The Synergistic Effect of Microwave Radiation and Hypergravity on Rats and the Intervention Effect of Rana Sylvatica Le Conte Oil

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Abstract

Aim: The phenomena of hypergravity and microwave radiation are widespread, which cause more and more concern for the hazards to human health. The aim of this study was to investigate the synergistic effect of microwave radiation and hypergravity on rats and observe the protective effect of Rana sylvatica Le conte oil.

Methods: Rats were exposed to microwave radiation and hypergravity, and the rat weight, the climbing pole height, serum enzyme activities, blood urea nitrogen concentration, and total antioxidant capacity were detected.

Results: The climbing pole height, the activities of choline acetyl transferase and cholinesterase, and the total antioxidant capacity decreased, whereas the activities of alanine aminotransferase, aspartate aminotransferase, creatine kinase, isocitric dehydrogenase, hydroxybutyrate dehydrogenase, and the blood urea nitrogen concentration increased in the hypergravity irradiation group as compared with the others.

Conclusion: These results imply that the motion and nervous system of rats might be affected critically by the synergistic effect of microwave radiation and hypergravity, and it causes damage to most rat organs, such as the bone, skeletal muscle, liver, heart, and kidney, and the antioxidant effect is also damaged, while the injury resulted from it could be protected by Rana sylvatica Le conte oil.

Keywords
hypergravity, microwave radiation, serum enzyme, Rana sylvatica Le conte oil

Introduction

With the wide use of microwave technology in medical, communications, radar, detection, television, nuclear physics, and other fields, it brings great benefits for people, but it also causes more and more concern for the pollution of the surrounding environment and the hazards to human health. The thermal effect of microwave radiation may cause death, disability, or injury to organisms upon the diversity of radiant intensity, time, and space. Nonthermal effects of microwave radiation on organisms cannot be ignored, such as the teenagers who use mobile phones for long may have fatigue, lethargy, tinnitus, memory decrease, decreased vision, and other phenomena due to the long-term and low-intensity microwave radiation.¹ Furthermore, with the advent of long-term interplanetary missions, involving increasing numbers of people, space biology is becoming an emerging area of research devoted to assess the effects of altered gravity conditions and microwave radiation on human health. Furthermore, the 2-factor synergistic effect of overweight conditions and microwave radiation exists in the accelerated up-flight process of aircraft which is common in daily and the hypergravity is about 6G.²,³ The necessity

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to develop reliable animal models to protect human health has led to a variety of animal studies such as motion sickness syndrome, which mimics a related disorder arising in astronauts during space missions. The damage of microwave radiation and the harm of hypergravity to organisms have been confirmed, respectively, but the current research focus has shifted to the 2-factor synergistic effect on body, and it has attracted more and more concerns due to the catholicity and complexity of the injury.

Rana sylvatica Le conte oil is the egg oil of Rana sylvatica Le conte that habitats in the forest swamp of Northeast China, and it is the dried oviduct of female Rana sylvatica Le conte, also known as Oviductus Ranae. Many studies have proved that Rana sylvatica Le conte oil has the antiaging and antioxidant effect, and it is useful to increase immunity. It has been reported that the Rana sylvatica Le conte oil without hypergravity irradiation has no effect on the weight, enzyme, oxygen radicals, and transforming growth factor-β (TGF-β) mRNA of rat. The animal experiment was designed to investigate the synergistic effect of microwave radiation and hypergravity on the activities of serum enzymes, the motion of rats, and the antioxidant capacity, and also to observe the influence of Rana sylvatica Le conte oil as an original radioprotector for the common microwave radiation contact persons and for the special population with injury resulted from radiation and hypergravity.

Materials and Methods

Animal Model

Adult Wistar rats (n = 36, grade II, initial weight 200 ± 20 g, Jilin University Animal Laboratories) were chosen which were housed under controlled environmental conditions with free access to food and water and were randomly divided into 3 groups for experiment. There were 12 rats in each group, which were equally divided between female and male. A first group was the control group. A second group was the hypergravity irradiation group (the microwave irradiation group in the hypergravity condition). A third group was the intervention group (the microwave irradiation group interfered with Rana sylvatica Le conte oil). Azalein solution and picric acid solution were used, respectively, to make identification numbers of animals in each group.

A replication animal model was selected that rats were exposed to microwave radiation of 200 mW/cm² power density and +6G hypergravity for 5 minutes. The animal centrifuge was used to simulate artificial hypergravity condition. Microwave was emitted by the microwave transmitter with frequency 2625 ± 15 MHz, wavelength 11.26 to 11.49 cm, and average power 2.4 kW. Rana sylvatica Le conte oil was provided by the Jilin Frog King Co Ltd, and the administration dose of it (dry weight) was 100 mg/kg/d which was dissolved in 2 mL ultrapure water. Rats in the intervention group were treated with 2 mL Rana sylvatica Le conte oil by intragastric administration once a day for 12 days continuously, whereas rats in the control group and the hypergravity irradiation group were treated with 2 mL isotonic Na chloride by intragastric administration once a day for 12 days continuously. All of the rats were anesthetized with 10% chloral hydrate after stopping intragastric administration for 1 day, and then blood was collected with asepsis syringe from the abdominal aorta and was centrifuged to obtain serum for detection.

Detection Items

The weights of rats in each group were measured before experiments and before they were killed. The determinant for climbing pole height was a plastics cylinder (height 80 cm, diameter 80 cm) in which center there was a wooden pole (height 150 cm; diameter 3 cm) with scale, the detection started with rat head was set at the height of 10 cm on the wooden pole and then the climbing pole height of each rat was recorded within 2 minutes.

The activities of choline acetyl transferase (ChAT), cholinesterase (ChE), hexokinase (HK), total superoxide dismutase (T-SOD), and the total antioxidant capacity (T-AOC) in rat serum were determined by detection kits according to the instructions that were provided by Jiancheng Biotechnology Co, Ltd, Nanjing, China. The concentration of blood urea nitrogen (BUN) was determined by BUN detection kit, which was provided by Boding Biological Engineering Co, Ltd, Beijing, China. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), isocitric dehydrogenase (ICDH), and hydroxybutyrate dehydrogenase (HBDH) in rat serum were determined by detection kits according to the instructions that were provided by Changzheng Biochemical Reagent Co, Ltd, Shanghai, China.

Ethics

The experiments were carried out in compliance with the relevant national laws relating to the conduct of animal experimentation. Certificate number of breeder is 3030010.

Statistics

Rat weight, climbing pole height, BUN concentration, the activities of ChAT, ChE, ALT, AST, CK, HK, ICDH, HBDH, T-SOD, and the T-AOC in rat serum were evaluated using the 1-way analysis of variance followed by Tukey HSD Post Hoc Test for repeated measures. All data analyses were performed using the SPSS 14.0 statistical package (SPSS Inc). P values <.05 were considered significant.

Results

Rat Weight Detection

Figure 1 shows the results of rat weight detection. Compared with the weight of rat before experiment, it increased in all 3 groups after experiment. The increase of rat weight in the hypergravity irradiation group and the intervention group was less than that of the control group, but there was no significant
Although the difference was not significant, the increase of rat weight in the intervention group was more than that of the hypergravity irradiation group ($ P > .05 $).

**Climbing Pole Height Detection**

Figure 2 shows the climbing pole height of rat. Compared with that of the control group, the climbing pole height of rats decreased significantly in the hypergravity irradiation group ($ P < .05 $). The climbing pole height of rats also decreased in the intervention group as compared with that of the control group, but it was not significant ($ P > .05 $). However, the height of the intervention group increased significantly as compared with that of the hypergravity irradiation group ($ P < .05 $).

**Discussion**

The phenomena of hypergravity and microwave radiation are increasing in daily life, and the microwave thermotherapy has been gradually used in radiotherapy. There are 2 types of theory about the biological mechanism of microwave radiation effect—the thermal effect and the nonthermal effect, and the latter one is a specific effect of microwave radiation by which the biological changes caused is not clear. It is demonstrated that the permeability of blood–brain barrier to sucrose is increased significantly by microwave radiation, but it has nothing to do with the thermal effect. It is reported that microwave radiation increases bone demineralization rate independently of temperature. At present, the damages caused by microwave radiation or hypergravity to organisms have been reported, respectively, but there are very few reports about the synergistic effect of microwave radiation and hypergravity, especially on rat motions, serum enzyme activities, and the T-AOC, and there is no report on the intervention effect of *Rana sylvatica* Le conte oil as a radioprotector.

The results of rat weight detection showed that there was no significant difference in the increase of rat weight among the 3 groups during experiments, but there was a suppressing tendency in weight increase of the hypergravity irradiation group as compared with that of the control group ($ P < .05 $). The activity of rat serum ChAT/ChE/ALT/AST/CK/ICDH/HBDH/HK/BUN/T-SOD/T-AOC decreased significantly in the hypergravity irradiation group as compared with that of the control group ($ P < .05 $). However, compared to that of the hypergravity irradiation group, the activity of rat serum ChAT/ChE/ALT/AST/CK/ICDH/HBDH/HK/BUN/T-SOD/T-AOC of the intervention group increased, significantly ($ P < .05 $).
Figure 3. Serum ALT/AST/BUN/ChAT/ChE/CK/ICDH/HBDH/HK/T-SOD/T-AOC detection. The serum ChAT/ChE/ALT/AST/CK/ICDH/
HBDH/HK/BUN/T-SOD/T-AOC activities of rats in all study groups, the control and the groups irradiated with microwave of 200 mW/cm²
power density under +6G hypergravity in the absence or presence of Rana sylvatica Le conte oil intervention. The activity of rat serum
cytokine decreased significantly in the hypergravity irradiation group as compared with that of the control group (P < .05). The activity of
rat serum cytokine increased significantly in the intervention group compared to that of the hypergravity + irradiation (P < 0.05).
*P < .05 versus control, #P < .05 versus hypergravity + irradiation. Values are mean activities of serum ChAT/ChE/ALT/
AST/CK/ICDH/HBDH/HK/BUN/T-SOD/T-AOC from 12 rats. Error bars indicate standard deviation of mean values. ALT indicates
alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; ChAT, choline acetyl transferase; ChE, choline-
terase; CK, creatine kinase; ICDH, isocitric dehydrogenase; HBDH, hydroxybutyrate dehydrogenase; HK, hexokinase; T-AOC, total
antioxidant capacity; T-SOD, total superoxide dismutase.
The nervous system is sensitive to reflect the damage caused by microwave radiation and hypergravity, and the microwave radiation, such as the mobile communication, mainly influences the simple reaction and short-term memory of brains.\(^1\) Acetylcholine (Ach) is a primary excitatory neurotransmitter of the central nervous system and automatic nervous system, and it is reported that Ach in brain hippocampus decreases 40% by microwave radiation.\(^17\) Choline acetyl transferase synthesized in nerve cells is an important enzyme to catalyze the synthesis of Ach which is degraded in synaptic cleft by ChE synthesized in liver. The results showed that the activities of serum ChAT and ChE decreased significantly in the hypergravity irradiation group as compared with those of the control group and the intervention group, so it demonstrated that the nervous system was affected critically by the synergistic effect of microwave radiation and hypergravity through inhibiting the activities of ChAT and ChE, while the damage could be protected by Rana sylvatica Le conte oil. It is reported that ChAT activity decreases in the brain hippocampus of patients with Alzheimer disease,\(^18\) so it might provide an inspiration for the treatment of patients having Alzheimer disease with Rana sylvatica Le conte oil for its protective effect to the ChAT activity.

Alanine aminotransferase that is widely distributed in tissues or organs mainly exists in the cytoplasm of liver cell and is one of the bone formation marks, so it is an indicator for bone and liver cell damage. Aspartate aminotransferase is an important transaminase that is distributed in liver, heart, skeletal muscle, and kidney, and the activity of AST is the highest in heart muscles, but it is also an indicator for cellular necrosis of liver. The experiment showed that the activities of ALT and AST in rat serum increased significantly in the hypergravity irradiation group as compared with those of the control group and the intervention group, so it proved that the synergistic effect of microwave radiation and hypergravity caused damage to liver and heart of rats, so the intracellular enzymes were released into blood, while the Rana sylvatica Le conte oil had protective effects to rats that were exposed to hypergravity and microwave radiation.

Creatine kinase is distributed in skeletal muscles, cardiac muscles, and brain tissues, while the majority of it is in muscle cells, so the enhancement of CK activity in rat serum might indicate the damage of muscle cells. When the physiological function of brain tissues is destroyed, CK also can be released into blood through the damaged blood–brain barrier.\(^20\) The results showed that the activity of rat serum CK increased significantly in the hypergravity irradiation group as compared with those of the control group and the intervention group, so it indicated that the synergistic effect of microwave radiation and hypergravity might cause damage to muscles and brains of rats, while the injury could be protected by Rana sylvatica Le conte oil.

Isocitric dehydrogenase, HBDH, and HK are major enzymes involved in the metabolism of carbohydrate, lipid, and energy. The activity of serum ICDH that is most abundant in rat liver is a sensitive indicator reflecting the severity of damage in liver cells. Hydroxybutyrate dehydrogenase is an important enzyme in the oxidation process of ketone bodies and is distributed in brain tissues, cardiac muscles, and kidneys, whereas the activity of HBDH in liver is very low, so the increase of serum HBDH activity indicates the damage of the brain, heart, and kidney. The experiment showed that the activities of serum ICDH and HBDH increased significantly in the hypergravity irradiation group as compared with those of the control group and the intervention group, so it proved that the synergistic effect of microwave radiation and hypergravity might cause serious damage to the liver, brain, heart, and kidney, for a large number of these serum nonfunctional enzymes were released into blood only when tissues and cells were damaged, while the injury could be reduced by intervention with Rana sylvatica Le conte oil. The synergistic effect of microwave radiation and hypergravity had no significant impact on the activity of serum HK that was a key enzyme in carbohydrate oxidation and decomposition, so the mechanism needs further study.

Urea nitrogen is a major end product of protein metabolism and is excreted by kidney, so the BUN concentration is one of the main indicators to measure renal function. The results showed that the BUN concentration of rat increased significantly in the hypergravity irradiation group as compared with those of the control group and the intervention group, so it provided further evidences that the renal function was damaged to a certain degree by the synergistic effect of microwave radiation and hypergravity.

Total superoxide dismutase and T-AOC are important indicators to measure the antioxidant effect, and the T-SOD reflects the ability of body to scavenge oxygen-free radicals, whereas the T-AOC reflects the total antioxidant capacities of enzymatic and nonenzymatic system. The results showed that there was no significant difference in serum T-SOD activities within the 3 groups, so the ability to scavenge oxygen-free radicals was not affected by the synergistic effect of microwave radiation and hypergravity. The results showed that the T-AOC of rat serum decreased significantly in the hypergravity irradiation group as compared with those of the control group and the intervention group, so it proved that the antioxidant effect was damaged by the synergistic effect of microwave radiation and hypergravity, while the injury could be protected by intervention with Rana sylvatica Le conte oil.

**Conclusion**

In summary, the motion and nervous system of rat might be affected critically by the synergistic effect of microwave radiation and hypergravity, for the climbing pole height and the activities of serum ChAT and ChE decrease significantly. Furthermore, the synergistic effect of microwave radiation and hypergravity causes damage to most rat organs, such as the bone, skeletal muscle, liver, heart, and kidney, so the activities of ALT, AST, CK, ICDH, and HBDH and the concentration of BUN increase apparently, and the antioxidant effect might also be damaged, for the T-AOC of rat serum is decreased significantly by it, while the injury resulted from it could be protected by intervention with Rana sylvatica Le conte oil, but it needs more in-depth study to recognize the mechanism.
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