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Protective effect of procyanidins extracted from the lotus seedpod on immune function injury induced by extremely low frequency electromagnetic field

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ABSTRACT

This study aimed to evaluate the protective effect of Lotus seedpod procyanidins (LSPCs) from extremely low frequency electromagnetic field (ELF-EMF) exposure (50 Hz, 8 mT, 28 days) and their protective mechanism against radiation damage. The results showed that LSPCs increased the organ index of mice and made the damaged blood-producing function and cytokine(INF-γ, TNF-α, IL-2, IL-6 and IL-10 in spleen) levels by ELF-EMF-irradiation recovered to normal appearance. And experimental results proved that dosing LSPCs inhibit more stagnation of splenocytes in G0/G1 phase caused by ELF-EMF, thus the spleen cells from G0/G1 phase to S phase shift, restore normal cell metabolism, promote the splenocytes proliferation, reduced the apoptosis of spleen cells, effective protect the damage induced by the ELF-EMF radiation. In addition, LSPCs prevented the decline of DNA content caused by ELF-EMF. Western blot determinated the levels of apoptosis genes including Bcl-2, Bax, Bcl-cl, Caspase-3 and Caspase-9. The results revealed that a significant suppression in Bcl-2 expression and increase in Bax, Caspase-3 and Caspase-9 expression in splenic cells in ELF-EMF group. However, LSPCs restored these changes. Taking these results together, it may be summarized that LSPCs could protect hematopoietic tissues and the immune system from ELF-EMF. And it may be hypothesized that ELF-EMF-induced apoptosis in splenocytes might occur via triggers the trans-activation of Bax and activates caspases-3 and −9, which then cleaves the death substrates, leading to apoptosis in splenocytes of mice treated with ELF-EMF.

1. Introduction

At present, there are many artificial sources of electromagnetic fields in our living environment. We are exposed to electromagnetic fields much more frequently than in the previous natural environments. Electric and magnetic fields in the extremely low frequency (ELF) range (3–300 Hz) are mostly associated with electric power systems that mostly operate at a frequency of 50–60 Hz [1,2]. ELF-EMF has been classified as “possibly carcinogenic to humans” by the International Agency for Research on Cancer (IARC) [3]. ELF-EMFs are mainly generated by power transmission lines, power equipment or appliances [4]. Because of the rapid development of industry and society, humans are surrounded by various electric devices, and exposure to ELF-EMFs is increasing. Currently, the biological effects induced by ELF-EMFs on human health have become a cause for concern [5,6]. Furthermore, some studies reported ELF-EMF radiation can cause cancer and impact the body's cells apoptosis [3,7].

Immune system performs many important functions in people and higher animals, such as: identify self and danger signals, trigger immune response, perform the immune effect and ultimately maintain its stability and so on, immune system maintain the necessary guarantee of uniform physiological functions. External factors (compounds or physical factors, etc.) on the body, can affect the immune system function, and this change often before other symptoms damage has occurred. ELF-EMF, as a kind of external physical factors acting on the organism, will inevitably have some impact on the body's immune system. Research planning revision of the WHO in 2003 also emphasized on conducting research on the effects of electromagnetic radiation on the immune system. And in recent years, research has shown that in certain circumstances, the electromagnetic field can produce certain effect on the body’s immune function.

Extremely low frequency electromagnetic radiation is used to damage the immune cells or tissues[8]. Therefore, non cells and/or tissues should be protected against radiation injury. In particular,

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the immune system is most sensitive to radiation, as the radiation induces a high level of lymphocyte apoptosis, causes damage to the hematopoietic system, etc[9].

The ELF-EMF can affect cell growth cycle and breeding, cause some cells early apoptosis, cause cell toxicity. Wolf, etc. [10] found that 50 Hz ELF-EMF makes people normal fibroblasts and lengthen of diploid fibroblasts G1 phase extended, slowed to enter S phase, make the abnormal cell differentiation and proliferation, and tip that cell differentiation and abnormal proliferation are relevant with free radicals, such as reactive oxygen species (ROS) produced by ELF EMF.

Many scholars have done a lot of research to study the electromagnetic radiation effects on immune cells and cytokines. Italy Gobba [11] tested the electromagnetic environment, determined and compared NK activity in peripheral blood lymphocytes exposure to extremely low frequency and intermediate frequency magnetic field. The results showed that the degree of exposure to electromagnetic had negative correlations with NK cell activity. Other studies have shown that the power frequency electromagnetic field (electric field intensity of 20–58 kV/m, magnetic flux density of 0.8–0.8 mT) damaged the body's immune function, raised the activation of T lymphocytes ability, obviously enhanced the activity of IL-2, significantly increased serum levels of IgG.

Though a large variety of compounds have shown promise as irradiation – protectors in laboratory studies, most of them failed even before reaching the preclinical stage due to their toxicity and side effects. In view of the exposure to irradiation and associated human health risk, people are trying to provide the experimental evidences to counteract and relieve the chronic cerebral injuries of irradiation by means of dietary components. Various natural irradiation-protective products have been discovered, including polyphenol, flavonoids and polysaccharides.

At present, procyanidins (proanthocyanidin, PC) as a kind of new functional food additive and plant drugs gradually aroused people's concern. Procyanidins have immunity, antioxidant capacities [12,13], lower blood cholesterol [14], reduce the changes of cardiovascular diseases [15,16], and can effectively prevent tumor [17–20], and other health benefits.

Studies confirmed that procyanidins have a significant effects on immunomodulatory [21]. Enhanced normal intercellular space connection communication, activated cell through secreting cell activation factor, finally performed to promote phagocytosis and lymphocyte transformation, thereby enhancing immune function. Procyanidins can play a role of anti-oxidation, prevent spontaneous mutations caused by endogenous antioxidant, protecting T and B lymphocytes and macrophages DNA, cell membranes from oxidative damage, promote T, B lymphocyte proliferation, enhance cellular immune function [22]. Procyanidins also have obvious promoting effect on nonspecific cellular immunity, increase the activity of NK cells, enhance the body's nonspecific immune function.

Lotus seedpod is an important natural source of oligomers and polymers of catechin and epicatechin, which are also denominated procyanidins [23,24]. LSPCs were constituted by a variable number of flavan-3-ols units linked together through C_4–C_8 (or C_6) interflavanoid bonds, and the oligomeric procyanidins are considered to be the main active constituents of LSPCs [25].

Our previous study has confirmed that LSPCs have very strong scavenging free radicals and antioxidant activity both in vivo and in vitro, and had a significant protective effect on nerve system and reproductive system damage from ionizing radiation [26,27], but the ELF-EMF cause the damage on the immune function has not been reported. Based on this, In the present study, we use ICR mice as the research object, observe the protective effect on the mice immune injury induced by ELF-EMF, aims to research the immune toxicity from ELF-EMF; At the same time using procyanidins improve the immune function, to exploit the theory basis for the immune function of LSPCs. In our research, we also investigated whether ELF-EMF exposure triggered the apoptosis of splenocytes and if it altered the expression of apoptosis regulating molecules such as Bcl-2, Bax, Bcl-xl, Caspase-3, and Caspase-9 at the protein level. And confirm whether LSPCs have a regulating effect in this process.

2. Materials and methods

2.1. Chemicals & reagents

Lotus seed pot procyanidins isolated from lotus seedpot, and purified. And lotus seedpod was collected from Honghu Lantian Lake (Hubei, China). RPMI 1640 was purchased from Gibco (NY, U. S.). Antibodies for IFN-γ, IL-2, IL-6, IL-10 and TNF-α were from Abcam Trading Company Ltd. (ShangHai, China). PVDF membranes were obtained from Shanghai Canspec Scientific Instruments Co., Ltd. The ECL western blotting system was from CWBIO (Beijing, China). Horseradish peroxidase (HRP)-conjugated anti-rabbit IgG Ab were obtained from EarthOx (San Francisco, CA. USA), and anti-β-actin mAb (AC-15) was purchased from Cell Signaling Technology (Beverly, MA).

Propidium iodide (PI) and an Annexin V-FITC Apoptosis Detection kit were purchased from Beyotime Biotechnology Co. (ShangHai, China). Polyclonal anti-Bcl-2, −Bcl-xl, −Bax, −Caspase-3, and −Caspase-9 antibodies, along with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibody, were bought from Santa-Cruz Biotechnology (SantaCruz, CA).

2.2. Preparation of LSPCs extract

Lotus seedpod was collected from Honghu Lantian Lake (Hubei, China). This variety of *N. nucifera* Gaertn. were named Number 2 Wuhan plant and authenticated by the Department of Botany, Wuhan Plant Institute of the Chinese Academy of Science. The procyanidin extract of lotus seedpod (LSPCs) was extracted, purified, and characterized by the method described previously [28]. LSPCs is a light red brown amorphous powder extracted with Me2CO/H2O and purified by Sephadex LH-20 column chromatography, with a purity of >98%.

2.3. Animals

ICR rats (18–22 g), purchased from the Experimental Animal Center of Jiangsu university were kept under conditions of controlled temperature ($23 \pm 2\,^{\circ}C$) and humidity ($50 \pm 5\%$), and a 12 h light/dark cycle. Certificate number: NO. 201508865. Food and water were availablead libitum. The care of the animals was in accordance to the Guidelines for the care and use of Animals in the Institutional Committee on the Use of Animals for Research and Teaching, Jiangsu University.

2.4. Animals grouped and treated with LSPCs

The rats were randomly divided into five groups with 10 rats in each group. LSPCs dissolved in normal saline were used for administration (i.g.) daily at doses of 30, 60 and 90 mg/kg to animals for 15 consecutive days before the ELF-EMF exposure. At the beginning of ELF-EMF exposure, mice continued to be given LSPCs orally by a gavage daily for 4 weeks. Five groups as follows: Group I was sham-exposed (control); Group II was ELF-EMF exposed alone and orally received normal saline but no LSPCs (ELF-EMF); Group III was ELF-EMF exposed plus LSPCs 30 mg kg-1 (ELF-EMF + LSPCs30); Group IV was ELF-EMF exposed plus LSPCs 60 mg kg^{-1} (ELF-EMF + LSPCs60); Group V was ELF-EMF exposed