J Occup Health 2002; 44: 226–229

Journal of Occupational Health

Effects of Electromagnetic Field on Free-Radical Processes in Steelworkers. Part I: Magnetic Field Influence on the Antioxidant Activity in Red Blood Cells and Plasma

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Abstract: Effects of Electromagnetic Field on Free-Radical Processes in Steelworkers. Part I: Magnetic Field Influence on the Antioxidant Activity in Red Blood Cells and Plasma: Bogusław KULA, *et al.* Department of Experimental and Clinical Biochemistry, Silesian School of Medicine, Poland—The purpose of the study was the evaluation of electromagnetic field (electric field strength of 20 V/m, f=50 Hz and magnetic field strength of 2 A/m, f=50 Hz) effects on the antioxydative activity in steelworkers' red blood cells and plasma. The plasma GSH-Px (EC 1.11.1.9) activity, MDA and ceruloplasmin levels as well as SOD (EC 1.15.1.1.), CAT (EC 1.11.1.6) and GSH-Px (EC 1.11.1.9) in red blood cells were measured. Statistically significant decreases in red blood cells SOD and GSH-Px activities, a CAT activity increase and plasma MDA increase and a ceruloplasmin decrease were found in workers exposed for 3–10 yr and for longer than 10 yr to electromagnetic fields. No statistically significant changes in the parameters evaluated were found among steelworkers employed for shorter than 3 yr. All changes observed among workers exposed to electromagnetic fields result in adaptative responses by activating systems controlling the balance of body oxidative mechanisms.
(J Occup Health 2002; 44: 226–229)

Key words: Electromagnetic field, SOD, CAT, GSH-Px, MDA, Ceruloplasmine

Numerous epidemiological studies evaluating the health effects of extremely low frequency (ELF) magnetic fields, especially power line frequency (50, 60 Hz) suggest an increased risk of functional disorders in the circulatory system, central nervous system and immunological system, and even the induction of neoplasms in so called "electric professionals" and persons living in the vicinity of power lines[1, 2]. For example, mean personal 24 h continual exposure levels of magnetic fields in transformer workers measured with magnetic field time-series data collected with EMDEX II instrument are generally 10 times higher than in office workers[3]. The epidemiological data are frequently contradictory, and owing to methodological reasons, it is almost impossible to establish a relationship between the electromagnetic fields and increased cancer incidence. But the possibility that power line frequency electromagnetic fields affect body functions is especially annoying[1, 4].

Considering the biophysical mechanism of the power line influence, one should take into account the coexistence of 2 factors:

— the physical mechanism as a primary element
— the biochemical mechanism as a secondary element.

Both mechanisms are similar at the molecular level. All biological effects resulting from these consecutive and inseparable mechanisms may finally result in particular physiological (health) outcomes.

Experimental data have confirmed that industry-frequency magnetic fields are important because of the biophysical influence on the cell[5–7]. One of the major molecular effects of magnetic fields is their influence on nuclear spins of paramagnetic molecules[8]. This mechanism plays an important role when in the course of a chemical reaction the chemical bond is disrupted and two molecules with unpaired electrons are formed (a pair of radicals). Depending on their electron spin orientations, radical recombination or diffusion and formation of free radicals (e.g. oxygen radicals) may take place. External magnetic fields with an intensity of several mT may change the reciprocal orientation and

Received Dec 8, 2001; Accepted April 15, 2002
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hence influence the reaction (changing the proportions of products, e.g. increasing the number of oxygen radicals). Theoretically, the mechanism applies to constant external magnetic fields. Due to a very short radical duration-period (several ms) this can, however, be evoked also by low-frequency magnetic fields, since in the case of 50 Hz frequency the period is 20 ms, we may assume that during the radical persistence the field remains nearly constant. Worthy of notice here are the results of experimental *in vitro* studies[6, 8], which revealed that the intracellular processes occurring under the influence of a power line magnetic field related to free radicals and signal transmission may determine its biological effects. An uncontrolled free oxygen radical release, termed oxidative stress, may cause protein oxidation, enzyme inactivation and lipid peroxidation within the cellular membranes, resulting in structural and functional abnormalities as well as in oxidative damage to the DNA and RNA. DNA damage may lead to increased mutation frequency and triggering of carcinogenesis[9].

The purpose of this study was presentation of anti-free radical protective mechanisms in the red blood cells and plasma of steel workers exposed to electromagnetic fields generated by induction furnaces.

Subjects and Methods

1. Exposure

The study was performed in steelworkers (men only) who worked in a tool shop and heavy processing shop, where two GSJ-50 induction heaters were located (induction of 1.3 mT). Electromagnetic fields were used for sintering turning cutters and hardening toothed wheels. The shops were closed rooms with appropriate humidity, ventilation and temperature. The indices of electromagnetic field exposure established by the National Inspection Committee for the Control of Radiation were as follows:

1. electric field intensity 20 V/m, f=50 Hz
2. magnetic field intensity 2 A/m, f=50 Hz

2. Subjects

Sixty-four steelworkers were selected for the study according to the following inclusion criteria:

a) no history of liver disease
b) no abnormalities on physical examination
c) no complaints on physical examination
d) no previous contact with toxic substances
e) no shift work

They were exposed to an electromagnetic field from Monday to Friday, 34 h a wk (mean 6.8 h a d). The workers were divided into 3 groups according to the duration of exposure (Table 1).

The control group consisted of 25 persons aged 20–45 yr (mean age 35.2 ± 9.9 yr) who worked at the steelworks but were not exposed to magnetic or electric radiation (accountants and engineers).

Table 1. Mean age and exposure duration in study groups

Group	Employment period	Age (in years ± SD)	Number of workers
1	up to 3 yr	24.2 ± 6.5	20
2	3 to 10 yr	36.2 ± 7.2	28
3	over 10 yr	38.8 ± 8.9	16

3. Biochemical parameters

Blood was obtained from the ulnar vein in heparinized test tubes. Blood samples were collected every 4 wk (on Fridays) at 8 a.m. (2 h after the start of work) after a 12 h fast. Blood was centrifuged (600 g; 10 min, 4°C). After separation of plasma, the red blood cells (RBCs) were rinsed 3 times with cold 0.9% NaCl, and then the RBCs were diluted 1:4 in water and hemolyzed by triple freezing in a dry ice-methanol mixture. The solution obtained was then centrifuged (8000 g; 10 min, 4°C).

The following activities were measured in the hemolizates obtained:

1. Superoxide dysmutase (SOD; EC 1.15.1.1.)[10]
2. Catalase (CAT; EC 1.11.1.6)[11]
3. Glutathione peroxidase (GSH-Px; EC 1.11.1.9)[12]

The following serum parameters were measured:

1. Glutathione peroxidase activity (GSH-Px; EC 1.11.1.9)[12]
2. Malone dialdehyde (MDA) level[13]
3. Ceruloplasmin level[14]

Total protein content was also measured in hemolisates and plasma[15].

Results

Steelworkers employed for a shorter period than 3 yr (group 1) did not have any significant differences in red blood cell enzymatic antioxidative activity. Differences in SOD and GSH-Px activities in red blood cells were observed in workers employed for longer than 3 yr. In group 2 (exposure period, 3–10 yr), the activity decrease was 13% and 12% respectively, while in group 3 (exposure period over 10 yr) the activity decreases were 19% and 12% respectively. In both groups, CAT activity increased significantly (19% and 32%, respectively) (Table 2). Plasma GSH-Px activity showed a tendency to decrease in steelworkers exposed to electromagnetic fields, but the decrease was not statistically significant. The plasma MDA concentration in group 2 was higher by 28% and by 56% in group 3, but the ceruloplasmin concentration was noticeably decreased (41% and 54%, respectively) (Table 3). The results were analyzed by *t*-test, $p<0.05$.

Table 2. Red blood cells antioxidant enzyme activities in steelworkers exposed to an electromagnetic field

Parameter	Control	Group 1	Group 2	Group 3
SOD [U/mg protein]	1.68	1.66	1.47	1.36
SD	± 0.13	± 0.13	± 0.15	± 0.38
p		–	+	+
CAT [U/mg protein]	0.16	0.15	0.19	0.21
SD	± 0.02	± 0.03	± 0.05	± 0.07
p		–	+	+
GSH-Px [μmol NADPH/min/mg protein]	11.81	11.02	10.38	10.45
SD	± 0.99	± 1.02	± 2.01	± 1.95
p		–	+	+

(+) $p< 0.05$, (–) insignificant.

Table 3. Plasma antioxidant parameters in steelworkers exposed to an electromagnetic field

Parameter	Control	Group 1	Group 2	Group 3
GSH-Px [μmol NADPH/min/mg protein]	2.61	2.43	2.39	2.27
SD	± 0.39	± 0.40	± 0.42	± 0.43
p		–	–	–
MDA [nmol/m*l*]	3.51	3.98	4.49	5.49
SD	± 0.48	± 0.52	± 0.72	± 1.69
p		–	+	++
Ceruloplasmine [mmol/*l*]	1.32	1.29	0.78	0.61
SD	± 0.13	± 0.14	± 0.17	± 0.20
p		–	+	++

(+) $p<0.05$, (++) $p<0.01$, (–) insignificant.

Discussion

Superoxide dismutase (SOD) plays a key role in the system protecting the body from destructive free-radical activity. Its absence or decreased activity may have noxious metabolic outcomes. A decrease in the SOD activity that was found in steelworkers exposed to electromagnetic field in groups 2 and 3 results in the accumulation of superoxide aninoradicals in red blood cells, but details of the mechanism remain obscure[7]. The mechanism of detoxication is not confined to the SOD, since its very toxic product, hydrogen superoxide, is then eliminated with catalase (CAT). Hydrogen superoxide, a product of SOD activity, is also a strong inhibitor of this enzyme[16]. That is why the effective detoxication of active oxygen forms takes place with concordant SOD and CAT action.

In our study SOD activity was decreased due to the effect of electromagnetic field, and CAT activity was higher also in workers in groups 2 and 3. This can be assumed to be a result of the processes of adaptation[2]. Glutathione peroxidase (GSH-Px) inactivates hydrogen superoxide and organic hydroxides together with glutathione reductase and glucose-6-dehydrogenase with reduced glutathione. The oxidized glutathione generated is then reduced again by NADPH-dependent glutathione reductase, in the process that maintains the peroxidation continuity[17]. Appropriate NADPH amounts are delivered by glucose-6-phosphate and 6-phosphogluconian dehydrogenases.

In our study, a significant red blood cell GSH-Px activity decrease was observed in groups 2 and 3. Such a tendency may indicate abnormal function of the antioxidative system caused by the electromagnetic field.

Groups 2 and 3 of steelworkers exposed to electromagnetic fields had lower ceruloplasmin activity, which seems to indicate that the free copper plasma concentration increases. Ceruloplasmin-conjugated copper makes a secure form of this element. Free copper binds to various low molecular ligands, which do not protect the body from its participation in oxygen generating processes[18]. One may assume that copper released by an electromagnetic field intensifies these processes leading to increased generation of active oxygen forms.

Plasma MDA levels reflect the unsaturated lipid peroxidation taking place not only in the blood but also in other tissues. In our study, a statistically significant MDA level increase was found in groups 2 and 3, that may indicate increased lipid peroxidation caused by the electromagnetic field.

We may state that electromagnetic fields had major effects on group 2 and 3 steelworkers. Decreased SOD and GSH-Px activities, concomitantly increased CAT activity in red blood cells, as well as an increased lipid peroxidation were observed. All these changes lead to some adaptative responses due to activation of systems controlling the body oxidative mechanism balance.

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