Effect of Magnetic Fields on the Na^+-K^+ATPase Activity of Erytrocyte Membrane and the Levels of Malondialdehyde and Glutathione

MANYETİK ALANLARIN ERİTROSİT MEMBRANI Na^+-KATPaz ENZİM AKTİVİTESİ, MALONDİALDEHİT, GLUTATYON DÜZEYLERİ ÜZERİNE ETKİSİNİN ARAŞTIRILMASI

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Summary

All members of modern societies constantly live in electromagnetic fields and waves whose intensities are much higher than those found in the nature.The potential negative effects of those on human health continue to be the subject of controversy.

Object: To obtain data related with the effects of magnetic fields on free radical of metabolism, we measured malondialdehyde (MDA), glutathione (GSH) levels and Na-K-ATPase enzyme activity on the human subjects.

Material and Methods: This study performed on the human subjects who were admitted, daily to MRI in the Department of Radiology .The human subjects were exposed to a magnetic field exposure period of 16.18 minutes and intensity of 1.5 Tesla (T). There were 59 subjects, of them 28 male and 31 female with an age ranged from 19 to 74 years. Nine of these subjects had received contrast agent before MRI. Blood samples were taken before MRI and there after. Blood samples which taken before MRI were used as controls. Analysis of MDA, GSH and erythrocyte membrane's Na-K-ATPase enzyme activity were performed immediately after blood collection at the clinical lab of Medicine. Na-K-ATPase activity was determined in erythrocyte membranes by the method of Kitao & Hattori. MDA level was measured by Draper and Hadley methods. GSH level was measured according to method of Beutler.

Results: We have demonstrated that the levels of GSH, MDA and erythrocyte membrane's Na-KATPase enzyme activities were not changed in the course of Magnetic Resonance Imaging (MRI).

Conclusions: Because this study performed in the patients who were admitted, daily to MRI in the Department of Radiology, the exposure time to the magnetic field was shorter than the ones used in most of other investigations in this area. Therefore, this shorter exposure time may affect the results of present study.

Key Words: MRI, Na/K-ATPase, Malondialdehyde, Glutathione

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Özet

Modern toplumlarda yaşayan hemen herkes sürekli olarak doğada bulunanın çok üstünde elektromanyetik alanlara maruz kalmaktadır.Bunların insan sağlığı üzerindeki olumsuz etkileri tartışma konusu olmaya devam etmektedir.

Amaç: Manyetik alanların insanlarda serbest radikal metabolizması üzerindeki etkilerini gözlemlemek için malondialdehit (MDA), glutatyon (GSH) seviyelerini ve Na-K-ATPaz enzim aktivitesini ölçtük.

Materyal ve Metod: Çalışma rutin olarak radyoloji bölümünde MRG ye alınan hastalar üzerinde yapıldı. Hastalar 1.5 T şiddetinde manyetik alana 16.18 dakika maruz kaldılar. Çalışmaya 28 erkek 31 kadın olmak üzere toplam 59 hasta katıldı. Uygulama için MRG'dan önce ve sonra kan örnekleri alındı. MRG'den önce alınan kan örnekleri kontrol grubu olarak kullanıldı. 9 hastaya MRG den önce kontrast ajan verildi. Eritrosit membranı Na-K-ATPaz enzim aktivitesi Kitao& Hattori, malondialdehit düzeyleri Draper- Hardley, glutatyon düzeyleri Beutler metodları ile ölçüldü.

Bulgular: Bu çalışma sonucunda MDA, GSH düzeylerinin ve Na-K-ATPaz enzim aktivitesinin Manyetik Rezonans Görüntüleme (MRG) uygulamasından sonra değişmediğini gösterdik.

Sonuç: Çalışma, rutin olarak MR testine alınan hastalarda yapılmıştır, dolayısıyla manyetik alana maruz kalınan sürenin kısa olması sonuçları etkilemiş olabilir. Bu alanda yapılan araştırmaların çoğunda uzun süreli manyetik alan etkileri incelenmektedir.

Anahtar Kelimeler: Manyetik Rezonans Görüntüleme (MRG), Na-K-ATPaz, Malondialdehit, Glutatyon

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For many years the biological effects of magnetic fields (MFs) have been the subject of great interest. Epidemiological studies since 1979 have raised some medical and much public concern that electromagnetic fields might have harmful biological effects (1). Electromagnetic fields of various frequencies and strengths are produced by high-voltage power lines, household appliances, radio and television transmitters, radars and industrial equipment (2). Issues associated with the exposure of patients to strong, static magnetic fields during magnetic resonance imaging (MRI) are discussed. The history of human exposure to magnetic fields is reviewed, and the contradictory nature of the studies regarding effects on human health is described.

Several studies reported in the literature have found enough evidence for a relationship between the exposure to electromagnetic fields and the increased incidence of some cancers in both children and adults (3,4). Effects have been reported on cell proliferation, ion transport, activation of several enzymes (Na-K-ATPase) in membrane signal transduction (5).

Na^+-K^+-ATPase has proved to be useful model for studying the effects of electric and magnetic fields on a membrane protein by enabling us to measure changes due to parameters, and to develop ideas about mechanism (6). Furthermore, since the Na^+-K^+-ATPase extends through the membrane, effects that occur on the cell can be transmitted inside the cell by normal biological transport mechanisms (7).

Magnetic fields (MFs) can increase the yields of some types of free radicals formed through homolytic cleavage, photoinduced processes, or random encounters (8). A number of studies reported that magnetic field (MF) perturb free radicals behaviour to increase their lifetime and concentration (9).

To see the effect of magnetic fields on free radical metabolism, we measured malondialdehyde (MDA), glutathione (GSH) levels and Na-K-ATPase enzyme activity on the human subjects.

Materials and Methods

Chemicals

The chemicals used were listed below: TCA (Merck);TBA (T-5500 Sigma);Tris (Sigma 8168); HCI (Sigma 920-1); NaCI(Sigma 9625); KCI(Sigma P4504); $MgCl_2$(Merck 8266); Na_2ATP(Sigma A7699); SDS (Sigma L4509); EDTA(SigmaE-1644); SDS(Merck)Tris-HCL buffer 10 mM pH=7.4, Heparine (Nevparin 5000 IU/ml).The solutions used in the experiments were prepared in deionized water.

Preparation of blood samples

This study performed on the human subjects who were admitted to MRI in the Department of Radiology, routinely. The human subjects were exposed to a magnetic field exposure period of 16.18 minutes and intensity of 1.5 Tesla (T). There were 59 subjects of them 28 male and 31 female with an age ranged from 19 to 74 years. Nine of these subjects had received contrast agent before MRI. Blood samples were taken before MRI and thereafter. Blood samples which taken before MRI were used as controls. Analysis of MDA, GSH and erythrocyte membrane's Na-K-ATPase enzyme activity were performed immediately after blood collection at the clinical laboratory of Medicine.

Measurement of Na-K-ATPase activity

Heparinized blood samples were centrifuged at 4500(g) to remove plasma. RBCs were lysed twice with isotonic sodium chloride, hypotonically in 5mM ice-cold phosphate buffer solution (pH= 8) and processed in a Kontron centrifuge at 20000g for 30 min at +4°C. The resulting membranes were washed with phosphate buffer of decreasing molarity in order to remove the hemoglobin completely. Na-K-ATPase activity was determined in the erythrocyte membranes by the method of Kitao & Hattori (10). The total ATPase activity was assayed by incubating ghost at 37°C in a medium containing 5 mM MgCl,140 mM NaCl and 14 mM KCL in 40 mM Tris-HCl, pH 7,7. The reaction was started by the addition of 3 mM Na_2ATP and stopped 20 min later by the addition of 14% trichloracetic acid. Inorganic phosphate (Pi), hydrolysed from the reaction, was measured according to

Fiske& Subbarow (11). The results were expressed as umol of Pi per mg of membrane proteins per 10 min.

Measurement of MDA and GSH concentrations

MDA level was measured by Drapper and Hardley (12) methods. To assess lipid peroxidation, MDA, an end product of lipid peroxidation was measured by the thiobarbituric acid reaction. Plasma was deproteinized with trichloracetic acid (TCA). 2500ul of TCA reagent was added to 500ul of plasma and heated in water bath at 90°C for 15 min. These samples were then centrifuged at 3000(g) for 10 min.lOOOul of TBA (0.675%) reagent was added to the supernatant and heated in water bath at 90°C for 15 min. Briefly, MDA level was estimated by measuring the characteristic absorbance at 532 nm after reaction with thiobarbituric acid. The concentration of MDA was calculated using extinction coefficent of $1.56x10^{5}xM^{-1}$. GSH level was measured according to method of Beutler (13). The amount of total GSH was determined from a standard curve obtained with known amounts of GSH standards.The results are finally expressed as mmol/gHb.

Statistical analysis

Comparisons of patient and control groups were performed by using Test of Mann-Whitney U and Wilcoxon Signed Ranks. Data are presented as mean ± SD, and a value of $P<0.01$ was considered significant.

Results

As shown in Table 1,erythrocyte Na^{+}-K^{+} ATPase activities were not significantly different between the all exposed subjects and control subjects (2.44±1.85; 2.82+1.90 umolPi.mgprr'10 min ',respectively), Table 2 shows the erythrocyte Na^{+}-K^{+} ATPase activities in exposed male and control subjects. When only male subjects were considered, comparable differences were not found in the erythrocyte Na^{+}-K^{+} ATPase activities. Similarly, comparable differences were not found in exposed female subjects and control subjects (Table 3).

Table 1. MDA, GSH Levels and Na^{+}-K^{+} ATPase activity in all groups

	Controls	Total Subjects	P values*
N	59	59	
MDA (nmol/ml)	5.65 ± 1.71	6,27+ 3.56	N.S
GSH (mmol/gHb)	4.35 + 1.59	4.16+ 152	N.S
Na^{+}-K^{+} ATPase (umol Pi.mgprt"' 10 min-')	2.82 ± 1.90	2.44 + 1.85	N.S

Values are means + standart deviation. N represents the number of study groups.
*As compared with control group.

Table 2. MDA, GSH Levels and Na^{+}-K^{+} ATPase activity in the exposed male and control subjects

	Controls	Male Subjects	P values*
N	28	28	
MDA (nmol/ml)	5.89 ± 1.75	6.23 ± 2.78	0.391
GSH (mmol/gHb)	4.25 ± 1,45	4,07 ± 1,54	0.230
Na^{+}-K'ATPase (umol Pi.mgprt"' 10 min-')	2,90 ± 2,22	2,39 ± 1,67	0.648

Values are means ± standart deviation. N represents the number of study groups.
*As compared with control group.

Table 3. MDA, GSH Levels and Na -K ATPase activity in the exposed female and control subjects

	Controls	Female Subjects	* P values
N	31	31	
MDA (nmol/ml)	5.43 + 1.69	6.31 ± 4.17	N.S
GSH (mmol/gHb)	4.44 ± 1.72	4.25 ± 1,53	N.S
Na^{+}-K^{+}ATPase (umol Pi.mgprt"' 10 min-')	2.77 ± 1.69	2,49 + 2.02	N.S

Values are means ± standart deviation. N represents the number of study groups.
*As compared with control group.

We examined the MDA concentrations showing the level of lipid peroxidation in subjects exposed to magnetic field and control subjects by measuring absorption at 532 nm. Table 1 shows the MDA levels in exposed subject and control subjects. MDA concentrations in exposed subjects

to magnetic field were not significantly different from those of control subjects (respectively; 6,27± 3.56; 5.65 ±1.71 nmol/ml). Table 2 shows the levels in exposed male and control subjects. MDA concentrations in male subjects exposed to magnetic field were not significantly different from those of control subjects. Also MDA concentrations for female subjects exposed to magnetic field were not significantly different than those observed for control subjects (Table 3).

Table 1 shows the GSH levels in exposed subject and control subjects. GSH levels in subjects exposed to magnetic field were not significantly different from those of control subjects (4.16 ± 152; 4.35 ± 1.59 mmol/gHb, respectively). Table 2 shows the GSH levels in exposed male and control subjects. GSH levels for the male subjects exposed to magnetic field were not significantly different from those of control subjects. Also, female subjects exposed to magnetic field were not significantly different from those tested control subjects in GSH levels (Table 3).

Table 4. Correlation between MDA, GSH Levels and Na^+-K^+ ATPase activity and exposure time in the exposed subjects

		Exposure time (16.18 min.)
MDA (nmol/ml)	r	-0.175
	P	0.205
	N	59
GSH (mmol/gHb)	r	-0.208
	P	0.197
	N	59
Na*-K* ATPase (umol Pi.mgprt" 10 min-')	r	-0.33
	P	0.784
	N	59

Correlation is significant at the 0.01 level (2-tailed)

Table 5. MDA, GSH Levels and Na -K ATPase activity in the exposed male and control subjects taken contrast agent

	Controls	Male Subjects	*P values
N	7	9	
MDA (nmol/ml)	6.04 ±1.63	6.23 ± 2.77	N.S
GSH (mmol/gHb)	4.74 ± 1.91	3.87 ± 0.47	N.S
Na'-K' ATPase (umol Pi.mgprt" 10 min-')	0.85 ± 0.62	1.47 ± 1.28	N.S

Values are means ± standart deviation. N represents the number of study groups.
*As compared with control group.

Table 6. MDA, GSH Levels and Na^+-K^+ ATPase activity in the exposed female and control subjects taken contrast agent

	Controls	Female Subjects	*P values
N	7	7	
MDA (nmol/ml)	5.32 ± 1.70	6.25 ±3.84	N.S
GSH (mmol/gHb)	4.35 ±1.71	4.31 ± 1.64	N.S
Na'-K* ATPase (umol Pi.mgprt" 10 min-')	3.17 ± 1.57	2.74±2.12	N.S

Values are means ± standart deviation. N represents the number of study groups.
*As compared with control group. MF: Magnetic Field

Table 4 shows correlation between MDA, GSH levels and Na+-K+ ATPase activity and exposure time in the exposed subjects. When all groups were considered, no correlation was found between parameters and exposure time.

Table 5 shows, MDA, GSH levels and Na^+-K^+ ATPase activity in subjects who received contrast agent before the MRI. GSH, MDA levels and Na^+-K^+ ATPase activity in male subjects received contrast agent before the MRI did not significantly different from those for control subjects. Similarly, they were not significantly different in exposed female subjects who received contrast agent before the MRI as compared to the control subjects (Table 6).

Discussion

The radical-pair mechanism provides a simple basis for the interpretation of the effects of magnetic fields in biological systems. External magnetic field perturbs spin evolution processes in radical pairs, ultimately causing an increase in radical concentration and lifetime. An extended disturbance in both homeostasis and the integrity of DNA by magnetic field enhanced free radical

attack would weaken the biological response to genotoxic stress and promote increased mutagenesis and cancer (14). To see the effects of magnetic fields of 1.5T on free radical of metabolism, malondialdehyde, glutathione levels and Na/K ATPase activity in human subjects we measured. The effects on the lipid peroxidation, antioxidant system and enzyme activity due to the exposure of subjects to the constant magnetic field associated with MRJ methods has been examined.

The first observation obtained from the study, magnetic field with exposure period of 16.18 minutes and strenght of 1.5T did not cause any changes in the level of MDA. Our results are consistent with those from other reports (15,16).

To elucidate the biological effect of static magnetic fields, Watanabe et al. (15) measured lipid peroxidation in the liver, kidneys, heart, lungs and brain of mice exposed to static magnetic fields. Lipid peroxidation in the liver was significantly increased by exposure to 4,7 T of static magnetic fields for 3, 6, 24, or 48 hours, whereas that in the kidneys, heart, lungs and brain was not changed compared to the control.

There are various studies in which the effect of a magnetic field on free radical metabolism has been investigated. Adair (16) reported that a field of 5 mT can not change the recombination rate by as much as % 1. The known role of spin orientation in the recombination of radical pairs may constitute a mechanism for the biological effects of magnetic fields. They suggested that environmental magnetic fields can not be expected to affect human biology significantly by modifying radical pair recombination probabilities. Kashkalda et al. studied influence of impulse magnetic field on lipid peroxidation and antioxidant defence in seminal tissue of rats. Monthly exposure to the expulse magnetic field (intensity of 30 k A/m; impulse frequency of 2,5 and 25 kHz) activated initial, intermediate and final stages of lipid peroxidation, increased non-enzymatic (ascorbic asid) defence systems and depressed the enzymatic (glutathione peroxidase,catalase) ones (17).

The effects of static magnetic fields of 150-155 mT on the activities of superoxide dismutase and the contents of malondialdehyde (MDA) in the serum and the activities of glutathione peroxidase (GSH-Px) in the whole blood were observed by Jin at al (18) in healthy volunteers. The results showed that the activities of superoxide dismutase and GSH-Px significantly increased and the content of MDA significantly decreased in short term at the observed magnitude of static magnetic fields.

Gonet (19) reported the effect on the ascorbate system due to the exposure of mice to the constant magnetic field associated with MRI methods. The mice were exposed to a magnetic field of 1,00 T strength for 0,2h, 3h or 24h. The ascorbyl free radical in tissues was measured in vitro which in vivo corresponds to the level of ascorbic acid. Only in a group of mice where the ascorbyl free radical was measured 24h following their exposure to a magnetic field of 1,00T strength for 24 hours, the level of free radical in tissues significantly decreased.

Regarding the GSH data, our results are consistent with those from other reports that GSH levels in the individuals who were exposed to magnetic field were not changed compared with the controls. The effects of magnetic fields of 50 Hz (4 hours) on the contents of reduced glutathione (GSH) were observed by Dacha et al (20) in the healthy volunteers. The results showed that the level of glutathione (GSH) was not changed compared with the controls. Fiorani et al. (2) investigated whether 50 Hz magnetic fields could influence the intracellular impairments that occur when erythrocytes or reticulocytes are exposed to this oxidant system. According to their reports the effect of magnetic fields investigated is able to potentiate the cellular damage induced in vitro by oxiding agents.

Recent studies of the effect of magnetic fields on the membrane Na-K-ATPase show a dependence on enzyme activation (20). To determine a threshold of the effect of magnetic fields on the Na/K ATPase, Blank and Soo (21) have studied changes in enzyme activity due to variations in the magnitude of the field at 60Hz. They reported that the threshold was between 2 and 3 mG.

Blank and Soo (22) have measured the frequency dependence of the magnetic field effect on the Na-K ATPase activity using a specially designed exposure system with a frequency range of 1-3000 Hz and range of magnetic fields from zero to 10G (0-1 mT).They reported that there was no effect of the magnetic fields on enzyme activity for 1Hz, but at higher frequencies in the range 3-3000 Hz there was a small effect.

Blank (23) measured the effect of low frequency magnetic fields on Na-K ATPase activity. They reported that magnetic fields increase Na-K ATPase activity. In the frequency range of 20-70 Hz and for amplitudes of 0,02-2 G, Na-K ATPase activity increases by about 5-10% with optima around 60 Hz. A large increase in the effect occurs below an enzyme activity of 0,05-0,1 jxmol Pi/ mg.protein/min. Contrary to this prediction, we were unable to find such a stimulation of Na-K ATPase.

In conclusions, we have demonstrated that the levels of GSH, MDA and erythrocyte membrane's Na-KATPase enzyme activity were not changed after MRI. This study performed on the human subjects who were admitted to MRI in the Department of Radiology, routinely. Consequently, short time exposure to the magnetic field may affect the results. Most of the investigations at this field of magnetic was elucidate effect which cover a long time.

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