

The effect of microwave radiation on osteogenesis and osteolysis of rats in the hypergravity condition and the role of *Rana sylvatica* Le conte oil as a radioprotector

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Background: Microwave Thermotherapy has been gradually used in radiotherapy and it is reported that microwave radiation increases bone demineralization rate independently of temperature. Furthermore, it becomes more and more widespread that the damage caused by the synergistic effect of microwave radiation and hypergravity. The experiment was designed to investigate the effect of microwave radiation on osteogenesis and osteolysis of rats in the hypergravity condition and to observe the role of *Rana sylvatica* Le conte oil as a radioprotector. **Materials and Methods:** Wistar rats were exposed to microwave radiation of 200mW/cm² power density and +6G hypergravity, and the concentrations of osteocalcin, calcitonin, calcium, phosphorus, and hydroxyproline and the activity of alkaline phosphatase were detected in serum. **Results:** The serum concentrations of osteocalcin and calcitonin and the activity of alkaline phosphatase decreased, while the calcium, phosphorus and hydroxyproline concentrations increased after the synergistic effect of microwave radiation and hypergravity. *Rana sylvatica* Le conte oil intervention inhibited significantly the decrease of osteocalcin concentration and restrained significantly the increases of hydroxyproline and calcium concentrations in serum. **Conclusion:** These results imply that the synergistic effect of microwave radiation and hypergravity inhibits osteogenesis and enhances osteolysis in rats causing bone metabolic disturbance, while the injury resulted from microwave radiation and hypergravity could be protected by intervention with *Rana sylvatica* Le conte oil and it provides an original direction in the investigation of radioprotectors. **Iran. J. Radiat. Res., 2011; 9(1): 1-8**

Keywords: Microwave radiation, hypergravity, osteogenesis, osteolysis, *rana sylvatica* Le conte oil.

INTRODUCTION

Microwave Thermotherapy (TRMT) has been gradually used in radiotherapy, because the combination of thermotherapy and radiotherapy has a good complementary anti-tumor effect, especially to the cells in the central part of tumor which is insensitive to radiotherapy. There is no ionization when microwave effects on organisms, while there are phenomena of reflexion, scattering, absorption and interference which belong to electromagnetic waves. 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. The necessity to develop reliable animal models to protect human health has led to a variety of animals studies such as motion sickness syndrome (1,2), which mimics a related disorder arising in astronauts during space missions (3-4). The damage of microwave radiation and the harm of hypergravity to organisms have been confirmed respectively (5-7), and it has attracted more and more concerns due to

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the catholicity and complexity of the injury. With the development of aerospace medicine, the current research focus has shifted to the two-factor synergistic effect on body ^(8,9). At present, there are very few reports about the synergistic effect of microwave radiation and hypergravity on serum osteocalcin and calcitonin, and there is no report on the role of *Rana sylvatica* Le conte oil as a radioprotector. *Rana sylvatica* Le conte oil is the egg oil of *Rana sylvatica* Le conte which habitats in the forest swamp of Northeast China and it is the dried oviduct of female *Rana sylvatica* Le conte, also known as *Oviductus Rannae*. Many studies have proved that *Rana sylvatica* Le conte oil has the anti-aging and antioxidant effect, and it is useful to increase immunity ⁽¹⁰⁾. Osteocalcin is the most abundant non-collagen protein in mature bone tissue secreted by osteoblasts. Usually, most osteocalcin combined with hydroxyapatite crystal deposits in the bone matrix, but only a little of it is released into the blood circulation, so the serum osteocalcin level is an important and sensitive index to reflect the bone metabolism and the osteoblast activity. Calcitonin is a peptide hormone secreted by the thyroid parafollicular cell whose main role is to promote the deposition of calcium and phosphorus in bone tissue, thereby promoting osteogenesis and decreasing the concentrations of calcium and phosphorus in serum, and thus it is an important peptide hormone to regulate calcium and phosphorus metabolisms and to adjust bone metabolism. The animal experiment was designed to investigate the effect of microwave radiation on the serum concentrations of osteocalcin, calcitonin, hydroxyproline (Hpro), calcium, phosphorus and the alkaline phosphatase (ALP) activity of rats in the hypergravity condition. Furthermore the protective role of *Rana sylvatica* Le conte oil was observed. This experiment might provide references for the investigation of original radioprotectors to the common microwave radiation contact persons and to the special population with

injury resulted from radiation and hypergravity.

MATERIALS AND METHODS

Animal groups

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. Adult Wistar rats (n=36, Grade II, initial weight 200±20g, 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 twelve 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 in the hypergravity condition). Azalein solution and picric acid solution were used respectively to make identification numbers of animals in each group.

Animal model ⁽¹¹⁾

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 (centrifugation of rats with 1.5m rotating arm radius, 27.5 kg single-ended static load, 0.5-40G normal acceleration, more than 3G tangential acceleration, and 0.1G accuracy). Microwave was emitted by the microwave transmitter with frequency 2625 ± 15 MHz, wavelength 11.26-11.49 cm, and average power 2.4 kW. Rats were fed for 5 days before experiments. The rats of the hypergravity irradiation group and the intervention group were adapted to the +6G hypergravity condition for 5 minutes, and then they were exposed

to the microwave radiation of 200 mW/cm² power density and the +6G hypergravity for 5 minutes, while the control group rats were fixed on the animal centrifuge which was turned off for 10 minutes.

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 ultra pure 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, while 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 one day and were fixed on the experimental operation platform. The abdominal cavity of rat was opened with surgical scissors, and the abdominal aorta was isolated with glass minute hands, and then blood was collected with asepsis syringe and was centrifuged to obtain serum for detection.

Detection items

Osteocalcin and calcitonin concentrations in rat serum were determined by the ELISA kits (Jiancheng Biotechnology Co., Ltd. of Nanjing, China) and the BIORAD-680 microplate reader following the manufacturer's instructions. Hpro detection kit, ALP detection kit, calcium detection kit and inorganic phosphorus detection kit were bought from Jiancheng Biotechnology Co., Ltd. of Nanjing, China. Serum Hpro concentration, ALP activity, calcium concentration, and phosphorus concentration were measured according to the instructions of detection kits by new century T6 UV-visible spectrophotometer (Purkinje General Instrument Co., Ltd. of Peking, China). The methyl thymol blue chromatometry was used in the detection of calcium concentration and the malachite green coloration method was used in the assay of

inorganic phosphorus concentration.

Statistical analysis

All data analyses were performed using the SPSS 14.0 statistical package (SPSS Inc.). Osteocalcin, calcitonin, Hpro, calcium and phosphorus concentrations and ALP activity in rat serum were evaluated using the One-way ANOVA followed by Tukey HSD Post Hoc Test for repeated measures. P values <0.05 were considered as significant.

RESULTS

Serum osteocalcin detection

Figure 1 shows the results of serum osteocalcin detection. Compared with that in the control group, the concentration of rat serum osteocalcin decreased significantly in the hypergravity irradiation group ($P < 0.05$). The concentration of rat serum osteocalcin in the intervention group appeared to decline, but there was no significant decrease as compared with that of the control group ($P > 0.05$). Compared with that of the hypergravity irradiation group, the concentration of rat serum osteocalcin in the intervention group increased significantly ($P < 0.05$).

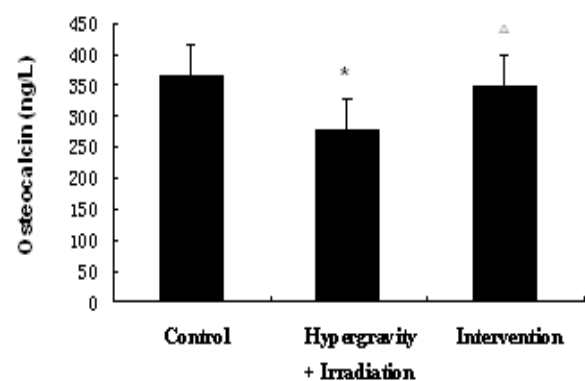


Figure 1. The serum osteocalcin concentration of rats in all study groups, controls, and irradiated with microwave of 200mW/cm² power density under +6G hypergravity in the absence or presence of *Rana sylvatica* Le conte oil intervention. * $P < 0.05$ vs Control, ^Δ $P < 0.05$ vs Hypergravity + Irradiation. Values are mean concentration of serum osteocalcin from 12 rats. Error bars indicate standard deviation of mean values.

Serum ALP detection

Figure 2 shows the results of serum ALP detection. Compared with that of the control group, the ALP activities of rats decreased significantly in the hypergravity irradiation group and the intervention group ($P < 0.05$). There was no significant difference between the hypergravity irradiation group and the intervention group ($P > 0.05$).

Serum calcitonin detection

Figure 3 shows the results of serum calcitonin detection. Compared with that of the control group, the concentration of rat serum calcitonin decreased significantly in the hypergravity irradiation group ($P < 0.05$). Compared with the serum calcitonin concentration of the control group, there was a decreasing tendency in the intervention group, but it was not significant ($P > 0.05$). Although the difference was not significant, the serum calcitonin concentration of the intervention groups was higher than that of the hypergravity irradiation group ($P > 0.05$).

Serum calcium detection

Figure 4 shows the results of serum calcium detection. Compared with that of the control group, the serum calcium concentrations of rats increased significantly in the hypergravity irradiation group and the intervention group ($P < 0.05$). Compared with that of the hypergravity irradiation group, the concentration of rat serum calcium in the intervention group already decreased significantly ($P < 0.05$).

Serum phosphorus detection

Figure 5 shows the results of serum phosphorus detection. Compared with that of the control group, the serum phosphorus concentrations of rats increased significantly in the hypergravity irradiation group and the intervention group ($P < 0.05$). Compared with the serum phosphorus concentration of the hypergravity irradiation group, there was a decreasing tendency in the intervention group, but it was not significant ($P > 0.05$).

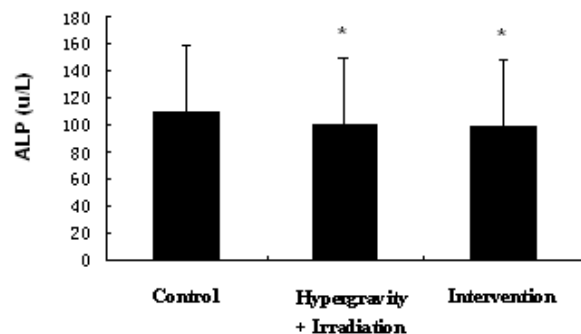


Figure 2. The serum ALP (alkaline phosphatase) activity of rats in all study groups, controls, and irradiated with microwave of 200mW/cm² power density under +6G hypergravity in the absence or presence of *Rana sylvatica* Le conte oil intervention. * $P < 0.05$ vs Control, $\Delta P < 0.05$ vs Hypergravity + Irradiation. Values are mean activity of serum ALP from 12 rats. Error bars indicate standard deviation of mean values.

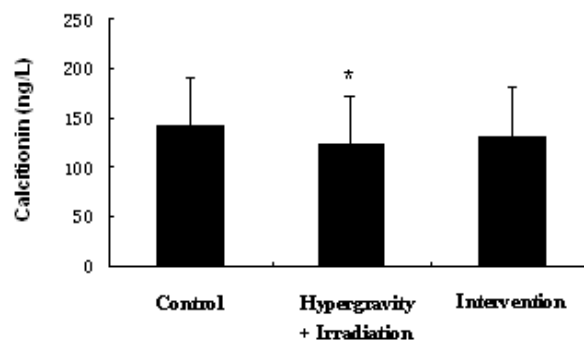


Figure 3. The serum calcitonin concentration of rats in all study groups, controls, and irradiated with microwave of 200mW/cm² power density under +6G hypergravity in the absence or presence of *Rana sylvatica* Le conte oil intervention. * $P < 0.05$ vs Control, $\Delta P < 0.05$ vs Hypergravity + Irradiation. Values are mean concentration of serum calcitonin from 12 rats. Error bars indicate standard deviation of mean values.

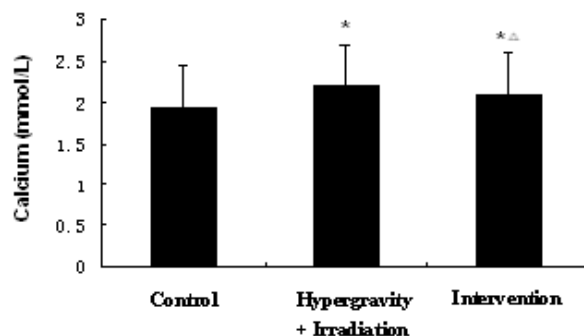


Figure 4. The serum calcium concentration of rats in all study groups, controls, and irradiated with microwave of 200mW/cm² power density under +6G hypergravity in the absence or presence of *Rana sylvatica* Le conte oil intervention. * $P < 0.05$ vs Control, $\Delta P < 0.05$ vs Hypergravity + Irradiation. Values are mean concentration of serum calcium from 12 rats. Error bars indicate standard deviation of mean values.

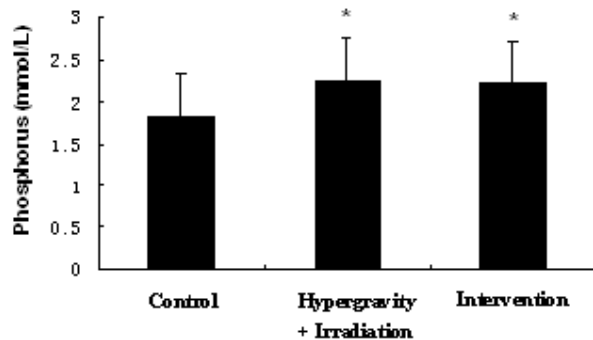


Figure 5. The serum phosphorus concentration of rats in all study groups, controls, and irradiated with microwave of 200mW/cm² power density under +6G hypergravity in the absence or presence of *Rana sylvatica* Le conte oil intervention. *P < 0.05 vs Control, ^P < 0.05 vs Hypergravity + Irradiation. Values are mean concentration of serum phosphorus from 12 rats. Error bars indicate standard deviation of mean values.

Serum Hpro detection

Figure 6 shows the results of serum Hpro detection. Compared with that of the control group, the serum Hpro concentration of rats increased significantly in the hypergravity irradiation group (P < 0.05). There was no difference in serum Hpro concentrations between the control group and the intervention group (P > 0.05). The serum Hpro concentration of the intervention group decreased significantly as compared with that of the hypergravity irradiation group (P < 0.05).

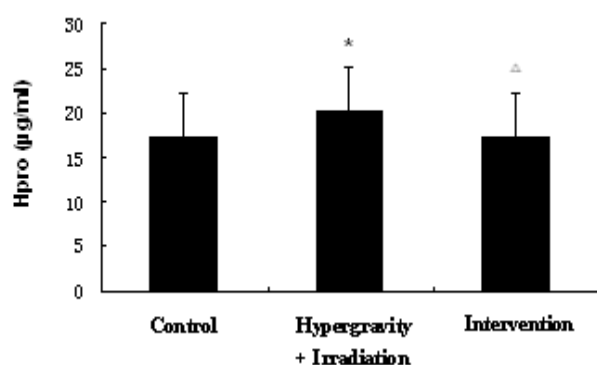


Figure 6. The serum Hpro (hydroxyproline) concentration of rats in all study groups, controls, and irradiated with microwave of 200mW/cm² power density under +6G hypergravity in the absence or presence of *Rana sylvatica* Le conte oil intervention. *P < 0.05 vs Control, ^P < 0.05 vs Hypergravity + Irradiation. Values are mean concentration of serum Hpro from 12 rats. Error bars indicate standard deviation of mean values.

DISCUSSION

It becomes more and more widespread that the damage caused by microwave radiation and hypergravity with the development of aviation and spaceflight, and the phenomena of microwave radiation and hypergravity are increasing in daily life (12,13), furthermore, the Microwave Thermotherapy has been gradually used in radiotherapy. The thermal effect of microwave radiation may cause death, disability, or injury to organisms upon the diversity of radiant intensity, time and space, so it is urgent to investigate effective measures of prevention and cure to microwave radiation. It is reported that microwave radiation increases bone demineralization rate independently of temperature (14). Non-thermal effects of microwave radiation on organisms can not be ignored, such as the teenagers who use mobile phones for long may suffer from fatigue, lethargy, tinnitus, memory decrease, decreased vision, and other phenomena due to the long-term and low-intensity microwave radiation (15), so it is essential to investigate more effective protection methods and radioprotector.

Osteocalcin is a vitamin K-dependent calcium-binding protein which is composed of 43 amino acid residues, and is the main component of noncollagen proteins in bone. The main role of osteocalcin is to regulate and maintain bone calcium. It is synthesized and secreted by the osteoblasts and the three amino acid residues of gamma-carboxyglutamic acid (Gla) inside its molecule promote its binding to hydroxyapatite and subsequent accumulating in bone matrix in the presence of calcium. About 2/3 of osteocalcin combines with hydroxyapatite crystal to accumulate in bone matrix, while only 1/3 of it is secreted into blood. The detection of serum osteocalcin concentration provides not only an important noninvasive specific marker reflecting the status of osteogenesis and osteolysis but also a

sensitive index which reflects the osteoblast activity. The results showed that the serum osteocalcin concentration of rats decreased significantly after the synergistic effect of hypergravity and microwave radiation, so it indicates that hypergravity and microwave radiation result in the inhibition of osteoblasts activity and cause damage to the whole process of osteogenesis. The *Rana sylvatica* Le conte oil greatly lessened the damage caused by hypergravity and microwave radiation for the serum osteocalcin concentration increased significantly after the intervention of *Rana sylvatica* Le conte oil as compared with that of the hypergravity irradiation group. So this study provides some references for the clinical orthopedics in the in-depth investigation of prevention and cure to the injuries resulted from microwave radiation and hypergravity. The osteolysis progressively exceeds the osteogenesis with age and thus body will be prone to suffer from osteoporosis. It might provide an inspiration for the aging and anti-aging research that microwave radiation and hypergravity have depressive effect on the osteogenesis which has been confirmed in the experiment.

Serum ALP activity is an important index to evaluate the function of osteoblast. ALP hydrolyzes a variety of phosphate esters to increase the concentration of inorganic phosphorus which provides materials for the deposition of bone salt, so it promotes osteogenesis effectively. The results showed that the serum ALP activities of rats decreased significantly in the hypergravity irradiation group and the intervention group as compared with that of the control group. It indicated that the synergistic effect of microwave radiation and hypergravity decreased serum ALP activity, thereby reducing materials of bone calcification, inhibiting osteogenesis and leading to bone metabolic disturbance.

Calcitonin secreted by the thyroid parafollicular cell is a single chain polypeptide hormone composed of 32 amino acid

residues. It functions mainly to promote the deposition of calcium salts by promoting osteoblast formation and to suppress the dissolve of bone salts by inhibiting osteoclast formation. Furthermore, calcitonin inhibits the absorption and increases the excretion of calcium and phosphorus, as a result, reducing the concentrations of serum calcium and phosphorus. Serum calcitonin level determines the concentrations of serum calcium and phosphorus, so it is an important index reflecting bone metabolism. The experiment showed that the concentration of rat serum calcitonin decreased significantly in the hypergravity irradiation group as compared with that of the control group, while the serum calcium and phosphorus concentrations of rats increased significantly in the hypergravity irradiation group and the intervention group as compared with that of the control group. It proved that the damage of bone metabolism was obvious and serious resulted from the synergistic effect of hypergravity and microwave radiation. There was an increasing tendency in the serum calcitonin concentration of the intervention group as compared with that of the hypergravity irradiation group although it was not significant, and furthermore there was already no significant difference between the intervention group and the control group. The serum phosphorus concentration of the intervention group trended to decrease, while the serum calcium concentration of the intervention group reduced significantly as compared with that of the hypergravity irradiation group. It is reported that *Rana sylvatica* Le conte oil has a good antiradiation effect [16]. It was demonstrated in this experiment that *Rana sylvatica* Le conte oil had protective effect on calcitonin and had influence on serum calcium and phosphorus concentrations in rats which were exposed to hypergravity and microwave radiation although it was finite.

The serum Hpro is an important index to assess the degree of osteolysis. The result

showed that the enhancement of serum Hpro concentration was significant as a result of the synergistic effect of hypergravity and microwave radiation, so it is confirmed that the synergistic effect of these two factors inhibits osteogenesis and enhances osteolysis not only by decreasing the serum osteocalcin concentration but also by increasing the serum Hpro concentration. The serum Hpro concentration of the intervention group decreased significantly as compared with that of the hypergravity irradiation group and there was no difference between the intervention group and the control group, so the protective effect of intervention with *Rana sylvatica* Le conte oil provides further evidences that it has antioxidant, antiradiation, and anti-aging biological effect, and this study might provide some cognitions for the investigation and development of *Rana sylvatica* Le conte oil as a radioprotector.

CONCLUSION

In summary, the serum concentrations of osteocalcin and calcitonin and the activity of ALP decreased, while the calcium, phosphorus and Hpro concentrations increased after the synergistic effect of microwave radiation and hypergravity. These results imply that the synergistic effect of microwave radiation and hypergravity inhibits osteogenesis and enhances osteolysis in rats causing bone metabolic disturbance. It provides cognitions and references not only for the study of radioprotection in the clinical Microwave Thermotherapy combined with radiotherapy, especially to bone tumors, but also for the investigation of etiological factor and the prevention and cure measures to common diseases such as osteoporosis. The intervention of *Rana sylvatica* Le conte oil inhibited significantly the decrease of osteocalcin concentration and restrained significantly the increases of Hpro and calcium concentrations in serum. This finding reveals that the injury resulted from microwave

radiation and hypergravity could be protected by intervention with *Rana sylvatica* Le conte oil and it provides an original direction in the investigation of radioprotectors.

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