Effect of Microwave Radiation at 2450MHz on the Oxidative Damage to the Mice Liver

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Abstract: To study the effect of the microwave radiation on the activities of superoxide dismutase (SOD) and catalase (CAT), as well as the contents of glutathione (GSH) and malondialdehyde (MDA) in the mice liver. Under the microwave frequency at 2450MHz, the lab mice were divided into four groups, including one blank control group, three other groups with radiation power density of 10mW/cm^2, 20mW/cm^2, and 40mW/cm^2, respectively. Each group was irradiated for 2 hours each day in one month. Activities of SOD and CAT in the liver were determined using NBT hydroxylamine method and ultra-violet spectrophotometry, respectively; meanwhile, contents of MDA and GSH were determined using TBA and DTNB development processes. The activity of SOD increased compared with that of the blank control group, in which a significant increase was observed corresponding to the group with 40mW/cm^2 irradiation. The contents of MDA and GSH both increased significantly, while the activity of CAT showed no obvious difference, but declined instead. The microwave radiation at frequency of 2450MHz can cause the oxidative stress in the mice liver, resulting in some certain damages.

Key words: microwave; damage; experiment

Microwave has been successfully applied to the science and technology, national defense, communications, and information industry, etc. The use of microwave ovens and the number of mobile phone owners increase rapidly. The influence of microwave on organisms has attracted much attention for domestic and abroad researchers[1]. Microwave at frequency of 2450MHz has been widely used in households and hospitals, which becomes the main method in the interventional therapy for liver cancer and in the abdominal operation for physiotherapy. In real environments, people can often been influenced by such electromagnetic radiations; besides, 2450MHz microwave has strong biological effects[2], which could cause damages to organisms in varying degrees. Moreover, such damages will be multidimensional to organisms. It is shown by many surveys and practices, when exposed by overdose microwave radiations for a long time, the functionality of nerve, vision, endocrine, and cardiovascular systems will be affected. Currently, there are many reports on the effects of microwave on the immunity, nerve, and reproduction systems, while a few number of reports relating with the liver. In order to investigate the damage mechanism of microwave to organisms, especially the damage to liver, we have observed and determined changes of SOD and CAT activities, as well as changes of GSH and MDA contents in the mice liver after irradiated by microwave, which aims to provide a preliminary theoretical basis for in-depth investigations further as well as the radiation protection.

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1 Materials and Methods

1.1 Lab Animals

Healthy Kunming mice 40, equal male and female, weighting 22±3g.

1.2 Drugs and Reagents

SOD kit, MDA kit, GSH kit, CAT kit, Coomassie brilliant blue protein (Institute of Nanjing Jiancheng Biological Engineering), and other reagents are all domestic analytically pure.

1.3 Instruments

STW-1B microwave therapy (Xuzhou Shengpu Medical Instrument Technical & Equipment Co., Ltd); 752-visible spectrophotometer (Shanghai Spectrum Equipment Co., Ltd); Adventurer millionth electronic balance (OHAUS Corporation); Low-speed centrifuge (Shanghai Anting Scientific Instrument Factory).

1.4 Microwave Irradiation Methods

Lab mice were divided into four groups, including one blank control group, three other groups with

radiation power density of 10mW/cm^2, 20mW/cm^2, and 40mW/cm^2, respectively. Each group contained 10 mice with equal number of males and females, which were irradiated at the microwave frequency of 2450MHz. for 2 hours each day in one month. We adopted free position total body irradiation of mice, put in a plastic box contained within the light box under the microwave irradiation.

1.5 Determination of SOD and CAT Activities, MDA and GSH Contents

The lab mice were killed through cervical dislocation the next day after microwave irradiation, then the mice abdomens were cut open immediately, the liver tissues were took out and further rinsed clean in the cold normal saline. After drying them using the filter paper and weighting, we took 0.5g from the liver, and made homogenates with a 10% ratio of weight to volume by adding the normal saline. Further through centrifuging them at a speed of 3000rpm for 15 minutes, we took the supernate for index determination.

Determination of oxidation indexes: operate according to kit instructions, SOD determined using hydroxylamine method[3]; CAT determined using ultra-violet spectrophotometry[4]; MDA determined using thiobarbituric acid method[5]; GSH: the reaction between dithiobis-nitrobenzoic acid and sulfhydryl compound producing one yellow compound, used for quantitative colorimetric determination.

1.6 Statistical Methods

The data were processed through the variance analysis using a statistics software, Pasw Statistics 18.0, and then represented using mean and standard deviation (x±s).

2 Results

After the microwave irradiation for one month, SOD activity in the mice liver is higher than that of blank control group, where the group with irradiation power of 40mW/cm2 has a significant increase (P<0.05), as shown in Table 1; MDA content also increases significantly compared with that of blank control group (P<0.05); Shows that the content of GSH decreases significantly (P<0.05), and a significant gradient appears with the radiation power increasing; CAT activity has no significant difference (P<0.05), but shows a declining trend.

Table 1 Effect of the microwave at frequency of 2450MHz with different irradiation powers on the oxidative damage to the mice liver (n =10, x±s)

Group	SOD (U/mgprot)	MDA (nmol/mgprot)	GSH (mg/gprot)	CAT (U/mgprot)
Blank group	20.45±1.28	5.33±1.04	19.71±1.26	1.22±0.13
10mW/cm^2	20.83±1.34	7.04±0.42*	17.85±1.14*	1.20±0.11
20mW/cm^2	21.29±0.97	7.03±1.11*	16.54±1.68*	1.14±0.17
40mW/cm^2	22.79±1.08*	8.07±0.72*	15.81±1.07*	1.09±0.09

Note: *P<0.05, a significant difference observed compared with blank control group

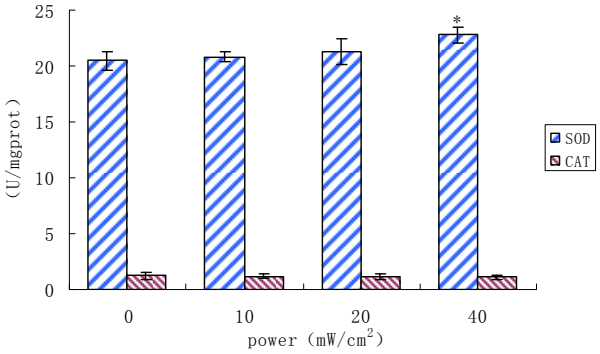

Figure. 1 SOD and CAT activity in the mice liver in groups with different irradiation powers

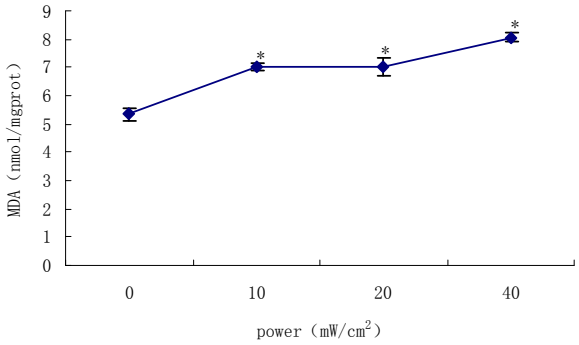

Figure. 2 MDA content in the mice liver in groups with different irradiation powers

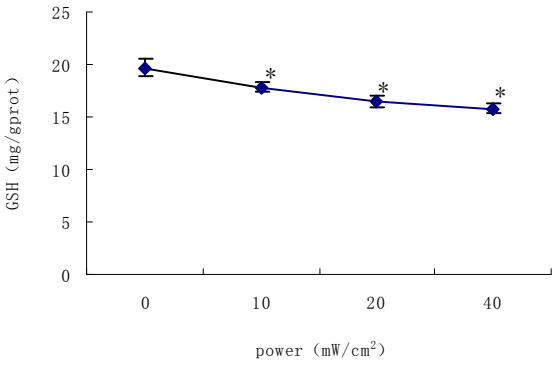

Figure. 3 GSH content in the mice liver in groups with different irradiation powers

Note: *P<0.05

3 Discussions

Currently, the biological effects of microwave can be divided into two kinds: the thermal effect and non-thermal effect. The mechanism of microwave radiation on organisms is quite complicated. When organisms absorb the microwave energy, the temperature of tissues will thus rise, termed as the thermal effect of microwave. Nonetheless, the controversy on the non-thermal effect of microwave didn't stop until 2000 when a landmark article (Pomerail D Nature [6]) was reported by Nature, the scientific community has finally recognized the non-thermal effect of electromagnetic field.

Electromagnetic field can influence the composite rate of paramagnetic free radicals, this amounting to influence the life of free radicals. In other words, this influences the instantaneous concentration of free radicals, thus causing a series of related biological reactions. Normally, there exists a dynamical equilibrium between the oxidation and antioxidant in the organism body. However, due to the endogenous or exogenous stimulus, the metabolism of free radicals will disorder and thus produce a large number of free radicals in the organism body, such as reactive oxygen species (ROS), nitric oxide (NO), etc. Reactions of free radicals are involved in many physiological and pathologic processes in the organism body, and there exist the free radical-mediated lipid peroxidation chain reaction and a defense system consisting of free Radical scavengers and antioxidants. Generally, these two systems keep in balance, until they are suffering from some external adverse factors or diseases, the system balance of oxidation and antioxidant in the organism body will be destroyed; meanwhile, superfluous free radicals in the organism body will affect polyunsaturated fatty acids causing chain reactions, termed as lipid peroxidation reaction[7].

Liver plays an important role in the metabolism of various tissues, and is also a place easy to produce free radicals and lipid oxidation[8]. Many researches show that there is a very close relationship between the damage of liver cells and the toxicity of free radicals[9]. The increased lipid in the liver consumes SOD through lipid peroxide stress response[10]; superabundant ROS can produce peroxide MDA through the reaction between lipid peroxidation, and macromolecules of biomembranes and nucleic acids[11].

GSH is an important non-enzymatic antioxidant in the organism body. Liver cells have a high content of GSH, which is the most important source of non-protein sulfhydryls within cells, as well as the most important micromolecule peptide. GSH can clear many free radicals in the organism body, thus protecting many proteins and enzymes. The content of GSH is an important indicator measuring the antioxidant capability in the organism body[12]. The experimental results show that, after irradiation for one month, The GSH content in the mice liver was significantly reduced, and an obvious gradient was observed with the radiation power increasing. Due to the decrease of GSH, it is bound to destroy the oxidative balance in the organism body, causing the oxidative stress.

MDA is the degradation product from lipid peroxide. The organism can produce free radicals through enzyme and non-enzyme systems, which could attack polyunsaturated fatty acids in the biomembranes, causing lipid peroxidation and hence generating lipid peroxides. MDA acts as a sign of lipid peroxidation, and its emergence in large numbers denotes that the lipid peroxidation occurs in the organism body. MDA Reflects not only the extent of free radical production, but also the degree of lipid peroxidation, while it indirectly reflects to what extent the organism cells have been damaged when attacked by free radicals. The experimental results show that, after microwave irradiation for one month, the content of MDA in the mice liver increased significantly, which shows that the lipid peroxidation occurred in the liver after irradiation, consuming many antioxidases, and hence causing damage to the mice liver to some extent.

The main role of CAT is to catalyze the decomposition of H2O2 into H2O and O2, making that the reaction of H2O2 and O2 does not generate hazardous -OH under the action of iron chelate. CAT is the marker enzyme of peroxisome. SOD is one of the most important enzymes against oxidative damage, widely spreading in aerobionts, oxytolerants, and some anaerobes, which is also one kind of enzymes clearing superoxide anion radicals[13]. CAT and SOD occupy a very important position in the antioxidant system, which constitute the first line of antioxidant defense in the organism body. The early generated free radicals will be reduced by them, ensuring that the organism will not be damaged. However, activities of CAT and SOD will decrease when the number of free radicals goes beyond their scavenging capabilities[14]. The experimental results show that, after microwave irradiation for one month, the activity of CAT in the mice liver has no significant change, but in a declining trend, while the activity of SOD was rising, especially in the group with high power, it would increase significantly. When combining with the changing contents of GSH and MDA, it shows that the peroxidation has occurred in the mice body, and thus proves that the number of free radicals increased due to the electromagnetic radiation, causing the oxidative stress in the mice body. However, the increase of SOD activity in the liver might be a stress protection against microwave radiation damage. The synthesis of SOD in the liver has a rapid increase induced by free radicals generated by microwave irradiation[15], alleviating the damage to the organism.

It is shown by both the experiments and analysis

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that, free radicals in the mice liver are produced when irradiated by microwave; meanwhile, the biosynthesis of SOD has been induced as well. If microwave radiation can cause the apoptosis of irradiated cells by the imbalance of antioxidant and oxidative system and the significance of the prevention and treatment remain to be further explored.

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