissues.
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1. Introduction

Epidemiological and results of experimental *in vitro* and *in vivo* studies carried out in recent years have given more attention to the biological effects of electromagnetic field (EMF). Previous data suggested the association between EMF exposure and the increased incidence of certain types of tumour, particularly leukemia and brain cancer (Wertheimer et al., 1995; Aldrich et al., 2001). Recently, Abdelmelek et al. (2005) reported that static magnetic field (SMF) (128 mT) increased the norepinephrine content in skeletal muscle associated to sympathetic hyperactivity in rats. Previous reports from our laboratory by Chater et al. (2004) demonstrated that sub-acute exposure to SMF stimu-

lated biosynthesis of plasma corticosterone and metallothionein in female rats and enhanced apoptosis. In part, the mechanism for this stress response by SMF is believed to be related to oxidative stress (Chater et al., 2005). There are a number of data implicated EMF in free radical production, like superoxide anion in different cells and organs, in macrophages, kidney, liver, and monocytes (Khadir et al., 1999; Kula et al., 2000; Simko et al., 2001; Lupke et al., 2004). Indeed, several findings concluded that magnetic field-induced changes in enzyme activity, gene expression, affects membrane structure and functions and causes DNA damage (Savitz, 1995; Lewy et al., 2003; Yokus et al., 2005). Previous data indicated an increase in DNA single and double-strand breaks in rat brain following EMF exposure (Lai and Singh, 2004).

During the last several years, many articles have been presented indicating important role of zinc in the therapy of depression (Frederickson, 1989; Levenson, 2006). Zinc is a trace element, essential for living organisms. More than 300

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enzymes require zinc for their activity. Zinc plays an important role in the DNA replication, transcriptions and protein synthesis, influencing cell division and differentiation (Frederickson, 1989). It has been noted that zinc has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant system (Powell, 2000; Ozturk et al., 2003; Ozdemir and Inanc, 2005). One study has shown that zinc deficiency in the diet paved the way for the cell damage in rat testicles (Oteiza et al., 1999). Furthermore, zinc deficiency increased the lipid peroxidation in various rat tissues, whereas the zinc supplementation corrected the impairment (Shaheen and El-Fattah, 1995; Ozdemir and Inanc, 2005).

Literature analysis showed a lack of data regarding the interaction between SMF and zinc in biological system. Our previous study demonstrated that zinc administration prevents hematological and biochemical alteration induced by SMF in rats (Amara et al., 2005). The present study performed an experimental approach firstly to investigate the effects of sub-chronic exposure to SMF on antioxidative response and DNA damage in rat tissues. Secondly, to estimate the protective role of zinc in rat tissues against damage induced by SMF.

2. Materials and methods

2.1. Animals

Adult Wistar male rats (SIPHAT, Tunisia), weighing 180–200 g were randomly divided into control rats ($n = 6$), SMF-exposed rats ($n = 6$), zinc-treated rats ($n = 6$) and co-exposed rats ($n = 6$). Animals were housed in group of six in cages at 25 °C, under a 12:12 h light/dark cycle, with free access to water and commercial wash.

Animals were cared for, under the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes. The experimental protocols were approved by the Faculty Ethics Committee (Faculté des Sciences de Bizerte, Tunisia).

2.2. Exposure system

Lake Shore Electromagnets (Lake Shore Cryotronic, Inc., Westerville, OH, USA) are compact electromagnets suited for many applications such as magnetic resonance demonstrations. Water-cooled coils provide excellent field stability and uniformity when high power is required to achieve the maximum field capability for the electromagnet (Abdelmelek et al., 2005).

2.3. Static magnetic field exposure

Static magnetic field (SMF) was measured and standardized in the total floor area of the Plexiglas cage (20, 10, 20 cm) at 128 mT. The two bobbins of the Lake Shore System were separated by 12 cm. Male rats were exposed to the SMF, 1 h/day (between 9 and 12 h) during 30 consecutive days. The cage in the Lake Shore contained two rats for each assay. The control rats were placed under the same conditions without applying the SMF.

2.4. Zinc treatment

Rats received zinc chloride solution (40 mg/l) for 4 weeks in drinking water. The co-exposed rats were exposed to SMF with zinc supplementation (40 mg/l, *per os*) during 30 consecutive days.

2.5. Tissue preparation

After 4 weeks of exposure all groups were sacrificed and their liver and kidney were immediately excised. The tissues were weighed, rinsed with ice-

cold deionized water and dried with filter paper. Liver or kidney fractions were homogenized using the appropriate buffer (Tris 10 mM, ethylenediaminetetraacetic (EDTA) 1 mM, phenylmethylsulfonyl fluoride (PMSF) 1 mM; pH 7.5). The homogenates were centrifuged at $600 \times g$ for 10 min and recentrifuged at $13,000 \times g$ for 20 min at 4 °C to obtain a postnuclear homogenate and postmitochondrial supernatant fractions (Ebru and Mesut, 2002).

2.6. Malondialdehyde (MDA) assay

Lipid peroxidation (LPO) in tissues was measured by the thiobarbituric acid reacting substance (TBARS) and was expressed in terms of malondialdehyde (MDA) content (Placer et al., 1966). Sample aliquots were incubated with 10% trichloroacetic acid and 0.67% thiobarbituric acid. The mixture was heated on a boiling water bath for 30 min, an equal volume of *n*-butanol was added, and the final mixture was centrifuged; the organic phase was collected for fluorescence measurements. Samples assayed for MDA contained 1 mM butylated hydroxytoluene (BHT) in order to prevent artefactual LPO during the boiling step. The absorbance of samples was determined at 532 nm. Results were expressed as µmol MDA/g protein.

2.7. Glutathione peroxidase (GPx) activity

The reaction was carried out at 25 °C in 600 µl of solution containing 100 mM of potassium phosphate buffer, pH 7.7, 1 mM of EDTA, 0.4 mM of sodium azide, 2 mM of glutathione, 0.1 mM of nicotinamide adenine dinucleotide phosphate, and 0.62 U of glutathione reductase. The activity of GPx was assayed by the subsequent oxidation of NADPH at 340 nm with *t*-butylhydroperoxide as a substrate (Puget and Michelson, 1977).

2.8. Catalase (CAT) activity

CAT activity was measured at 20 °C according to Aebi (1984). The homogenate was incubated with ethanol (10%) and Triton (10%). Activity was assayed at 25 °C by determining the rate of degradation of H_2O_2 at 240 nm in 10 mM of potassium phosphate buffer (pH 7.0). The extinction coefficient of 43.6 mM/cm was used for calculation. One unit is defined as 1 pmol of H_2O_2 consumed per minute and the specific activity is reported as units per milligram of protein.

2.9. Superoxide dismutase (SOD) activity

The method described by Paolotti and Mocali (1990) was used for the assay of SOD activity. This method consists of purely chemical reactions sequence, which generates superoxide from molecular oxygen in the presence of EDTA, manganese(II) chloride and mercaptoethanol.

2.10. DNA extraction from tissues and HPLC-EC analysis

DNA was extracted using a previously reported chaotropic method that prevents spurious oxidation of DNA bases (Ravanat et al., 2002). Tissues samples were weighted still frozen and divided into fractions of 200 mg. Each portion of tissues was homogenized in 1.2 ml of buffer (320 mM sucrose, 10 mM Tris, 5 mM $MgCl_2$, 0.1 mM desferroxamine mesylate, 1% Triton, pH 7.5). After homogenization, the sample was centrifuged. The supernatant was discarded and the nuclear pellet obtained was suspended in 600 µl of extraction buffer (10 mM Tris, 5 mM EDTA, 0.15 mM desferroxamine mesylate). SDS (10% in water) was added. RNase A and RNase T1 were then added and the resulting suspension gently vortexed for 10 s. The sample was incubated for 15 min in a water bath set at 50 °C. Then, Qiagen proteinase (30 µl) was added and the sample gently vortexed for 10 s prior to be incubated 60 min at 37 °C. A DNA was precipitated by addition of sodium iodide (NaI) solution (1.2 ml). The sample was vigorously vortexed for 30 s. Isopropanol (2 ml) was added and the samples gently shaken until complete homogeneity. After centrifugation, the DNA pellet was rinsed by 1 ml of 70% ethanol. The DNA pellet was then solubilized into 100 µl of deionized water containing 0.1 mM deferroxamine mesylate. The DNA solution was incubated with 2 units of nuclease P1, the sample was held at 37 °C for 2 h. Then,

Table 1
Effects of zinc administration on the antioxidant enzymatic system and glutathione level in liver of SMF-exposed rats

	GPx (U/mg pt)	CAT (U/mg pt)	CuZn-SOD (U/mg pt)	Mn-SOD (U/mg pt)	Glutathione (U/mg pt)
C	1580.4 ± 100.16	334 ± 21.41	23.96 ± 1.37	1.16 ± 0.03	59.26 ± 4.08
Zn	1727.66 ± 102.39	389.33 ± 10.86	26.31 ± 1.63	1.1 ± 0.08	56.88 ± 7.39
SMF	1231.4 ± 86.28[*]	274.66 ± 13.71[*]	16.5 ± 0.8[*]	0.93 ± 0.02[*]	42.46 ± 3.12[*]
SMF + Zn	1444.83 ± 125.70	327 ± 38.96	20.8 ± 1.84	1.03 ± 0.06	48.48 ± 5.15

Data represent the means ± S.E.M. of six animals per group. [*]$p < 0.05$, compared to control (C). SMF: static magnetic field, Zn: zinc, GPx: glutathione peroxidase, CAT: catalase, SOD: superoxide dismutase, and pt: protein.

Table 2
Effect of zinc administration on the antioxidant enzymatic system and glutathione level in kidney of SMF-exposed rats

	GPx (U/mg pt)	CAT (U/mg pt)	CuZn-SOD (U/mg pt)	Mn-SOD (U/mg pt)	Glutathione (U/mg pt)
C	1452.6 ± 45.84	186.16 ± 4.02	13.85 ± 0.51	0.72 ± 0.03	1.63 ± 0.21
Zn	1268.6 ± 103.29	150.5 ± 8.46	13.36 ± 0.94	0.81 ± 0.02	2.4 ± 0.36
SMF	1118.66 ± 104.3[*]	134.16 ± 10.5[*]	10.82 ± 0.61[*]	0.69 ± 0.03	1.6 ± 0.18
SMF + Zn	1179 ± 15.81[*]	150.66 ± 18.23	11.85 ± 0.1[*]	0.74 ± 0.06	1.38 ± 0.14

Data represent the means ± S.E.M. of six animals per group. [*]$p < 0.05$, compared to control (C). SMF: static magnetic field, Zn: zinc, GPx: glutathione peroxidase, CAT: catalase, SOD: superoxide dismutase, and pt: protein.

4 units of alkaline phosphatase were added together with palk buffer (Douki et al., 2000). After incubation 1 h at 37 °C, the samples were centrifuged and the aqueous layer collected and analysed by HPLC-EC. The resulting solution contained normal bases and 8-oxodGuo as nucleosides. The procedure described by Kasai (1997) was applied for the measurement of the 8-oxo-7,8-dihydro-2′-desoxyguanosine (8-oxodGuo). Typically, the HPLC system consisted of a Merck Hitachi HPLC pump, model 6200, connected to a SIL 9A automatic injector (Shimadzu, Kyoto, Japan). The isocratic mobile phase constituted of 50 mM KH_2PO_4 that contained 8% methanol. The flow-rate was 1 ml min^{-1}. Separation of nucleosides was performed using a C18 reversed-phase Uptisphere ODB octadecylsilyl silica gel column (5 μm, 4.6 mm × 250 mm) from Iterchim (Montluçon, France) maintained at 30 °C. The retention time of 8-oxodGuo is 21.5 min. The amount of DNA analysed was determined from the area of the peak of 8-oxodGuo after appropriate calibration. A Coulochem II. model 5200 A, electrochemical detector (ESA, Chemlsford, MA, USA) was used for the detection of 8-oxodGuo. Elution of unmodified nucleosides was monitored using an UV detector (model 2151, LKB Bromma) set at 280 nm. For each sample, the amount of DNA injected onto the column was estimated using the UV signal of dGuo after appropriate calibration.

2.11. Data presentation and statistical analysis

Statistical test was performed using Statistica Version 5.0 (StatSoft, Tusla, OK, USA). Data is reported as the mean ± S.E.M. Differences between means were evaluated by one-way analysis of variance (ANOVA). Statistical significance of the differences between means was assessed by Student's t-test. The level of significance was set at $p < 0.05$.

3. Results

Activities of hepatic and renal GPx, CAT and the cytosolic CuZn-SOD were reduced in SMF-exposed rats (Tables 1 and 2). On the other hand, the mitochondrial Mn-SOD activity and glutathione level were decreased in liver, but not in kidney. Following zinc administration in SMF-exposed rats there was a restoration of GPx, CAT, CuZn-SOD, Mn-SOD and glutathione level in liver to those of control group (Table 1). However, only CAT activity was restored in kidney (Table 2). The level of MDA was increased in

hepatic (0.26 ± 0.01 μmol/g pt versus 0.16 ± 0.01 μmol/g pt, $p < 0.05$) and renal tissues (0.310 ± 0.009 μmol/g pt versus 0.230 ± 0.001 μmol/g pt, $p < 0.05$) following SMF exposure. Zinc supplementation in SMF-exposed animals resulted in
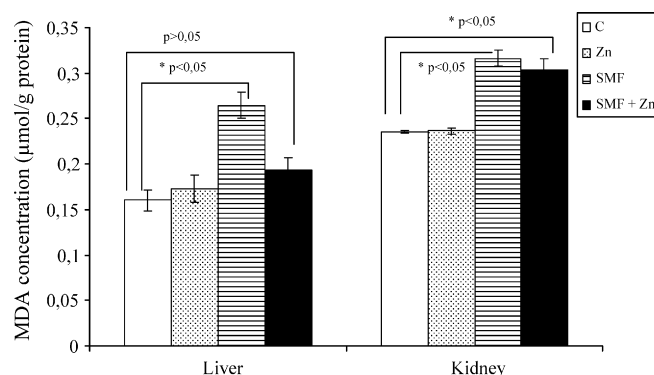

Fig. 1. Effect of zinc administration on the MDA level in liver and kidney of SMF-exposed rats. Data represent the means ± S.E.M. of six animals per group. [*]$p < 0.05$, compared to control (C). MDA: malondialdehyde, SMF: static magnetic field, and Zn: zinc.
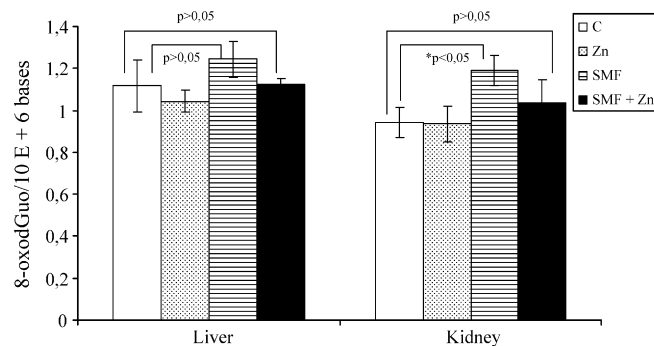

Fig. 2. Effect of zinc administration on DNA oxidation in liver and kidney of SMF-exposed rats. Data represent the means ± S.E.M. of six animals per group. [*]$p < 0.05$, compared to control (C). SMF: static magnetic field and Zn: zinc.

the restoration of the liver level of MDA to the control value (0.19 ± 0.01 μmol/g pt versus 0.16 ± 0.01 μmol/g pt, $p > 0.05$), but not in kidney (0.30 ± 0.01 μmol/g pt versus 0.230 ± 0.001 μmol/g pt, $p < 0.05$) (Fig. 1).

In the present study 8-oxodGuo level in cellular DNA has been used as a biomarker of oxidative DNA damage. Our data indicated that 8-oxodGuo content was increased in kidney under SMF exposure (1.18 ± 0.07 versus $0.94 \pm 0.07/10^6$ bases, $p < 0.05$). However, sub-chronic exposure to SMF did not exert any base damage in hepatic cellular DNA of rats (1.24 ± 0.08 versus $1.11 \pm 0.12/10^6$ bases, $p > 0.05$). Zinc administration in SMF-exposed animals attenuated DNA damage in the kidney (1.03 ± 0.11 versus $0.94 \pm 0.07/10^6$ bases, $p > 0.05$) (Fig. 2).

4. Discussion

The present study indicated that, sub-chronic exposure to SMF (128 mT, 1 h/day during 30 consecutive days) induced oxidative stress in rat tissues and DNA oxidation in kidney. Interestingly, zinc supplementation minimizes the adverse effect of SMF in rat tissues.

Results of the present study demonstrated that sub-chronic exposure to SMF is able to influence oxidative–antioxidative balance in rat tissues. We showed a decrease of GPx, CAT and the cytosolic CuZn-SOD activities in liver and kidney of rats under SMF. However, the mitochondrial Mn-SOD activity and glutathione level were decreased in liver, but remained unchanged in kidney. By contrast, SMF exposure increased the MDA level in liver and kidney of rats. Thus, the increase of MDA concentration following SMF application could be explained by lipid peroxidation. Our data showed that SMF exposure induced a decrease of antioxidant enzymes activity associated to a lipid peroxidation indicating oxidative stress in rat tissues. The mechanism by which SMF induced oxidative stress in rat tissues is not well understood. There are at least two possible explanations for the oxidative stress induction by SMF. Firstly, we hypothesized that SMF exposure could be followed by free radical production in rat tissues. Our results are consistent with previous findings regarding the hypothesis that EMF exposure is associated with free radical production. Lee et al. (2004) suggested that EMF could deteriorate antioxidant defensive system by ROS production. More invasive studies have shown that magnetic field influenced lipid peroxidation and antioxidant defence system in rat tissues (Kashkalda et al., 1995; Zwirska-Korczala et al., 2004; Aksen et al., 2006). It has been proposed that moderate levels of reactive oxygen species (ROS) can induce an increase in antioxidant enzyme activities, whereas very high level of these reactants was shown to attenuate antioxidant enzyme activities (Brydon et al., 2000).

Secondly, SMF exposure induced probably the perturbation of mineral element homeostasis (divalent), contributing to their deficiency in tissues. Duda et al. (1991) reported a change in liver and kidney concentration of cooper, manganese, cobalt and iron in rats exposed to static and low-frequency magnetic field. Previously, it has been reported that oxidative stress induced by EMF-exposure was related to zinc deficiency in tissues of exposed animals (Ozturk et al., 2003). On the other hand, Lai and

Singh (2004) showed that magnetic field exposure affects iron homeostasis in certain cells, leading to an increase in free iron in the cytoplasm and nucleus, which in turn leads to an increase in hydroxy radicals, via the catalytic activity of the Fenton reaction.

Regarding the fate of zinc administration in SMF-exposed rats, it may be assumed that this element minimize the oxidative stress induced by SMF in rat tissues. Our data showed that zinc supplementation in SMF-exposed animals restored the activities of GPx, CAT, CuZn-SOD and Mn-SOD in liver to those of control group. However, only CAT activity was restored in kidney. Several findings concluded that zinc has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant system (Powell, 2000; Ozturk et al., 2003; Ozdemir and Inanc, 2005). Moreover, we noted that zinc administration was able to attenuate the level of MDA in liver but not in kidney. Our data suggested that zinc is believed to stabilize membranes structure and protect cells against free radical injury. Accordingly, Ozturk et al. (2003) indicated that testis and kidney damage caused by periodic exposure to electromagnetic field are ameliorated or prevented by zinc supplementation.

In the current study, our results showed that sub-chronic exposure to SMF increased 8-oxodGuo level in kidney but not in the liver, indicating DNA oxidation in renal cells. These results are similar to those previously demonstrated by Yokus et al. (2005). It can be hypothesized that DNA oxidation might be related to overproduction of ROS under SMF. Our data suggested that renal cellular DNA was the most sensitive to SMF than that of hepatic cells; this result may be explained in part by the low basic level of antioxidant enzymes in the kidney in comparison to the liver. The severity of oxidative stress in the kidney could inactivate the DNA mismatch repair systems (Christina et al., 2002). Moreover, the MDA concentration in the kidney was higher than in the liver. MDA is one of the major aldehyde products of lipid peroxidation, known to react with DNA bases to form adducts, which found to be highly mutagenic when introduced in renal cells (Pandya and Moriya, 1996).

Interestingly, our finding reported that zinc administration in SMF-exposed rats minimize DNA damage in renal cells. Several mechanisms have been proposed to be involved in the antioxidant action of zinc in biological systems. One of these mechanisms is the capacity of zinc to replace transition metals (Fe, Cu) from binding sites in lipids, proteins, and DNA (Bettger and Dell, 1993; Powell, 2000). Our previous data correlated the preventive effect of zinc to metallothioneins induction and zinc accumulation in liver and kidney of SMF-exposed rats (Amara et al., 2005).

In conclusion, data from this study revealed that sub-chronic exposure to SMF induced oxidative stress in liver and kidney of rats. However, DNA oxidation was observed only in kidney. Interestingly, zinc supplementation minimizes the adverse effect of oxidative stress induced by SMF in rat tissues.

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