J. Rakuno Gakuen Univ., **37** (2) : 189～194 (2013)

Effects of Radiofrequency Radiation at 40 kHz on Stress-Induced Immune Response in Mice

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マウスにおけるストレス誘発免疫反応に対する 40 kHz のラジオ波の効果

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(Accepted 20 December 2012)

Introduction

The question of whether electromagnetic fields (EMF) in the radiofrequency (RF) range constitute a health hazard in exposed individuals has gained broad public interest because of the widespread applications of RF-radiation-based technology[8]. RF radiation is a portion of the electromagnetic spectrum below frequencies of visible light and above those of extremely low-frequency fields. RF radiation is produced by many man-made sources, including mobile phones and base stations, television and radio broadcasting facilities, radar, medical equipment, microwave ovens and radiofrequency heaters as well as a diverse assortment of other electronic devices within our living and working environments[8]. RF is categorized into three groups according to its frequency: extreme low frequency (ELF) (< 300 Hz), intermediate frequency (IF) (300 Hz －10 MHz) and high frequency (HF) (>10 MHz)[18].

Sufficiently intense RF radiation can cause heating of materials with finite conductivity, including biological tissues. A number of well-established biological effects and adverse health effects from acute exposure to intense RF radiation in a range of HF have been documented[1,8]. It has been reported that chronic exposure to RF in a range of ELF may also act as a stressor. Marino *et al*.[19] exposed rats to RF at 60 Hz for 1 month and attributed the reduced body weights to stress. Bruyn *et al*.[4] also suggested that RF acted as a chronic stressor in adult male mice. Besides the interest in health hazards, clinical trials of ELF in a variety of fields have been carried out[5,15－17,21]. Several lines of evidence resulting from both preclinical[9,24,25] and clinical [2,11,12] studies support the notion that repetitive transcranical magnetic stimulation (rTMS) may have antidepressant properties. Czeh *et al*.[7] showed that chronic psychosocial stress resulted in a significant increase in stress hormones and that treatment with rTMS at 20 Hz normalized the stress-induced elevation of stress hormones. Frederiks *et al*.[10] reported that pulsed ELF treatment could accelerate callus formation and bone healing after tibial osteotomy in rabbits in a dose-dependent manner. Furthermore, Food and Drug Administration (FDA) has endorsed the use of pulsed ELF for therapeutic purposes. However, there is still a profound lack of knowledge concerning the putative effects at molecular and cellular levels underlying the observed biological effects. Furthermore, biological effects of RF radiation in the range of IF are unknown. Recently, we reported that RF radiation at 40 kHz induced hepatic injury in LEC rats, an animal model for human Wilson disease[23].

It has been reported that stress including restraint stress results in a significant increase in

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stress hormones and immune suppression[3]. In the present study, we examined the effects of exposure to RF with IF range at 40 kHz on immune response using a stress-induced immune suppression method[3] and on serum concentrations of corticosterone in mice.

Materials and Methods

Mice: Specific pathogen-free male Balb/cCr Slc mice at 11-13 weeks of age were obtained from Japan SLC Inc., Japan. All research protocols were approved by the Animal Research Committee of Rakuno Gakuen University. The mice were housed at a temperature of 25 ± 2℃ and exposed to a daily cycle of 14-hr light and 10-hr darkness. A solid diet (MF-Food, Oriental Yeast Co. Ltd., Tokyo, Japan) and water were provided *ad libitum*.

Treatments: Anti-sheep red blood cells (SRBC) antibody-forming cell assay was carried out according to the method of Jerne and Nordin[14]. Fifteen mice were intraperitoneally (i.p.) injected with $2x10^8$ SRBC that were obtained from Nippon Biotest Lab., Japan and the mice were divided into 3 groups. Mice in the control group were not restrained and were not exposed to RF. Two groups of mice were kept under restraint for 20 min twice per day for 5 days. Mice in one of those groups were exposed to RF at 40 kHz during restraint (RE mice) and mice in the other group were not exposed to RF (RS mice). We used a restraint apparatus (Natsume Seisakusho Inc., Japan) that was made from a clear acrylic tube (inside diameter of 25 mm) with the parallel electrodes (widths of 25 mm). Mice were exposed to 40 kHz of RF radiation at 6 kV DC and 100 mV AC using an EMF apparatus (Serumi Co. Ltd., Japan).

Antibody-forming cell (AFC) assay: AFC assay was performed according to the method of Jerne and Nordin[14]. Briefly, spleens were obtained from mice at 6 days post-immunization with SRBC. Each spleen was excised and splenocytes were isolated with filtration using 50 μm nylon mesh. The cells were washed three times with RPMI-1640 medium (Sigma-Aldrich, Japan Co.) containing 10% fetal calf serum and 0.5% penicillin-streptomycin. After 100 μl suspension of splenocytes ($1x10^5$ cells) had been mixed with 100 μl suspension of 17% SRBC, 1 ml of 2 x MEM medium (Life Technologies Japan Ltd.) and 1 ml of 0.8% (w/v) agar (Sigma-Aldrich, Japan Co.), the mixture was incubated at 37℃ for 1 hr under a 5% CO_2 atmosphere. After incubation, the complement of guinea pig (Nippon Biotest Lab., Japan) was added to the plates and the plates were incubated at 37℃ for 30 min. The number of plaques was counted.

Measurement of concentrations of serum corticosterone: After mice had been kept under restraint for 30, 60 and 120 min with or without exposure to RF, blood samples were obtained from the mice. The samples were incubated at 4℃ for 16 hr and centrifuged at 2,000 rpm for 5 min at 4℃. Concentrations of serum corticosterone were measured using an AssayMax Corticosterone ELISA Kit (Assaypro Co., Japan).

Statistical analysis: All data were expressed as means ± standard deviation. Differences between means were analyzed statistically by Student's *t*-tests. Values of $p < 0.05$ were considered significant.

Results and Discussion

Since the AFC assay is a sensitive method for evaluating immune response, we examined the effects of RF radiation at 40 kHz on immune response in mice using this assay. The number of plaques formed by splenocytes from RS mice (125.0 ± 44.6) significantly decreased compared to those formed by splenocytes from control mice (197.0 ± 46.3) (Fig. 1). The result showed that restraint stress induced suppression of immune response in mice. This result is in good agreement with the report by Boranic *et al*.[3]. The number of plaques (258.3 ± 134.9) formed by splenocytes from RE mice showed a marginally significant increase compared to those from RS mice ($p = 0.06$). There was no difference in the number of plaques between control and RE mice ($p = 0.37$) (Fig. 1). These results showed that exposure to RF at 40 kHz induced improvement of immune suppression in the restrained mice.

It has been reported that a variety of stresses

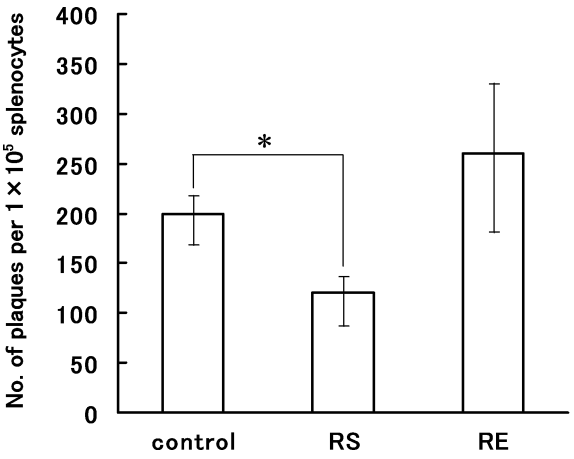

Fig. 1 Effects of RF radiation on number of plaques in AFC assay.

Numbers of plaques were counted using splenocytes from control, RS and RE mice. Each bar represents the average of five mice with standard deviation. *represents significant difference at $p<0.05$ when compared with control.

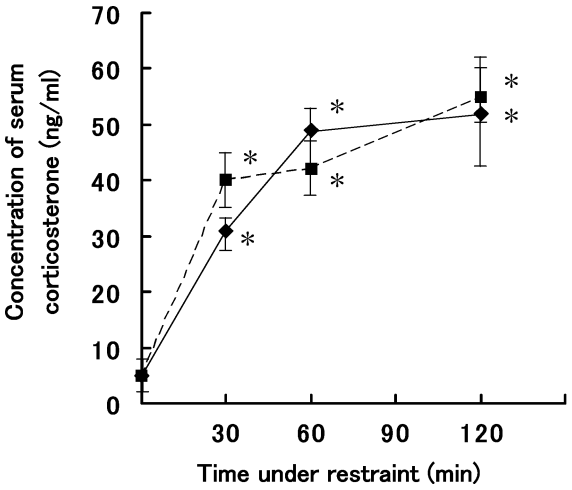

Fig. 2 Effects of RF radiation on concentrations of serum corticosterone.

Concentrations of corticosterone were measured in serum of mice that were kept under restraint from 30 to 120 min with (■) or without (◆) exposure to RF at 40 kHz. Each point represents the average of 3 to 4 mice with standard deviation. *represents significant difference at $p<0.05$ when compared with control.

including restraint stress result in a significant increase in stress hormone[3]. The concentration of corticosterone in serum of RS mice (32.0 ± 7.4 ng/ml) was significantly increased compared to that in control mice (3.8 ± 3.3 ng/ml) ($p<0.001$) at 30 min post-restraint (Fig. 2). The concentration increased in a restrained time-dependent manner (48.2 ± 3.5 ng/ml at 60 min and 54.0 ± 19.0 ng/ml at 120 min). These results showed that restraint induced an increase in stress hormone (corticosterone) in serum of mice. The concentrations of corticosterone in serum of RE mice significantly increased compared to those of control mice (Fig. 2). The concentrations were 40.3 ± 7.4, 44.5 ± 8.6 and 56.1 ± 6.8 ng/ml at 30, 60 and 120 min of restraint time, respectively. There were no significant differences in concentrations of serum corticosterone between RS and RE mice (Fig. 2). These results showed that exposure to RF at 40 kHz did not affect the concentrations of serum corticosterone induced by restraint stress in mice.

Cook and Persinger[6] suggested that the effect of RF on immune response was dependent on frequency. House *et al.*[13] reported that T cell function increased after exposure of mice to ELF. Although exposure of rodents to RF in a range of ELF could affect the immune response, effects of RF radiation in a range of IF on immune response are unknown. The present study showed that exposure to RF at 40 kHz induced improvement of immune suppression in restrained mice. It has been reported that exposure of rats to RF radiation at 20 Hz normalized the stress-induced elevation of stress hormones[7]. However, RF radiation of rats at 60 Hz did not change the concentrations of serum corticosterone[20,22]. Thus, the effects of RF radiation on concentrations of serum corticosterone might be dependent on the frequencies of RF. In the present study, no significant differences were found in concentrations of serum corticosterone between RS and RE mice. Therefore, the improvement of immune suppression by RF radiation at 40 kHz in restrained mice seemed not to be directly associated with changes in concentrations of serum corticosterone.

The present study is the first study showing that RF radiation in the range of IF affected immune response in mice, although the mechanisms by which RF radiation interacts with the immune system of mice remain unknown.

ACKNOWLEDGEMENTS

We thank the Management of Serumi Hospital for financial support. We acknowledge Assistant Professor Mori of Doshisha University for scientific advice.

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Abstract

In the present study, we examined the effects of exposure to radiofrequency (RF) with intermediate frequency (IF) range at 40 kHz on immune response using a stress-induced immune suppression method and on concentrations of serum corticosterone in mice. We used antibody-forming cell (AFC) assay to evaluate the effects of RF radiation at 40 kHz on immune response in mice. The number of plaques formed by splenocytes from RS mice that were restrained and were not exposed to RF significantly decreased compared to those formed by splenocytes from control mice that were not restrained and were not exposed to RF. Thus, restraint stress induced suppression of immune response in mice. The number of plaques formed by splenocytes from RE mice that were restrained and were exposed to RF at 40 kHz increased compared to those from by splenocytes from RS mice. There was no difference in number of plaques between control and RE mice. These results showed that exposure to RF at 40 kHz improved immune suppression in restrained mice.

It is well known that a variety of stresses including restraint stress result in a significant increase in stress hormones. Concentrations of corticosterone in serum of RS mice significantly increased in a restrained time-dependent manner compared to those of control mice. The concentrations of corticosterone in serum of RE mice also significantly increased in a restrained time-dependent manner compared to those of control mice. There were no significant differences in concentrations of serum corticosterone between RS and RE mice. These results showed that exposure to RF at 40 kHz did not affect concentrations of serum corticosterone induced by restraint stress in mice. Therefore, the improvement of immune suppression in restrained mice by RF radiation seemed not to be directly associated with concentrations of serum corticosterone. This is the first report showing that RF radiation in a range of IF affected immune response in mice.

和 文 要 約

本研究では中間波領域である 40 kHz ラジオ波（RF）のマウスにおけるストレスによる免疫抑制法を使用した免疫反応と血清コルチコステロン濃度に対する影響を検討した。抗体形成細胞（AFC）アッセイは免疫反応を評価する感度の高い方法であるので，私共はマウスの免疫反応に対する 40 kHz RF の効果を検討するために AFC

アッセイを使用した。拘束し，RF 非暴露群のマウス（以下 RS）からの脾細胞によって形成されたプラーク数は非拘束，非暴露群の対照マウスの脾細胞と比較して有意に減少した。この様に拘束ストレスはマウスに免疫反応の抑制を引き起こした。拘束し，40 kHz RF 暴露群のマウス（以下 RE）からの脾細胞によるプラーク形成数は RS 群と比較して増加し，対照群と有意差は見られなかった。これらの結果は 40 kHz RF での暴露がマウスにおいて拘束ストレスによって誘発される免疫抑制を改善することを示した。

拘束を含む様々なストレスはストレスホルモンの有意な増加を起こすことが報告されている。RS マウスにおける血清コルチコステロン濃度は対照マウスに比較して，拘束時間に依存して有意に増加した。RE マウスにおいても血清コルチコステロン濃度は対照マウスに比較して，拘束時間に依存して有意に増加し，RS マウスの濃度とは有意差は見られなかった。これらの結果はマウスにおける拘束ストレスによる血清コルチコステロン濃度の増加に対して 40 kHz RF 暴露が影響を与えないことを示した。したがって，拘束マウスにおける免疫抑制における RF 暴露による改善と血清コルチコステロンレベルは直接関連していないことが示唆された。本研究結果は中間波 RF 暴露がマウスにおける免疫反応に影響することを示した初めての報告である。