Letter to the Editor

Pathological Changes in the Sinoatrial Node Tissues of Rats Caused by Pulsed Microwave Exposure

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To observe microwave induced dynamic pathological changes in the sinus nodes, wistar rats were exposed to 0, 5, 10, 50 mW/cm^2 microwave. In 10 and 50 mW/cm^2 groups, disorganized sinoatrial node cells, cell swelling, cytoplasmic condensation, nuclear pyknosis, and anachromasis, swollen, and empty mitochondria, and blurred and focally dissolved myofibrils could be detected from 1 to 28 d, while reduced parenchymal cells, increased collagen fibers, and extracellular matrix remodeling of interstitial cells were observed from 6 to 12 months. In conclusion, 10 and 50 mW/cm^2 microwave could cause structural damages in the sinoatrial node and extracellular matrix remodeling in rats.

In recent years, several public concerns have emerged regarding the potential health problems associated with exposure to microwave. Heart is one of the most sensitive organs to microwaves[1-3]. Epidemiological studies have demonstrated that people exposed to microwaves had a higher likelihood of suffering from sinus tachycardia, sinus bradycardia, and sinus arrhythmia[4]. Animal experiments have also indicated that moderate and high power microwaves could induce myocardial tissue injury, causing more severe damage in the sinus node, atrioventricular node, and conduction fibers[5]. The sinus node functions as a pacemaker of the heart and initiates the heartbeat and controls the rate and rhythm of contractions. However, few studies have investigated the dynamic pathological changes of the sinus node in response to microwave exposure. In this study, we studied acute pulsed microwave exposure induced dynamic injuries to the sinus node tissues of rats.

160 male Wistar rats (200±20 g, 8 weeks of age) from the Laboratory Animal Center (Beijing, China) were divided into four groups (n=40 per group). Rats were exposed whole body with 2.856 GHz exposure source for 6 min at an average power density of 5, 10, 50 mW/cm^2 for exposure groups, respectively, or without exposure for the sham group. The key parameters of the pulsed microwave system were list in Supplement Table 1 (see the website of this journal). All the experimental procedures used in this study were approved by the Animal Care and Use Committee of our institute.

At 1, 7, 14, and 28 d and 3, 6, 9, and 12 months after exposure, the rats (n=5 per group) were anesthetized by sodium pentobarbital, and sinus node samples were dissected from the junction between the superior vena cava and the right auricular appendage. The structural changes of sinus nodes were observed after staining with hematoxylin and eosin (H&E) and the ultra-structures were detected by a transmission electron microscope (TEM; HITACHI Ltd., Tokyo, Japan) after heavy metal staining. Moreover, the collagen fibers were detected by using picrosirius red (0.1% sirius red in saturated aqueous picric acid) staining. The integrated optical density of collagen was analyzed from three randomly image per group by using the CMIAS-II image software. Data were presented as means±SEM. Statistical differences between the groups were determined by analysis of variance (ANOVA) using the Statistical Package for the Social Sciences software. Differences at P<0.05 were considered statistically significant.

HE staining revealed pale sinus node tissue, which was composed with spherical or oval shaped P cells and cylindrical and rod-shaped T cells. Normal histological structures were observed (Figure 1A-B) in the sham and 5 mW/cm^2 exposure groups, while dynamic injuries could be detected in 10 and 50 mW/cm^2 groups with the time extending. 1 d after exposure, increased P cells and T cells were observed. Moreover, vacuoles in the cytoplasm increased, and the T cells exhibited a wavy pattern (Figure 1C-D). 7 d after exposure, ballooned P and T cells with paler staining cytoplasm and off-center

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nucleus moved were detected (Figure 1E). 14-28 d after exposure, the structure of the sinus nodes became loose with disordered cell arrangement; the cytoplasm of some cells showed coacervation, acidophilic staining strengthened, and a small number of nuclei started to exhibit pyknosis and anachromasis (Figure 1F). And, the cellular injuries tended to recover at 3-6 months (Figure 1G). 9 months after exposure, increased collagen fibers between cells and around the sinus node artery, and decreased parenchyma cells were observed (Figure 1H). 12 months after exposure, the parenchyma cells reduced significantly, and the interstitial cells and collagen fibers increased along with infiltration of lipid droplets (Figure 1I). Furthermore, 50 mW/cm^2 microwave induced more severe damages to rats' SAN tissues.

It has been reported that microwave ranging from 1.004 mW/cm^2 to 0.974 kW/cm^2 could induce damage throughout the heart, and the sinus node and Purkinje fibers exhibited more severe and persistent damage than the myocardial fibers[6]. Furthermore, our data suggested that microwave had different effects on rats' SAN tissues at different stages after exposure (both short-term and long-term effects), and presented a certain dose-and-effect relationship, which will provide benefits to explore the mechanisms for microwave induced damage of heart function.

Ultrastructure detection showed that T cells had more organelles in the cytoplasm than P cells. In P cells, myofilaments were arranged regularly and neatly, incomplete with Z lines and had no M lines; mitochondria showed diffuse distribution; and the nucleus was big and round with smooth and neat nuclear membrane (Figure 2A). In T cells, Z lines and M lines appeared distinct and with complete muscle segments on the surface of myofibrils (Figure 2A-B). 5 mW/cm^2 microwave had no significant influence on the ultrastructure of SAN cells (Figure 2C), while

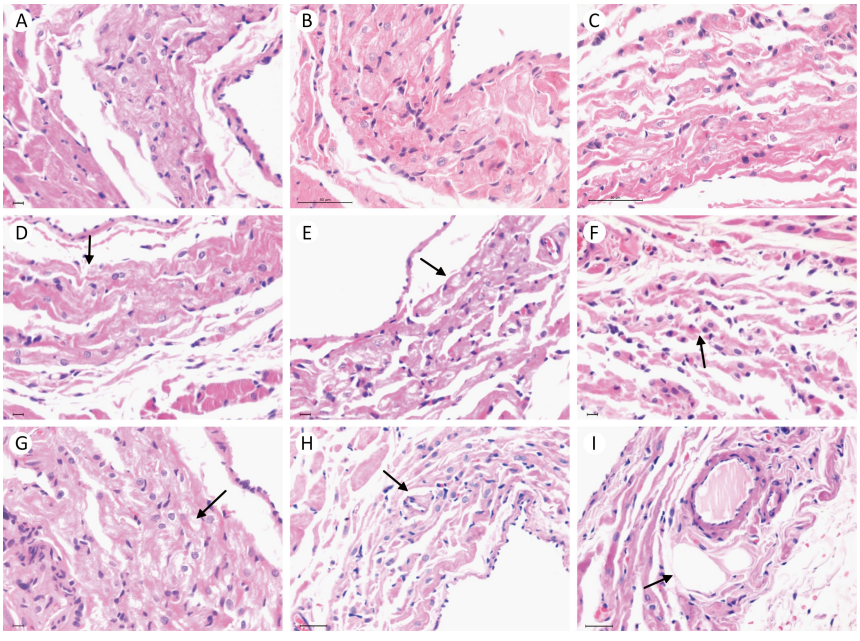

Figure 1. The development of damage to rats' sinus node after microwave exposure (HE staining, A-I scale bar=10 μm; F, G scale bar=25 μm; B, C scale bar=50 μm). Normal structures were observed in both sham group (A) and 5 mW/cm^2 group (B). Slightly disorder, including partly condensed cytoplasm and enhanced acidophilia staining could be detected in 10 mW/cm^2 group (C). In 50 mW/cm^2 group: cellular edema, increased cytoplasm vacuoles and T cells of wavy pattern (↑) could be detected at day 1 after exposure (D); worsen cellular edema, and pale cytoplasm staining with balloons formation (↑) were found at day 7 (E); disordered cell arrangement, coacervated cytoplasm (↑), strengthened acidophilia staining with karyopyknosis and anachromasis were observed at day 14 (F); some cells still in edematous state (↑) at month 6 (G); collagen fibers increased between cells and around sinoatrial node artery (↑), and parenchyma cells decreased at month 9 (H); parenchyma cells decreased significantly in interstitial tissues along with infiltration of lipid droplets (↑) at month 12 m (I).

10 and 50 mW/cm^2 microwave damaged mitochondria and myofibrils regressively. At 1 d after exposure, focal swelling and cavitation in mitochondria, thinned and chaotic arranged myofibrils, and widened perivascular spaces were detected (Figure 2D-F). 28 d after exposure, there appeared much more swollen, broken or cavitated mitochondria (Figure 2G); broken, reduced or absent cristae, and blurred, focally dissolved and broken myofibrils (Figure 2H). Some nuclear chromatin were condensed and off-center (Figure 2I); the perivascular spaces were remarkably widened; and vascular endothelial cells exhibited the presence of pinocytic vesicles (Figure 2J). 6 months after exposure, parenchyma cells exhibited degeneration with deformed or swollen mitochondria, or degenerated vacuoles or medullary sheath. The myofibrils exhibited narrowing and the muscle segments were shorter and of varied lengths. The Z lines became blurred or condensed, and the collagen fibrils among the interstitial tissue increased (Figure 2K). At 9-12 months after exposure, a proportion of P and T cells continued to exhibit regressive changes, and the mitochondria degenerated like medullary sheath (Figure 2L). Collagen fibrils among the interstitial tissue increased significantly, and many fibroblasts in a state of active hyperplasia were observed, along with infiltration of lipid droplets (Figure 2M).

Mitochondria are considered as the main center that handle internal and external risk signals[7], and is one of the sensitive organelles to microwave. Our data from this study confirmed microwave induced mitochondria injuries both in P cells and T cells in sinus nodes, including swollen inner compartment; disordered, shorter, fewer, broken, and absent cristae. Furthermore; myelin sheath degeneration, cavitation, and even lysis. Defects in the respiratory chains, increased production of reactive oxygen species (ROS), changes in the permeability of the mitochondrial membrane, and the release of apoptosis-promoting substances such as cytochrome C might cause injuries in mitochondria[8-9].

Extracellular matrix remodeling (ECM) plays key roles in maintaining the structures and functions of organs or tissues, and collagen fiber is the main content. The synthesis and degradation of collagen

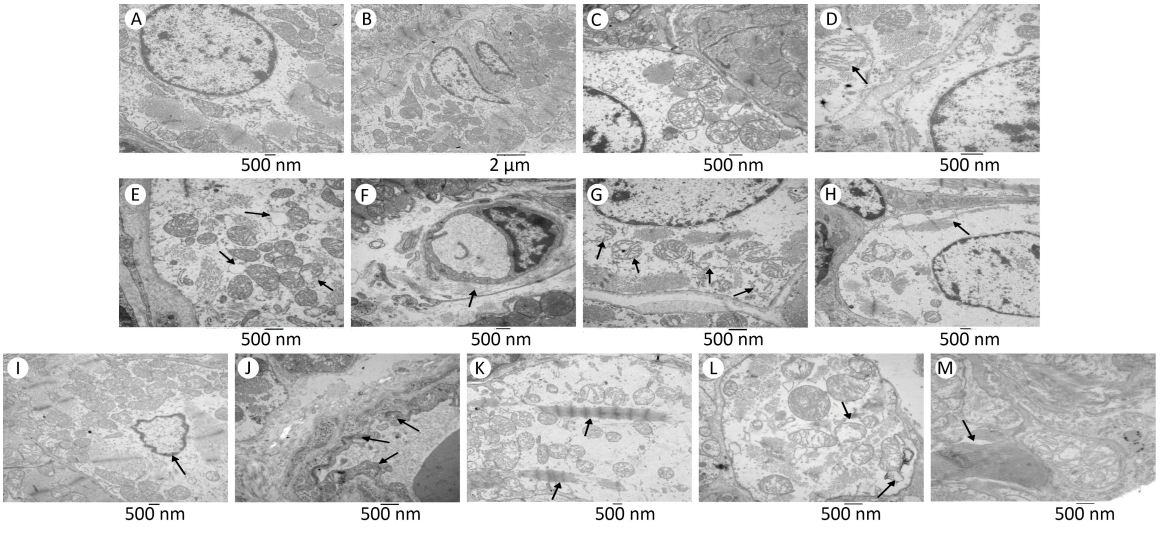

Figure 2. Effect of microwave exposure on ultrastructure changes in the sinus nodes TEM, scale bar=500 nm, A, C-M; scale bar=2 μm, B. Normal P (A) and T cells (B) were shown in sham group, and no significant abnormality were observed in 5 mW/cm^2 group (C). Swollen, focal cavitated, and ridges broken or decreased mitochondria (↑), and blurred myofibrils could be detected in 10 mW/cm^2 group. In 50 mW/cm^2 group: focal swelling and cavitation in some mitochondria (↑) (E), and widened perivascular spaces could be observed (↑) at 1 d after exposure; swollen, cavitated and broken mitochondria (↑) (G), broken and dissolved myofibrils (↑) (H), irregular nucleus, and condensed and moved off chromatin (I), significantly widened perivascular spaces, and vascular endothelial cells with many pinocytosis vesicles(↑) (J) were detected at 28d after exposure; the mitochondria of T cells appeared focal swelling, cavitation and even breaking and dissolving, and the muscle segments were shorter and Z lines were blurred or condensed (↑) (K) at 6m after exposure; degeneration of organelles and medulla sheath in mitochondria (↑) (L) could be detected at 9 m after exposure; increasing collagen fibers and lipid droplets infiltration could be found in the interstitial substance (↑) at 12 m (M) after exposure.

fiber always sustains a balance status under normal physiological condition. This balance is disrupted when the cells are damaged, resulting in excessive ECM with collagen fiber content, preventing damaged tissues from recovering to their normal physiological structure but converts to pathological structures and tissues. This is termed ECM repatterning[10]. Our results showed that microwave increased collagen fibers in the sinus nodes obviously at 9-12 months in the 10 mW/cm^2 group (P<0.05) and at 6-12 m in the 50 mW/cm^2 group (P<0.01), indicating that certain doses of microwave exposure could induce ECM repatterning in the sinus nodes of rats (Figure 3).

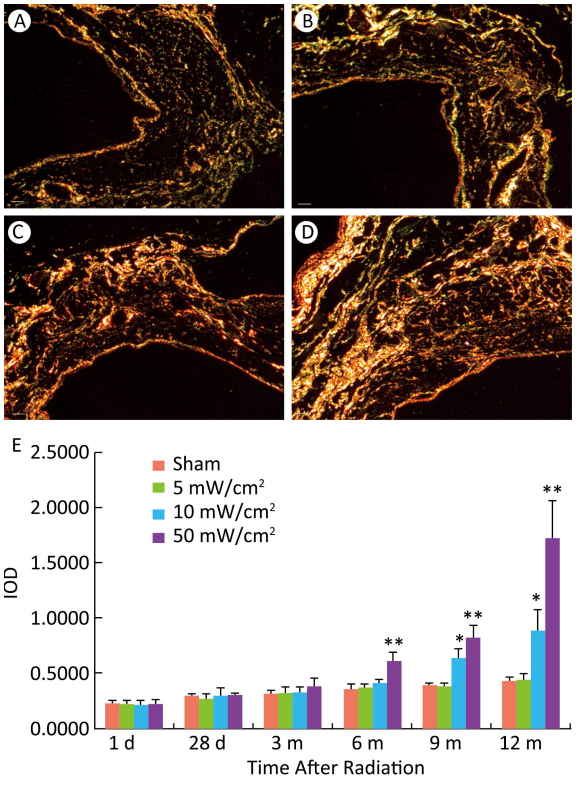

Figure 3. Type I and III collagen content in the sinoatrial node of rats. Type I (red) and III (yellow) collagen were stained with Sirius red. The representative images at 12 m after microwave exposure from sham (A), 5 mW/cm^2 (B), 10 mW/cm^2 (C), and 50 mW/cm^2 (D), groups were shown (scale bar=50 μm). The statistical analysis of integral optical density (IOD) values of collagen fibers in rats' sinus nodes at different times after microwave exposure were shown in E (n=5). $^*P$<0.05, $^{**}P$<0.01 *vs.* Sham group at the same time point after microwave exposure.

In summary, our results demonstrate that exposure to pulsed microwave could affect the structure of the sinus node in rats. We found that microwave exposure (≥10 mW/cm^2) adversely affected the structure of the sinus node and induced injuries therein. Our results established the foundation for further exploration into the mechanism of impairments following pulsed microwave exposure and preventive measures.

Author Contributions Conceived and designed the experiments: PENG Rui Yun. Performed the experiments: LIU Yan Qing, GAO Ya Bing. Analyzed the data: LIU Yan Qing, ZHAO Li. Contributed reagents/materials/analysis tools: DONG Ji, YAO Bin Wei. Wrote the paper: LIU Yan Qing.

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