Effect of Simulated Geomagnetic Activity on Myocardial Ischemia/Reperfusion Injury in Rats

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Abstract
Objective: To study the response of myocardial ischemia/reperfusion injury (MI/RI) in rats to simulated geomagnetic activity.

Methods: In a simulated strong geomagnetic outbreak, the MI/RI rat models were radiated, and their area of myocardial infarction, hemodynamic parameters, creatine kinase (CK), lactate dehydrogenase (LDH), melatonin, and troponin I values were measured after a 24-hour intervention.

Results: Our analysis indicates that the concentrations of troponin I in the geomagnetic shielding+operation group were lower than in the radiation+operation group (P<0.05), the concentrations of melatonin in the shielding+operation group and normal+operation group were higher than in the radiation+operation group (P<0.01), and the concentrations of CK in the shielding+operation group were lower than in the radiation+operation group and normal+operation group (P<0.05). Left ventricular developed pressure (LVDP) and ±dP/dtmax in the radiation+operation group were lower than in the shielding+operation group and normal+operation group (P<0.01). Left ventricular end-diastolic pressure (LEVDP) in the shielding+operation group was higher than in the normal+operation group (P<0.05). There was no significant difference in area of myocardial infarction and LDH between the shielding+operation group and the radiation+operation group.

Conclusion: Our data suggest that geomagnetic activity is important in regulating myocardial reperfusion injury. The geomagnetic shielding has a protective effect on myocardial injury, and the geomagnetic radiation is a risk factor for aggravating the cardiovascular and cerebrovascular diseases.

Keywords: Hemodynamics. Myocardial Reperfusion Injury. Creatine Kinase. Troponin I. L-Lactate Dehydrogenase. Disease Outbreaks. Animal. Rats.

INTRODUCTION

The Earth’s magnetic field is a protective barrier to life. The growth, development, and migration of living things require this magnetic field. Human activities are inextricably linked to the Earth’s magnetic field as well. At present, about 3.5 million people die of cardiovascular and cerebrovascular diseases each year in China, accounting for over 40% of the total number of deaths due to various causes[1]. Studies have shown that the occurrence and development of cardiovascular and cerebrovascular diseases are closely related to the geomagnetic field, but the specific mechanism of influence is not yet clear[2,3]. The traditional research methods can’t explain the mechanism of geomagnetic activity on cardiovascular and cerebrovascular diseases. Therefore, this project intends to simulate geomagnetic activity and study the response of myocardial ischemia/
reperfusion injury (MI/RI) in rats to this geomagnetic activity. It will provide a new way to study the relationship between geomagnetic activities and cardiovascular diseases. And the results of this study can provide a new idea to treat the patients with MI/RI, when there is a geomagnetic outbreak.

METHODS

Animal and Ethical Statement

The present study used eight to ten-week-old male Sprague-Dawley rats (Charles River), weighing 200±20 g, which were obtained from the Experimental Animal Center of the Kunming Medical University (Kunming, Yunnan, China) and were housed in the Laboratory Animal Center of the Kunming Medical University. Animal experimental protocols were approved and performed according to the guidelines of the Institutional Medical Experimental Animal Care Committee of the Kunming Medical University. Guidelines for Laboratory Animal Care and Safety from the United States National Institutes of Health (Bethesda, Maryland, USA) were also followed.

Reagents

Triphenyl tetrazolium chloride (TTC) was obtained from the Sigma-Aldrich Corporation (Missouri, USA). Kits for detecting lactate dehydrogenase (LDH), creatine kinase (CK), and troponin I (cTnI) were purchased from AU Clinical Chemistry Systems of the Beckman Coulter Inc. company (California, USA). The kit for detecting melatonin was purchased from the Abcam Inc. company (ab213978, Abcam, Cambridge, Massachusetts, USA).

Equipment

The geomagnetic experimental platform consists of four partial compositions, including a metal shielding experiment cage, radiation antenna, programmable signal generator, and control computer. The signal generator controlled by the main control computer can produce below 50 Hz and an arbitrary combination of signal spectra, especially analog Schumann resonances and geomagnetic burst, in the space of electromagnetic radiation (Figure 1). Patent No.: ZL201520208744.2.

Animal Grouping and Treatment

The male rats were randomly divided into the geomagnetic shielding place, geomagnetic radiation place, and normal place (rats living in general environment without intervention). The rats in each place were divided into sham operation group and operation group, being nine rats in each group. The shield+sham group, radiation+sham group, normal+sham group, and normal+operation group were the control group. The shield+operation group and radiation+operation group were the experimental group.

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| Geomagnetic shielding place | Geomagnetic radiation place | Normal place |
|----------------------------|-----------------------------|--------------|
| Shield+sham group          | Radiation+sham group        | Normal+sham group |
| Shield+operation group     | Radiation+operation group   | Normal+operation group |
in different places for 24 hours of reperfusion, and then their physiological and biochemical indicators were tested.

**Myocardial Infarct Sizes**

Following reperfusion, the heart was then excised, both atria and the right ventricle were removed, and the left ventricle was cut into five equal slices to create cross sections from apex to base. The slices were separated into normal zone and area at risk, both followed by incubation in 1% TTC to measure the viability of myocardial tissue. Viable tissue stained red, while nonviable tissue remained unstained or was white (Figure 2A). Infarct size as a percentage of area at risk was determined gravimetrically.

**Left Ventricular Pressure Test**

Cardiac function was measured by left ventricular cannulation. Rats were fasted for 12 hours and anesthetized with 3.6% chloral hydrate (1 ml/kg). The left common carotid artery was isolated, then the left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), and ± dP/dtmax were recorded by electrophysilograph (BIOPAC 150).

**Serum Biochemistry Test**

The blood was collected from the abdominal aorta artery catheter and added to procoagulant tube to centrifuge for 3000 min at 4°C. The serum was stored in an ultra-low temperature refrigerator at -20°C. The automatic blood biochemical analyzer (Beckman AU480, USA) detect cTnI, CK, and LDH in the blood with specific reagents from AU Clinical Chemistry Systems.

**Melatonin Test**

The serum level of melatonin was detected by using the enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s instructions. The results were determined spectrophotometrically at 450 nm.

**Statistical Analysis**

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 19 (SPSS Inc., Chicago, Illinois, USA). The data were presented as MEDIAN ± INTERQUARTILE RANGE and were compared using Kruskal-Wallis test. P<0.05 was considered statistically significant.

**RESULTS**

**Myocardial Infarction Area**

The results showed that there was no significant difference in the myocardial infarction area between the shielding + operation group, radiation + operation group, and normal + operation group (P>0.05), but there was significant difference in infarct size between the sham group and operation group (P<0.05) (Figure 2B).

**Left Ventricular Pressure**

The results showed that LVDP in the shield + operation group and normal + operation group were higher than in the radiation + operation group (P<0.05) (Figure 2C). There was no significant difference in LEVDP in the shielding + operation group and radiation + operation group (P>0.05), but in the shielding + operation group it was higher than in the normal + operation group (P<0.05) (Figure 2D). The ± dP/dtmax of the left ventricle in the shielding + operation group and normal + operation group were higher than that in the radiation + operation group (P<0.05) (Figure 2E).

**Troponin I**

The results showed that cTnI in the radiation + operation group was higher than in the shielding + operation group, and in the operation group it was higher than in the sham group (P<0.05) (Figure 3A).

**Creatine Kinase**

The results showed that CK in the shielding + operation group was lower than in the radiation + operation group and normal + operation group (P<0.05) (Figure 3B).

**Lactate Dehydrogenase**

The results showed that LDH in the shielding + operation group had no significant difference from the radiation + operation group (P>0.05), but LDH in the shielding + operation group and radiation + operation group were lower than in the normal + operation group (P<0.05) (Figure 3C).

**Melatonin**

The concentrations of melatonin in the radiation + operation group were significantly lower than in the shielding + operation group and normal + operation group (P<0.05) (Figure 3D).

**DISCUSSION**

Among the many causes of MI/RI, humans began to notice the potential impact of geomagnetic activity on human health[6,7]. A vast number of large-scale clinical observational experiments show that when there was a geomagnetic outbreak, arrhythmia, hypertension, myocardial infarction, cerebral infarction, stroke, and other cardiovascular and cerebrovascular events increased significantly[3,9-12]. The mechanisms of the geomagnetic field on the cardiovascular function are studies that became very meaningful. Based on the discovered mechanism, we will prevent the geomagnetic effects and reduce the probability of cardiovascular diseases.

MI/RI lead to ventricular systolic and diastolic dysfunction[13,14]. From our experimental results, there was no significant difference on the myocardial infarction area between the shielding + operation group and radiation + operation group, but the LVDP and ± dP/dtmax in the radiation + operation group were lower than in the shielding + operation group and normal + operation group, and the LEVDP in the shielding + operation group was higher than in the normal + operation group, indicating that geomagnetic shielding can help to improve heart function and geomagnetic radiation can increase the damage in the heart function.
Fig. 2 – Heart physiological indexes. A) results of TTC staining of the myocardium of rats; B) The infarct areas of the left ventricular tissue were measured by TTC staining (n=6); C, D, and E) the left ventricular pressures were recorded by electrophysiolograph (n=9).

LEVDP=left ventricular end-diastolic pressure; LVDP=left ventricular developed pressure; TTC=triphenyl tetrazolium chloride
During MI/RI, myocardial oxygen free radicals accumulate, calcium overload occurs, and the myocardium produces a large amount of oxygen free radicals. These oxygen free radicals convert to hydroxyl free radicals, which act on the cell membrane and change the myocardial cell membrane structure and function. The cells release large amounts of LDH and CK, and they are specific indicators of myocardial injury\cite{14,15}. From our experimental results, there are significant differences on CK between the shielding + operation group and radiation + operation group. This shows that geomagnetic activity has an impact on CK. We speculate that geomagnetic activity affects myocardial energy metabolism, muscle contraction, and adenosine triphosphate regeneration, because CK is correlated to those.

The cTnI is considered as the key biochemical marker in the diagnosis of myocardial injury. Patients with myocardial infarction have much higher concentrations of cTnI than healthy people\cite{16}. From the experimental results, cTnI in the radiation + operation group is higher than in the shielding + operation group, indicating that geomagnetic radiation has an increasing effect on cTnI, while geomagnetic shielding has an inhibitory effect on cTnI, which proves that geomagnetic radiation has the potential to aggravate myocardial injury. Geomagnetic shielding has a protective effect on myocardial injury.

Melatonin has a powerful antioxidant effect and a high degree of diffusive penetration ability, which can exert its own antioxidant effect on the cell membrane, cytoplasm, and nucleus to protect the myocardial tissue against oxidative damage. From the experimental results, the concentration of melatonin in the radiation - operation group is lower than in the shielding + operation group and normal + operation group, indicating that geomagnetic radiation can reduce its protective effect on myocardial injury by inhibiting melatonin secretion. At the same time, the geomagnetic shield can promote the secretion of melatonin and enhance the protective effect of melatonin on myocardial injury\cite{12,14,16}.

\textbf{Fig. 3} – Serum biochemical indexes. A, B, and C). The cTnI, CK, and LDH were measured by automatic blood biochemical analyzer (n=3), which are specific indicators of myocardial infarction; D) melatonin was measured by ELISA kit, which protects the myocardial tissue (n=4). CK=creatine kinase; cTnI=troponin I; ELISA=enzyme-linked immunosorbent assay; LDH=lactate dehydrogenase.
CONCLUSION

The geomagnetic activity-related heat exposure is associated with an increase in cardiac events. This report describes the effect of geomagnetic outbreaks on the heart and how these outbreaks can aggravate damages from myocardial infarction. These findings identify the geomagnetic radiation damages to cardiovascular disease, which suggests that the impact of the space weather changing on human health cannot be ignored. In the view of the damage of geomagnetic outbreaks to the myocardium, we can predict the time of the geomagnetic outbreak in advance through early warnings from the astronomical observation, which may help to protect people from these outbreaks’ effects.

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No conflict of interest.

Authors’ roles & responsibilities

HW  Substantial contributions to the conception or design of the work; drafting the work or revising it critically for important intellectual content; final approval of the version to be published

WC  The acquisition, analysis, or interpretation of data for the work; final approval of the version to be published

YD  The acquisition, analysis, or interpretation of data for the work; final approval of the version to be published

XC  The acquisition, analysis, or interpretation of data for the work; final approval of the version to be published

YD  Final approval of the version to be published

XL  Substantial contributions to the conception or design of the work; drafting the work or revising it critically for important intellectual content; final approval of the version to be published

LD  is responsible for the commissioning of geomagnetic experimental platform; final approval of the version to be published

REFERENCES

1. Barquera S, Pedroza-Tobías A, Medina C, Hernández-Barrera L, Bibbins-Domingo K, Lozano R. et al. Global overview of the epidemiology of atherosclerotic cardiovascular disease. Arch Med Res. 2015;46(5):328-38. doi:10.1016/j.arcmed.2015.06.006.

2. Feigin VL, Parmar PG, Barker-Collo S, Bennett DA, Anderson CS, Thrift AG, et al. Geomagnetic storms can trigger stroke evidence from 6 large population-based studies in Europe and Australasia. Stroke. 2014;45(6):1639-45. doi:10.1161/STROKEAHA.113.004577.

3. Vencloviene J, Babarskiene R, Slapikas R, Sakalyte G. The association between phenomena on the sun, geomagnetic activity, meteorological variables, and cardiovascular characteristic of patients with myocardial infarction. Int J Biometeorol. 2013;57(5):797-804. doi:10.1007/s00484-012-0609-8.

4. Yu L, Sun Y, Cheng L, Jin Z, Yang Y, Zhai M, et al. Melatonin receptor-mediated protection against myocardial ischemia/reperfusion injury: role of SIRT1. J Pineal Res. 2014;57(2):228-38. doi:10.1111/jpi.12161.

5. Gao E, Lei YH, Shang X, Huang ZM, Zuo L, Boucher M, et al. A novel and efficient model of coronary artery ligation and myocardial infarction in the mouse. Circ Res. 2010;107(12):1445-53. doi:10.1161/CIRCRESAHA.110.223925.

6. Berg H. (Solar and magnetic disturbances in relation to the medicometeorological prognoses). Arch Phys Ther (Leipzig). 1954;6(3):216-28. German.

7. Friedman H, Becker RO, Bachman CH. Geomagnetic parameters and psychiatric hospital admissions. Nature. 1963;200:626-8. doi:10.1038/200626a0.

8. Mikulecký M, Strestík J. Cerebral infarction versus solar and geomagnetic activity: a cross-regression study. Isr Med Assoc J. 2007;9(12):835-8.

9. Simko F, Reiter RJ, Pechanova O, Paulis L. Experimental models of melatonin-deficient hypertension. Front Biosci (Landmark Ed). 2013;18:616-25. doi:10.2741/4125.

10. Lewczuk B, Redlarski G, Zak A, Zidókowsia N, Przybilska-Gornickib B, Krawczuk M. Influence of electric, magnetic, and electromagnetic fields on the circadian system: current stage of knowledge. Biomed Res Int. 2014;2014:169459. doi:10.1155/2014/169459.

11. Li JH, Yang P, Li AL, Wang Y, Shi ZX, Ke YN, et al. The preventive effect of garlicin on a porcine model of myocardial infarction reperfusion no-reflow. Chin J Integr Med. 2014;20(6):425-9. doi:10.1007/s11655-012-1091-1.

12. Soh S, Jun JH, Song JW, Shin EJ, Kwak YL, Shim JK. Ethyl pyruvate attenuates myocardial ischemia-reperfusion injury exacerbated by hyperglycemia via retained inhibitory effect on HMGB1. Int J Cardiol. 2018;252:156-62. doi:10.1016/j.ijcard.2017.11.038.

13. Bartsch H, Mecke D, Probst H, Küpper H, Seebold E, Salewski L, et al. Search for seasonal rhythmicity of pineal melatonin production in rats under constant laboratory conditions: spectral chronobiological analysis, and relation to solar and geomagnetic variables. Chronobiol Int. 2012;29(8):1048-61. doi:10.1080/07420528.2012.719958.

14. Burch JB, Reif JS, Yost MG. Geomagnetic activity and human melatonin metabolite excretion. Neurosci Lett. 2008;438(1):76-9. doi:10.1016/j.neulet.2008.04.031.

15. Aimó A, Januzzi JL Jr, Vergaro G, Ripoli A, Latini R, Masson S, et al. Prognostic value of high-sensitivity troponin T in chronic heart failure: an individual patient data meta-analysis. Circulation. 2018;137(3):286-97. doi:10.1161/CIRCULATIONAHA.117.031560.

16. Böhmer AE, Souza DG, Hansel G, Brum LM, Portela LV, Souza DO. Long-term cyclopenthiazide treatment in non-transplanted rats and metabolic risk factors of vascular diseases. Chem Biol Interact. 2010;185(1):53-8. doi:10.1016/j.cbi.2010.02.029.