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Changes in NK Activity and CD57-CD16+ Level by Frontal Exposure to Red Photodiode Light

Tsutomu Kamei[1,2*#], Yoshitaka Toriumi[3], Hiroaki Kumano[4], Satoshi Ohno[2] and Mitsuo Yasushi[5]

[1]Shimane Institute of Health Science, Izumo 693-0021, Japan
[2]Department of Alternative and Complementary Medicine, Kanazawa University Graduate School of Medical Science, Kanazawa 920-0934, Japan
[3]Department of Pediatrics, Shimane Medical University, Izumo 693-8501, Japan
[4]Department of Psychosomatic Medicine, University of Tokyo, Tokyo 113-8655, Japan
[5]Corporate Research & Development Laboratories, Pioneer Corporation, Tsurugashima 350-2288, Japan

In zoological research, penetration of light has been reported of the frontal bones of fish, birds, and reptiles, suggesting the existence of physiologically direct photic routes to frontal lobes and/or deep parts of the brain. We studied the influences of frontal exposure to photodiode light on frontal alpha wave and peripheral NK cells. Repetitive exposure of the subject's forehead to a red light diode (660nm) significantly increased the effective amplitude of the frontal alpha waves (using a mean frequency with a range of +1.0 Hz), peripheral NK activity, and the level of CD57-CD16+. Frontal alpha wave activity and the level of CD57-CD16+ increased, suggesting the possibility of a non-invasive procedure for the activation of the frontal lobe and the increase of NK cells. This light is considered to penetrate the frontal bones of humans directly, and to act on the frontal lobe and/or other immunological regulatory centers in the brain, resulting in some neuro-immunological changes.

Key words: NK cell, NK activity, CD57, CD16, alpha wave, forehead, photodiode light

INTRODUCTION

The existence of a immune-regulatory mechanism modurated by the psychoneurosystem have been reported in several studies [1,2]. Correlation between visible light and immune response through an eye-brain mechanism has also been suggested [3].

Our previous study showed an in vivo correlation between activation of alpha waves from subjects' foreheads by photic driving response and increases of peripheral natural killer (NK) cell activity and CD4 levels; however, photic stimulation by photic driving was deemed to be a physical stressor which offsets such activation [4].

*To whom correspondence should be addressed.
E-mail: kamei@nanken.or.jp
#Present address: Shimane Institute of Health Science, Izumo, Japan

Further, a significant negative correlation was reported between the degree of mood and behavioral difficulties in the fall and winter (seasonality) and the total number of circulating NK cells [5]. This report suggested that continuous light in the daytime might be beneficial for the maintenance of the human immune surveillance. In zoological research, penetration of light has been reported of the frontal bones of fish, birds, and reptiles, suggesting the existence of physiologically direct photic routes to frontal lobes and/or deep parts of the brain. We recently suggested a direct immunological change by a red light diode exposure penetrating the frontal bone [6], but the direct physiological response of the frontal lobes and/or deep parts of the brain is unclear.

On the other hand, the effectiveness of clinical use of light has been already reported regarding seasonal affective disorder [7,8] and also non-seasonal depression [9,10]. It was recently reported that clinical depression is associated with several

large alterations in cellular immunity, including lowered parameters of NK cells [11,12].

Thus, we exposed each subject's forehead to a continuous red lightdiode, which apparently is non-invasive, and we examined the change in the amplitude of alpha wave and both the change in NK activity and the number of peripheral NK cells, the most representative parameter among these combinations being CD57-CD16+.

SUBJECTS AND METHODS

In this study, a red light (660nm) diode was used on the subject's forehead, with application of the light from approximately 10 cm in front of the subject's forehead. The illumination on the subject's forehead was 70-80 lx. The subject's eyes were completely shielded from the light by an eye mask.

Eight normal males aged 18-21 years old were each subjected to 22 sessions of exposure to the red light diode. Each session lasted 15 minutes, the experiment was carried out over 8 days. Before the 21st session there was a 15minute rest period (preliminary period, P-P). The 21st and 22nd exposure sessions were carried out with a 15minute rest period between the two sessions (lingering period 1, L-P-1), and there was another 15minute rest period after the 22nd session (lingering period 2, L-P-2).

A multichannel bioamplifier was used for brain wave measurement and Fast Fourier Transformation was performed. The effective amplitude (a square root of the power) of alpha waves (using a mean frequency with a range of plus or minus 1.0 Hz) was obtained, and the changes in the effective amplitudes appearing in the first six minutes of each of the five periods were calculated.

The NK activity and the level of CD57-CD16+ in peripheral blood were checked during the 21st and 22nd exposure sessions.

Each blood sample was devided to tubes containing citrate phosphate dextrose (CPD). NK activity was measured using ^{51}Cr labeled K562 targets. Effector and target cells were incubated at 37℃ for 4 hours in microtiter plates. Wells contained $1x10^4$ K562 cells and effector cells at a ratio of 50:1. Wells with K562 in medium alone or with 0.1N HCl were used to assess spontaneous and maximum release. Supernatants were harvested and the percentage of cytotoxicity was then calculated.

Lymphocytes were prepared for cytometoric analysis. Each blood sample was washed by phosphate-buffered saline solution (PBS) diluted 1:50. It was centrifuged at 1500 rpm for 10 min in room temperature and the supernatant was aspirated. The 100 μ l of washed blood and re-suspended in 100 μ l of PBS, and 10 μ l of fluorescein isothiocyanate (FITC)-labeled and phycoerythrin permit (PE)-labeled MAbs were poured.

Monoclonal antibodies (MAbs) were used in simple and dual immunofluorescence; these included the Leu7 FITC (CD57), Leu11c PE (CD16) (Becton Dickinson) MAbs. Cells were incubated with the MAbs at 4℃ for 30 min. Labeled cells were added 0.2ml of lysing reagent, shaked well immediately, and were placed in room temperature. They were washed in PBS, diluted to a final volume of 1 ml in refrigerated PBS, and stored in icebath until analysis. The proportion of lymphocytes was determined using the Cytron (Ortho D.S.). The flow cytometer was finely turned to obtain the sharpest peaks for fluorescence and scatter with bright fluorospheres (Ortho D.S.).

Isotype-matched monoclonal antibodies that did not react with human cells were used as controls to exclude nonspecific binding.

Double labeling was performed using the correlation of FITC and PE fluorescence. The count of each lymphocyte subset was obtained by the product of lymphocyte enumeration and the percentage of positive cells for the phenotype. The final count corresponds to the mean of duplicate analysis. Data were analyzed using absolute numbers of cells expressing a given phenotype. In this study, titers of CD57-CD16+ were examined for the parameter of NK cell count.

Statistical analysis was performed using the t-paired test.

RESULTS

The effective amplitude of alpha wave significantly increased in the left frontal pole (Fp1) ($p<0.002$) and in the right frontal pole (Fp2) ($p<0.001$) from the measurement in P-P to the measurement in L-P-2. Significant increases were also observed in left parietal (C3) ($p<0.02$), in the left occipital (O1) ($p<0.01$) and in the right occipital (O2) ($p<0.05$), but significant increases were not observed in the remaining 11 poles of the

brain.

Fifteen minutes after the 21st session, the increase of NK activity was 1.23 times compared to just before the 21st session ($p<0.05$). A gradual increase in the level of CD57-CD16+ was observed during 21st and 22nd sessions. Fifteen minutes after the 21st session, the increase was over 1.3 times compared to just before the 21st session ($p<0.02$). It was over 1.4 times just after the 22nd session ($p<0.02$), and near 1.5 times 15 minutes after the 22nd session ($p<0.02$), compared to just before the 21st session.

DISCUSSION

This study indicates that exposure of the subject's forehead to continuous light appears to increase the amplitude of alpha wave and both NK activity and the number of peripheral NK cells soon after exposure.

The effect of seasonal change on the numbers of circulating NK cells has been previously reported [5]; however, except for the effect of daylight, no other moderating factors were considered in the seasonality study. Additionally, the span of that study was quite long compared to our experiment. Previous experiments in vitro indicated that visible light increases the number of blast-transformed cells, even in a lymphocyte culture without PHA [13]. This study suggests the possibility of a proliferative system of NK cells, probably arising from some activated immune network induced by the visible light, but the details of the cause are not clear.

Recent results have demonstrated the relationship between alpha band power and processes of attention and semantic memory [14-17]. In addition, Basar et al. reported that middle-aged adults showed more expressed frontal spontaneous alpha activity in comparison with young adults, suggesting the alpha response system might be specifically related to frontal lobe functioning during aging [18]. The increase of the effective amplitude of the frontal alpha waves observed in this study may indicate the direct activation of this frontal lobe functioning. The frontal exposure to the red light diode increased both sides of the frontal functioning, suggesting that it may correct an anterior EEG alpha asymmetry often observed in patients with depression [19]. Phototherapy is already known as a method for healing depression [9,10]. But, from our results, it is difficult to consider the increases spread from occipital. There was a significant increase in effective amplitude of alpha waves in the bilateral frontal pole rather than in the occipital, and only a small increase was recognized in the parietal and temporal. In addition, it is known that the occipital alpha wave amplitude increases when the eyes are closed. For this reason we believe the main mechanism might have been independent of the function of the visual organ. Development of phototherapy restricted to the frontal region may be generally useful for healing a depressive state.

Focusing on NK cells, several disease states are associated with persistently low NK cell activity [20]: cancer, certain viral infections (including AIDS), depression, and so on. A recent study reported that a subset of NK cells may play a major roll in immune surveillance against cancer and in elimination of metastases in tissue, as well as in circulation [21]. In addition, it was shown that in a population of young, essentially healthy adults, about 14% had chronically low NK cell activity [22]. These individuals had a significantly higher frequency of viral illnesses and longer durations of fever associated with such infections [23]. These reports may support the theory humans with high NK activity will be protected against certain viral infections, indicating an effective state for the prevention of cancer, depression, and some viral infections.

In this study, the exposure of the subject's forehead to a continuous red light diode increased frontal alpha wave activity and the level of CD57-CD16+ in an apparent non-invasive fashion, suggesting the possibility of a procedure for the non-invasive activation of the frontal lobe and NK cells. This type of light exposure may contribute to the activation and maintenance of immune surveillance and to adjunct therapy for depression.

This study suggests that the red light diode penetrates the frontal bones of a human and stimulates some immunological regulatory center in the brain. Some neurological regulation of the immune system may be stimulated, but the details are unclear.

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