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Metformin Ameliorates 2.856 GHz Microwave-Radiation-Induced Reproductive Impairments in Male Rats via Inhibition of Oxidative Stress and Apoptosis

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Abstract: The reproductive system has been increasingly implicated as a sensitive target of microwave radiation. Oxidative stress plays a critical role in microwave radiation -induced reproductive damage, though precise mechanisms are obscure. Metformin, a widely used antidiabetic drug, has emerged as an efficient antioxidant against a variety of oxidative injuries. In the present study, we hypothesized that metformin can function as an antioxidant and protect the reproductive system from microwave radiation. To test this hypothesis, rats were exposed to 2.856 GHz microwave radiation for 6 weeks to simulate real-life exposure to high-frequency microwave radiation. Our results showed that exposure to 2.856 GHz microwave radiation elicited serum hormone disorder, decreased sperm motility, and depleted sperm energy, and it induced abnormalities of testicular structure as well as mitochondrial impairment. Metformin was found to effectively protect the reproductive system against structural and functional impairments caused by microwave radiation. In particular, metformin can ameliorate microwave-radiation-induced oxidative injury and mitigate apoptosis in the testis, as determined by glutathione/-oxidized glutathione (GSH/GSSG), lipid peroxidation, and protein expression of heme oxygenase-1 (HO-1). These findings demonstrated that exposure to 2.856 GHz microwave radiation induces obvious structural and functional impairments of the male reproductive system, and suggested that metformin can function as a promising antioxidant to inhibit microwave-radiation-induced harmful effects by inhibiting oxidative stress and apoptosis.

Keywords: microwave radiation; metformin; male reproductive damage; oxidative stress; apoptosis

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1. Introduction

With the rapid development of electrical equipment and wireless transmission technology, microwave devices have been increasingly used in daily life and industries such as telecommunications, medical devices, and military weapons, causing increasing concern for human health and environmental safety. Since the first report by Wertheimer showing the impact of electromagnetic radiation on fertility [1], significant efforts have been made to investigate the effects of microwave radiation on reproductive functions following occupational and environmental exposure [2]. Compared to other tissues, the testis with spermatogenic cells has been found to be more sensitive to microwave radiation, as the testis has lesser tissue protection and is more easily penetrated by microwave radiation [3,4]. According to epidemiological surveys and experimental studies, microwave radiation can damage testicular function, semen quality, and testicular structure [5–7].

The microwave band ranges in frequency from 300 MHz to 300 GHz [8], but the majority of studies of microwave-radiation-induced reproductive impairment have focused on the frequencies of 900 MHz, 1800 MHz and 2.45 GHz [7,9–11]. Emitters with a frequency of 2.856 GHz have been widely used in radar and other communication devices [12]. Our previous studies and other reports showed spatial memory and cognitive impairment and dose-dependent cardiomyocyte injury in rats, calcium efflux in hippocampal neurons and cardiomyocytes, and potential cytotoxicity in bone marrow mesenchymal stem cells following 2.856 GHz microwave exposure [13–19]. However, few studies have investigate the potential deleterious effects and the underpinning mechanisms of 2.856 GHz microwave exposure on reproduction, especially after long-term exposure.

Emerging evidence suggests that oxidative stress is critically involved in the pathogenesis and development of microwave radiation-induced damage to the reproductive system. It has been revealed that microwave radiation increases reactive oxygen species (ROS) in spermatozoa, which can deteriorate antioxidant enzymes and induce mitochondrial dysfunction, eventually damaging male fertility [20–23]. Under oxidative conditions, spermiogenesis becomes defective and defective gametes form, leading to unusual apoptosis and reproductive impairment [24].

Metformin, a biguanide compound widely used for type 2 diabetes mellitus, has been increasingly shown to be a promising radioprotective agent. Karam et al. reported that metformin protected against cardiac damage caused by ionizing-radiation-induced oxidative stress, inflammatory mediators, and endothelial dysfunction [25]. However, the precise effects of metformin on a reproductive system exposed to microwave radiation are unclear.

Metformin can stimulate cellular antioxidant enzymes, to indirectly scavenge free radicals and decrease lipid peroxidation, as well as to modulate cellular metabolism and mitochondrial function [26–29]. Recently, emerging evidence suggests that metformin protection is closely associated with its antioxidant capacity in the reproductive system. Metformin was found to increase sperm concentration and sperm viability, enhance antioxidant enzyme activity, and improve testicular tissue sperm quality [30,31]. These studies suggest that metformin may be a useful countermeasure against microwave radiation, but to date the effects of metformin on microwave radiation have not been investigated.

We established an animal model of microwave-radiation-induced damage that is closer to real-life exposure and demonstrated that metformin attenuated 2.856 GHz microwave-radiation-induced alteration in rats' sperm motility and structure, serum hormones, and mitochondrial function. Our results suggest that metformin can function as a promising antioxidant in inhibiting microwave-radiation-induced oxidative stress and apoptosis and in protecting male fertility.

2. Results

2.1. Metformin Relieved the Decrease in Sperm Motility Caused by Microwave Radiation

Sperm motility is one of the most widely used indicators to reflect reproductive function. Progressive sperm motility was defined as grade A + B; as shown in Figure 1, the proportion of grade A + B sperm in the radiation group was significantly lower than in the control group at 6 h, 7 d, and 14 d after microwave radiation ($p < 0.01$). However, in the metformin groups, the proportions of grade A + B sperm were significantly higher compared to the radiation group in a dose-dependent manner ($p < 0.05$ or $p < 0.01$) (Figure 1A). There was no statistical difference in the proportion of nonprogressive sperm (grade C) between the groups at all four time points after microwave radiation (Figure 1B). The proportion of immotile sperm (grade D) showed the opposite results to grade A + B sperm. Compared with the control group, the proportion of grade D was increased at 6 h, 7 d, and 14 d after microwave radiation ($p < 0.01$). After metformin administration, the proportion of grade D was decreased dose-dependently compared with the radiation group ($p < 0.05$ or $p < 0.01$) (Figure 1C). Collectively, the results showed that microwave radiation

impaired sperm motility, and metformin increased sperm motility in radiated rats in a dose-dependent manner.

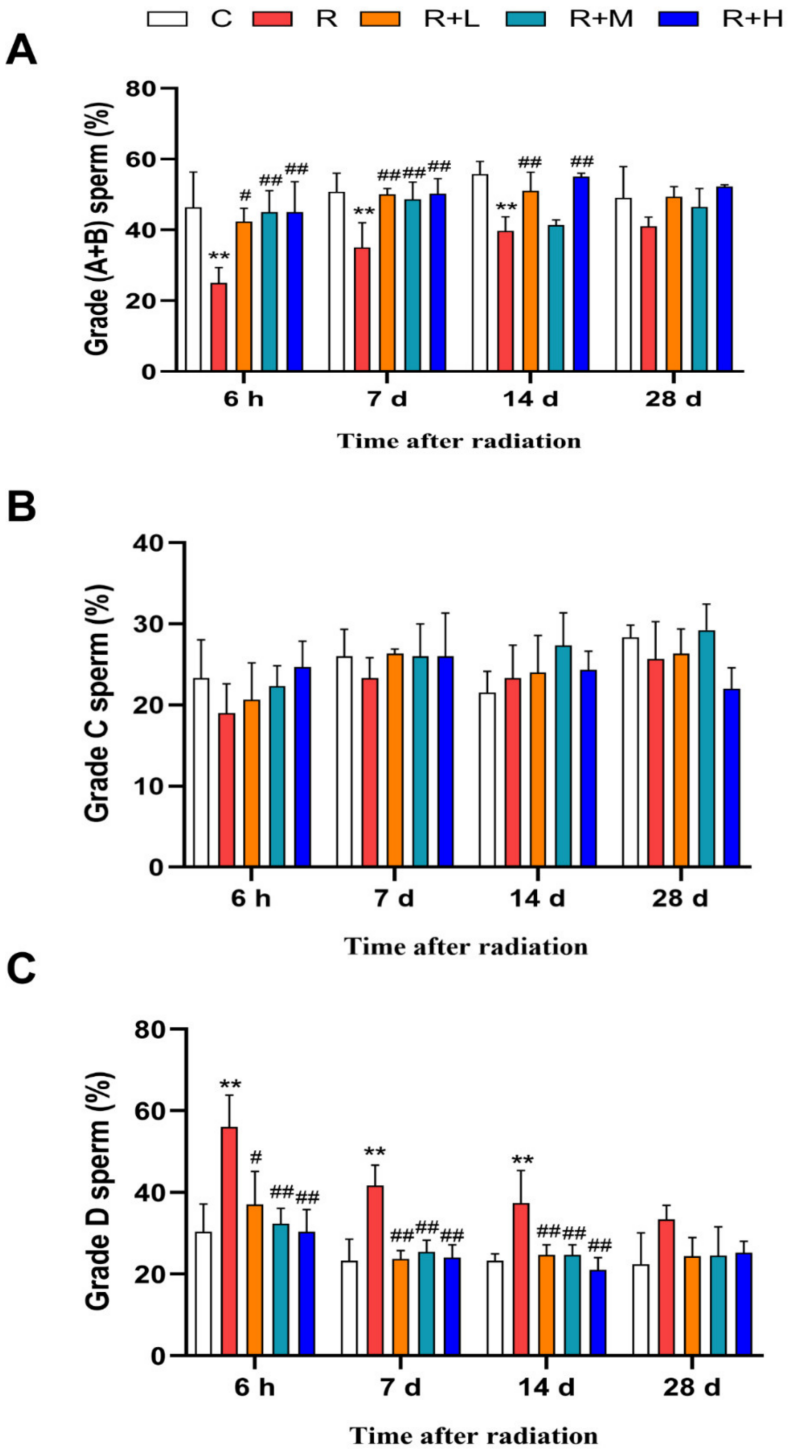


Figure 1. Metformin relieved the decrease in sperm motility caused by microwave radiation (n = 5 for each group). (**A**) Changes in the proportion of grade A + B sperm. (**B**) Changes in the proportion of grade C sperm. (**C**) Changes in the proportion of grade D sperm. ** shows $p < 0.01$ for the R group compared to the C group; and # shows $p < 0.05$ for the R + L, R + M, and R + H groups compared to the R group, and ## shows $p < 0.01$.