THE VARIATION OF PTERIDINE LEVELS IN MOUSE TISSUES

DURING EXPOSURE TO MAGNETIC FIELDS

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ABSTRACT

We investigated the alteration of pteridine levels in mouse tissues under the exposure to 50Hz circularly and linearly polarized magnetic fields. Neopterin (NP), biopterin (BP), tetrahydrobiopterin (BH4), pterin (AHP) and GTP-CHI activity were measured. Five week-old mice were exposed continuously in the period of 1,3 and 6 weeks. The circularly polarized magnetic fields may affect the immune system and biosynthetic pathway from q-BH2 to BH4 in mouse due to both changes of NP level and BH4/BP ratio. In horizontally polarized fields, almost the same results were obtained. From these results, magnetic field may affect the pteridine levels in mouse which indicate the indirect estimate of the degree of stress emerging during immune response.

INTRODUCTION

In the last two decades, many public concerns have been raised over the possible biological and health effects of extremely low frequency (ELF) magnetic fields. Research topics focused on leukemia and brain tumor in children and adults as a epidemiology. In vivo and in vitro experiments have included signal transduction, DNA synthesis, RNA transcription, nervous system, physiology, reproduction, immunology and endocrinology. A large number of studies concluded that ELF magnetic fields below 0.1mT do not produce any significant effects. Above 0.1mT, possible effects could be available. One of the main issues in ELF research still unsolved is the melatonin hypothesis. It was proposed that the reductions in melatonin production due to ELF electromagnetic fields lead to the suppression in the development of breast cancer. In order to investigate this hypothesis, several laboratory studies have been conducted. A series of experiments have been published to investigate the effects of exposure to 50Hz magnetic fields on melatonin in rat [1]. These results showed the melatonin secretion depends on the polarity of magnetic field. Linearly polarized magnetic fields did not suppress melatonin. By contrast, circularly polarized magnetic field suppressed melatonin in both plasma and pineal gland. Elliptically polarized magnetic fields with axis ratio of 4:1 did not suppress melatonin. The reason why there are differences between the result of circularly polarized magnetic field and that of linearly polarized magnetic field is not still clear. Other several laboratory studies showed no replication of earlier reported results. There are inconsistencies. The effect varies according to animal, human, the exposure period, strength and polarity of applied fields. The another topic in ELF research is to clarify the change of the immune system in animals and humans.

There are only two reports focusing on the effects of electromagnetic fields on the change of pteridine contents in human. To obtain possible effects of RF electromagnetic fields, 1mW/m^2, 900MHz/217Hz on the endocrine and immune systems in humans, the salivary concentrations of melatonin and neopterin [NP] were measured [2]. The results show they did not differ significantly between exposure and sham exposure. The another study was to investigate the possible effects of exposure to ELF electromagnetic fields on physiology in linemen on 400kV-power line, 2.8kV/m and 23.3uT [3]. The blood samples were also used to analyze the changes of hormones during two workdays: thyroid stimulating hormone, testosterone, prolactin, cortisol, NP etc. In this study, NP was analyzed as a key marker for the activation of the cellular immune system. The results showed no statistical difference between exposure and control conditions.

In order to analyze possible correlation between the pineal gland and immune system, Bartsch et al [4] carried out to investigate the parallel changes of melatonin and total biopterin [BP] during different stages of malignant growth, mammary tumor induced by DMBA in rat. BP can serve to estimate the state of cellular immunity. A cross links between the melatonin and cellular immunity is obtained due to the parallel changes of both parameters. BP and NP are the kind of pteridine compound.

The question arises whether the effects of magnetic field on pteridine contents are correlated with the effects of magnetic fields on the changes of melatonin secretion. In order to address this problem, we first tried to study the changes of pteridine levels in mouse tissues related to the exposure to magnetic fields.

OBJECTIVE

The objective of this study is to examine the effects of exposure to 50Hz magnetic fields on the contents of pteridine in mouse tissues (blood, liver, kidney, cerebrum and cerebellum). We investigated the alteration of pteridine levels in mouse under the exposure to 50Hz magnetic fields (B_h=B_v=0.35mTrms), circularly polarized and linearly polarized fields.

MATERIAL AND METHODS

Five week-old male mice at the start (Jcl:ICR) were obtained from CLEA Japan Inc. Five mice per group were used in this study. One group of five mice was kept in commercial cage. Mice were exposed continuously to 50Hz circularly and linearly polarized magnetic fields (B_h=B_v=0.35mTrms) in the period of 1, 3 and 6 weeks. Mice become six, eight and eleven week-old after each exposure. Because of exposure and sham-exposure systems were located in the same room, sham-exposed mice received a stray magnetic field from the exposure system and its values was measured to be about 0.14uTrms or less. Mice were adapted to a 12:12h light-dark cycle with light off at 19:00h. The average of temperature was 23 degrees, and relative humidity was 33.3%. Water and feed were given ad libitum. Mice were weighted two times per week and cage cleaning was done also two times per week. At the end of each exposure, mice were sacrificed in order to analyze the pteridine contents. NP, BP, tetrahydrobiopterin [BH4], pterin [AHP] levels and GTP-cyclohydrolase I [GTP-CH I] activity in various tissues are measured. Tissue weights of each mouse were measured at the end of each exposure. Mouse tissues were obtained after decapitation and stored at –80 degrees until assayed. For comparison, we referred to the data of cage-controlled groups (6, 8 and 12 week-old mice) [5].

BP and NP present mainly in tissues of mouse. They are almost types of reduction and unsettled. Reducible pteridine are generally oxidized by iodine in order to be settled and analyze by HPLC. BH4 change into BP when sample was oxidized in acid solution. On the other hand, when sample was oxidized in alkali solution, BH4 change into AHP. The above sample The preparation and determination of pteridine contents were performed by the method of Masada [6] and Fukushima [7]. Student's t test was used for comparison between two groups. A $p<0.05$ was considered as significant. For further comparison, cage control group was used to compare with other groups. The same exposure experiment was repeated three times at each polarized field.

RESULTS AND DISCUSSION

Pteridine, NP, AHP, BP, BH4 etc are general term which has 2-amino-4-oxo-pteridine ring and the group of pyrazinopyrimidine derivates. BH4 is a putative co-factor for the hydroxylation of aromatic amino acid, such as phenylalanine, tyrosine and tryptophan which are intermediate metabolite to regulate the initial and rate-limiting enzymes in the pathway for biosynthesis of the neurotransmitters dopamine and serotonin, the latter also being a precursor of melatonin. BH4 biosynthesis is regulated by the level of GTP-CHI which is most important enzyme in the pteridine biosynthesis. NP and AHP are postulated to correlate with cellular immune systems and aging.

There were no differences of body weights and growth curves among exposed and sham-exposed groups. Tissue weights at the decapitation have no difference among two groups at each exposure stages. The BP, NP, AHP and BH4 levels were measured in mouse tissues at each exposure stages.

In case of circularly polarized magnetic field, 0.5mTrms, the levels of NP and AHP in cerebellum in exposed group were increased compared to sham-exposed and cage-controlled groups. The increase of NP level suggests that the exposure may affect the activity of cellular immune system in mouse. By contrast, there were no differences of BP level among three groups. BH4 contents and the ratios of BH4/BP (BH4/BP) were significantly decreased in the exposed group compared to other groups. The levels of NP and AHP in cerebrum, liver and kidney in exposed groups were increased compared to other two groups. BH4 levels in above three tissues were decreased in exposed groups. BP levels are not affected by exposure. In case of pteridine levels in plasma, NP levels in one and three weeks-exposed groups were increased compared to other two groups and NP in six weeks-exposed groups became almost same level among three groups. BP levels in exposed groups were decreased slightly compared to other groups. In the same way, BH4 levels in exposed group were suppressed compared to

other groups. BH4/BP ratio in exposed groups was lowered compared to other two groups.

The ratio of neopterin to biopterin, N/B ratio in the cerebellum and plasma in exposed group at each exposure was increased compared to other two groups. The N/B ratio is constant in healthy individual and the changes of N/B ratio in tissues are observed as a result of disorders and/or aging. The increases of N/B ratio in exposed groups suggest the magnetic field may affect the metabolism of pteridine which indicate the activation of cell-mediated immune system. Activity of GTP-CH I was not different among three groups.

These results suggest that all biosynthesis of total BP is not affected by the exposure to circularly polarized magnetic field, but lead to that the magnetic fields may affect the biosynthetic pathway from quinonoid-dihydrobiopterin (q-BH2) to BH4 due to the decrease of BH4/BP ratio. BH4 is co-factor required for the catecholamine and indolamine. NP is released from activated macrophages in the cellular immune system, the T-lymphocyte-macrophage system and plays a key role in immunity. The circularly polarized magnetic field may affect the immune system and biosynthesis pathway from q-BH2 to BH4 in mouse due to both changes of NP level and BH4/BP ratio.

In the case of horizontally polarized magnetic fields, almost the same results were obtained. So, in this study, we compare the results of exposure experiment to circularly polarized magnetic fields with that of linearly polarized fields. In cases of circularly and horizontally polarized magnetic fields, the finding has been replicated consistently in three experiments. From these results, magnetic field may affect the pteridine levels in mouse which indicate the indirect estimate of the degree of stress emerging during immune response. Further experiment with vertically polarized magnetic fields is needed.

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