Letter to the Editor

AduoLa Fuzhenglin Down-regulates Microwave-induced Expression of β_1-adrenergic Receptor and Muscarinic Type 2 Acetylcholine Receptor in Myocardial Cells of Rats

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This paper is aimed to study the effect of ADL on expression of β_1-AR and M_2-AchR in myocardial cells of rats exposed to microwave radiation. Immunohistochemistry, Western blot and image analysis were used to detect the expression of β_1-AR and M_2-AchR in myocardial cells at 7 and 14 d after microwave exposure. The results show that the expression level was higher in microwave exposure group and 0.75 g/(kg·d) ADL group than in sham operation group and significantly lower in 1.5 and 3.0 g/(kg·d) ADL groups than in microwave group. So we have a conclusion that the expression of β_1-AR and M_2-AchR is down-regulated in myocardial cells of rats exposed to microwave radiation. ADL can protect rats against microwave-induced heart tissue injury.

Microwaves, widely used in many domains such as communications and medicine, may produce adverse biological effects on the cardiovascular system at the integrated system and cellular levels[1]. It was reported that heart is one of the most sensitive organs to microwave[2]. Microwaves can injure the function and structure of heart. It has been shown that the most obvious damage occurs in myocardial structure 7 d after radiation and does not recover 14 d after microwave exposure[3].

It was reported that β_1-adrenergic receptor (β_1-AR) and muscarinic type 2 acetylcholine receptor (M_2-AchR) in the heart play an important role in regulating myocardial contractile force and contraction frequency. Their expression levels are significantly elevated in pathological or stressful conditions. It was reported that 50 mW/cm^2 radiation can injure heart tissue and increase the expressions of caspase-3 and β_1-AR[4]. Microwave radiation can result in elevated serum levels of myocardial enzymes and Ca^{2+} in rats[3].

AduoLa Fuzhenglin (ADL), a new plant extract, can protect rats against microwave-induced heart tissue damage, increase the number of white blood cells in cancer patients[4-5], and improve cardiac structure and function in rats exposed to microwave[3]. However, no study is available on whether β_1-AR and M_2-AchR expressions are related to the protective effect of ADL against microwave-induced heart damage.

The effect of ADL on expressions of β_1-AR and M_2-AchR in myocardial cells of rats exposed to microwaves was studied in the present study.

140 male Wistar rats weighing 200±10 g obtained from Laboratory Animal Center (Beijing, China) were housed at 22±2 °C with a 12-h light-dark cycle. All protocols were approved by the Institutional Animal Care and Use Committee. The rats were randomly divided into sham operation group (*n*=25), microwave exposure group (*n*=25), 0.75 g/(kg·d) ADL group (*n*=30), 1.5 g/(kg·d) ADL group (*n*=30), and 3.0 g/(kg·d) ADL group (*n*=30).

The animals in 3 ADL groups were given ADL daily for 14 d at the doses of 0.75 g/(kg·d), 1.5 g/(kg·d) and 3.0 g/(kg·d), respectively. The animals in the sham operation group and microwave exposure groups received an equal volume of intragastric distilled water.

After the last session of intragastric administration, experimental groups were immediately exposed to microwaves for 15 min with an average power density of 30 mW/cm^2. The sham operation group was not exposed to microwaves.

On days 7 and 14 after microwave exposure, the left ventricle was removed from the rats after they were anesthetized with sodium pentobarbital (50 mg/kg IP), fixed in a 10% buffered formalin solution for a week, cut into 5 μm thick sections which were embedded in paraffin, deparaffined, soaked in 3% H_2O_2 for 10 min, heated in a microwave for antigen repair, kept at 4 °C with rabbit antibodies against β_1-AR and M_2-AchR (1:200)

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overnight, placed in biotin-goat anti-rabbit IgG (1:200), HRP-SA (1:200) and DAB coloration for 45 min at 37 °C. Nuclei were stained with hematoxylin. Negative controls were set up without adding the 1:200 rabbit antibody. Brown and light blue granules in cytoplasm and nuclei were observed. The vedorted above processes were washed 3 times in PBS, 5 min each time. Biotin-goat anti-rabbit IgG, HRP-SA, DAB were purchased from Santa Biotechnology and β_1-AR and M_2-AchR antibodies were bought from Cell Signaling Technology Company (USA).

The densities of β_1-AR, M_2-AchR were detected by Western blot. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. An entire heart was triturated in the presence of liquid nitrogen. Approximately 75 mg of ground heart tissue was diluted in homogenate buffer (0.05 mol/L tris buffer, pH 7.4, 0.05 mol/L, 2% protease cocktail inhibitor), followed by 1 min of trituration on ice. The protein concentration in supernatant was measured. Heart protein samples (loading, 45 μg) were prepared with loading buffer and placed into boiling water for 10 min. Proteins were separated by SDS-polyacrylamide electrophoresis on a 7.5% gel and blotted onto a polyvinylidene difluoride membrane (0.45 μm, Millipore). Non-specific antibody binding was reduced by blocking for 2 h at room temperature or overnight at 4 °C in ablocking solution (5% skim milk/ 2% bovine serum albumin/ 0.1% Tween in tris-buffered saline). Membranes were incubated with primary antibody diluted in tris-buffered saline containing 0.1% Tween for β_1-AR (1:750) and M_2-AchR (1:1 000), respectively. Horse radish peroxidase-conjugated secondary antibodies (GAR or GAM) (1:15 000) were diluted in blocking solution and incubated at room temperature for 1 h. Secondary antibodies were visualized using chemiluminescense and captured using gel imaging system (Abcam, USA). The β_1-AR and M_2-AchR proteins were assessed by densitometry using Image J software (National Institute of Health, Bethesda, MD). The density of interested proteins was corrected for loading variations.

The temperatures of rats were measured before and after microwave exposure. The rectal temperatures of rats in all groups were recorded on days 7 and 14.

The integral optical densities (IOD) in the interested proteins and GAPDH straps were analyzed by one-way ANOVA using the SPSS 13.0 statistical software. $P<0.05$ was considered statistically significant.

Body temperature was significantly higher in the microwave exposure group (R), 0.75 g/(kg·d) ADL group (S), 1.5 g/(kg·d) ADL group (M), and 3.0 g/(kg·d) ADL group (L) than in the sham operation group 15 min after exposure to microwaves, revealing that thermal effect may not exist at the average power density of 30 mW/cm^2.

The expression of β_1-AR was detected in rat myocardial cells. On days 7 after microwave exposure, the β_1-AR was weakly expressed in the sham operation group (Figure 1a-A), and the β_1-AR expression level was significantly higher in the microwave exposure group (Figure 1a-B) and significantly lower in 0.75 g/(kg·d) ADL, 1.5 g/(kg·d) ADL and 3.0 g/(kg·d) ADL groups than in the microwave exposure group (Figures 1a-C, 1a-D, and 1a-E). Microwave exposure at 30 mW/cm^2 increased the expression of β_1-AR. ADL at the doses of 1.5 g/(kg·d) and 3.0 g/(kg·d) curtailed the increased expression of β_1-AR after microwave exposure.

The expression of M_2-AchR was detected in in rat myocardial cells. On days 7 after microwave exposure, it was weakly expressed in the sham operation group (Figure 1b-A) and strongly expressed in the microwave exposure group (Figure 1b-B). No significant difference was found in the expression of M_2-AchR between 0.75 g/(kg·d) ADL group and microwave exposure group (Figure 1b-C). The M_2-AchR was weakly expressed in 1.5 g/(kg·d) ADL group and 3.0 g/(kg·d) ADL group (Figures 1b-D and 1b-E). Microwave radiation at 30 mW/cm^2 increased the expression of M_2-AchR while 0.75 g/(kg·d) ADL had no obvious effect on M2-AchR expression. ADL at the doses of 1.5 g/(kg·d) and 3.0 g/(kg·d) curtailed the increased expression of M2-AchR after microwave exposure.

On days 7 and 14 after microwave exposure, the expression levels of β_1-AR and M_2-AchR in rat myocardial cells were significantly higher in microwave exposure group than in other groups (Figures 2A-R and 2D-R). No significant difference was found in the expression of β_1-AR and M_2-AchR between 0.75 g/(kg·d) ADL group and the microwave exposure group (Figures 2A-S and 2D-S). The expression levels of β_1-AR and M_2-AchR were significantly in 1.5 and 3.0 g/(kg·d) ADL groups than in sham operation group (Figures 2A-M~L and 2D-M~L). ADL at the doses of 1.5 and 3.0 g/(kg·d) curtailed the increased expression of β_1-AR and M_2-AchR after microwave exposure (Figures 2B and 2E).

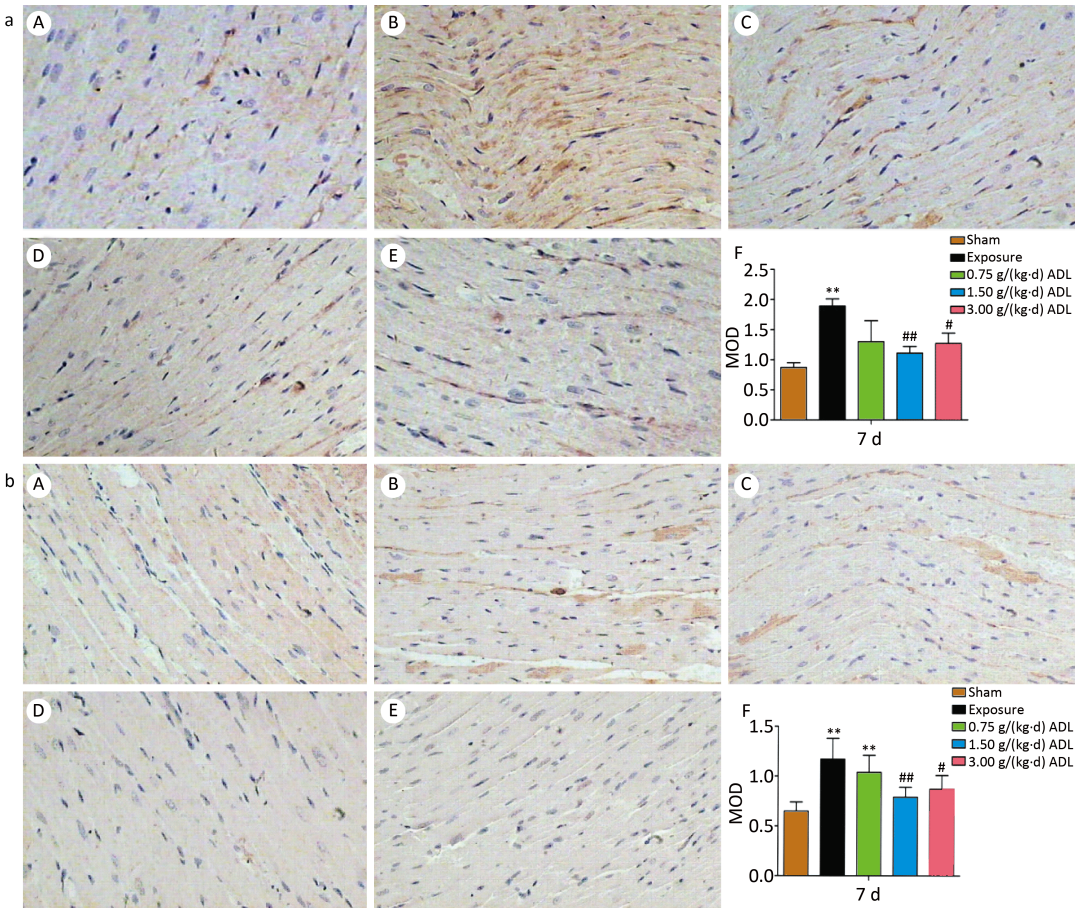

Figure 1. β_1-AR expression in sham operation group(a-A), microwave exposure group (a-B), 0.75 g/(kg·d) ADL group (a-C), 1.5 g/(kg·d) ADL group (a-D), and 3.0 g/(kg·d) ADL group (a-E), β_1-AR expression in 5 groups (a-F). M_2-AchR expression in sham operation group (b-A), microwave exposure group (b-B), 0.75 g/(kg·d) ADL group (b-C), 1.5 g/(kg·d) ADL group (b-D), and 3.0 g/(kg·d) ADL group (b-E), M_2-AchR expression in 5 groups (b-F). (SP×200) $^{**}P<0.01$ *vs* sham operation group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* microwave exposure group.

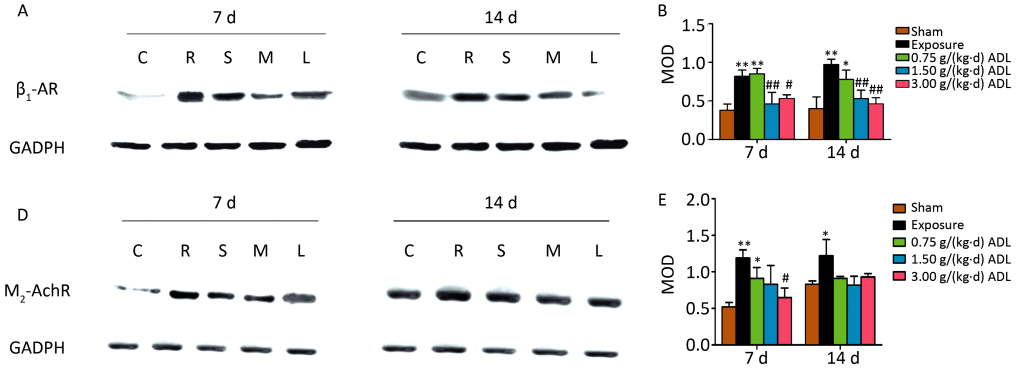

Figure 2. The protein of β_1-AR in rat myocardial cells (A), the expression of β_1-AR in sham operation group (C), microwave exposure group (R), 0.75 g/(kg·d) ADL group (S), 1.5 g/(kg·d) ADL group (M), and 3.0 g/(kg·d) ADL group (L). The protein of β_1-AR in 5 groups (B). The protein of M_2-AchR in rat myocardial cells (D), the expression of M_2-AchR in sham operation group (C), microwave exposure group (R), 0.75 g/(kg·d) ADL group (S), 1.5 g/(kg·d) ADL group (M), and 3.0 g/(kg·d) ADL group (L). The protein of M_2-AchR in 5 groups (E). $^{*}P<0.05$, $^{**}P<0.01$ *vs* sham operation group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* microwave exposure group.

The public have shown a growing concern for the potential human health hazards of exposure to microwave fields[6]. As the harmful effect of highintensity electromagnetic fields on human health has been confirmed, more studies are available on how to prevent microwave exposure-induced health problems.

The cardiovascular system is one of the most sensitive targets to microwave radiation. However, the underlying mechanisms for microwave radiation contributing to heart injury remain unclear. β-adrenergic receptor (β-AR) is the major receptor in heart tissue and plays an important role in regulating cardiac function. β_1-AR comprises approximately 70%-80% of the total β-AR population in healthy heart tissue[7]. It has been shown that specific stimulation of β_1-AR can induce apoptosis in cardiac myocytes *in vitro* and *in vivo*[8]. However, microwave exposure can injure the function and structure of the rat heart[3]. The exposure protocol did not produce any significant changes in rectal temperature, and the effects observed were non-thermal in nature in the present study. The relation between heart damage and expression of β_1-AR after microwave exposure remains unknown. The β_1-AR was over-expressed in rat cardiac tissue after microwave exposure in the present study, indicating that β_1-AR plays an important role in the regulation of cardiac function and cardiac structure.

Muscarinic acetylcholine receptors regulate the central and peripheral functions[9]. M_2-AchR is believed to be the only functional subtype of muscarinic acetylcholine receptor in the heart, although recent studies have provided evidence for the presence of other subtypes. As the main receptor regulating heart function, M_2-AchR plays an important role in myocardial contractile force and contraction frequency. However, whether it causes heart damage following microwave exposure remains unclear. In the present study, microwave exposure up-regulated the expression of M_2-AchR. Studies are available on expression of β_1-AR or M_2-AchR in heart tissues[8-10]. β_1-AR and M_2-AchR mediates norepinephrine and acetylcholinesterase and regulate myocardial contractile force and contraction frequency, thus protecting animals or humans against microwave-induced heart injury.

Microwave exposure at 30 mW/cm^2 can cause turbulence of Ca^{2+}, AST and CK, and changes in the ultrastructure of heart tissue. ADL can prevent heart damage after microwave exposure. However, the underlying mechanism for decreasing β_1-AR and M_2-AchR contributing to heart damages remains unclear. It was reported that catecholamine participates in electromagnetic radiation-induced cardiovascular system damage. In the pathological condition or in times of stress, the heart function is regulated by the sympathetic nervous system[2]. The expressions of β_1-AR and M_2-AchR in heart tissues were up-regulated and the expression level of M_2-AchR reached its peak earlier than that of β_1-AR in the present study, suggesting that β_1-AR and M_2-AchR can protect the heart against microwave exposure-induced injury. ADL showed its definite effect on the microwave-induced expression of β_1-AR and M_2-AchR in rat myocardial cells.

The heart function can be adjusted by regulating the expression of β_1-AR and M_2-AchR in cardiac muscle cells. Drugs for the prevention and treatment of microwave radiation-induced injuries are lacking. ADL can increase the number of white blood cells in cancer patients, and can thus be used in prevention and treatment of microwave radiation-induced injuries.

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