Cerebral vessels reactivity disorders in microgravity and its pharmacological correction with new derivatives of 1,2,4-triazole

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Abstract. Objective: study of vascular reactivity disorders under the action of a pathological model of microgravity and study of the pharmacological effect of 1,2,4-triazole derivatives on the reactivity of the cerebral vessels of various structures of the brain when simulating circulatory disorders under the influence of a pathological model of microgravity. Methods: the anti-orthostatic position was used as a pathological model. A series of experiments was carried out with laboratory animals, which were previously implanted with platinum electrodes in various structures of the brain. Dilator reactivity of vessels to hypercapnia (CrCO2), and constrictor reactivity of blood vessels to hyperoxia (CrO2) were studied by the hydrogen clearance method. Investigated substances were injected into the marginal vein of the rabbit ear in a dose of 25 mg/kg just before the onset of the action of the experimental models. Results: new pharmacologically active substances reduce oppression of constrictor reactivity and dilator reactivity of vessels caused by head down tilting model (HDTM) in all studied structures of the brain. Conclusion: the investigated substances are interested for further study of their effects in correction of cerebral hemodynamic with decrease in gravity.

1. Introduction

The concept that space flight has a negative impact on human physiology, having an adverse effect on every organ or system, which is mainly associated with the effects of microgravity, space radiation, lack of movement, and circadian rhythm disturbance, is increasingly widely recognized [1].

During spaceflight, the human cardiovascular system undergoes major changes primarily associated with the effects of reduced gravitational force, or microgravity, on the human body. Microgravity can disrupt the hydrostatic pressure gradients that normally exist on Earth due to the effects of gravity. Most of the reported neurological symptoms following exposure to microgravity can be caused by changes in cerebral hemodynamics. Head-ward fluid shift is deemed as a primary contributor to altered hemodynamics and disorders of cerebral blood circulation autoregulation [2]. These phenomena might formally represent the prime initiating factors that account for observed
neurologic symptoms including post-flight headache, dizziness, syncope, impaired ability to execute cognitive functions, and the appearance of visual impairment and intracranial pressure (VIIP) syndrome [3, 4, 5, 6]. Also these changes present challenges to human adaptation and work in space. With the booming development of manned spaceflight projects, the resolution of these problems has become an urgent task.

2. Cerebral blood circulation: normal and during spaceflight
The brain is only about 2% of body weight, but it receives 15-20% of the blood flow from the total cardiac output, which makes the brain one of the most highly perfused organs in the body. The high metabolic requirements of the brain, largely dependent on oxidative metabolism, necessitate not only a high fraction of cardiac output but also relatively constant blood flow.

Cerebral autoregulation is a process, which aims to maintain adequate and stable cerebral blood flow. Autoregulation maintains a steady oxygen supply to the brain by adjusting cerebral arteriolar caliber and resistance to minimize the effects of changes of perfusion pressure. Cerebral autoregulation maintains cerebral blood flow at a relatively constant level of 60 to 150 mmHg [7].

Normally, carbon dioxide (CO₂) has a profound and reversible effect on cerebral blood flow, such that hypercapnia causes marked dilation of cerebral arteries and arterioles and increased blood flow, whereas hypocapnia causes constriction and decreased blood flow [8].

In space, the hydrostatic pressure gradients commonly found on Earth disappear. This leads to a disruption in the work of autoregulation of cerebral circulation. Factors including duration, partial pressures of carbon dioxide and individual adaptability contribute to this process and are unpredictable.

Long-term residence on the International Space Station (ISS) has been shown to be associated with a chronic increase in blood pressure in the brain compared to the normal vertical posture on Earth and an increased level of inhaled CO₂. Seven astronauts spent 3-4 months on ISS. Preflight testing (30-60 days before launch) was compared with postflight testing on landing day after return to Earth. Cerebrovascular autoregulation and CO₂ reactivity were estimated in a supine position from an autoregressive moving average (ARMA) model of data obtained during a test where two breaths of 10% CO₂ were given four times during a 5-min period. The results indicate that long duration missions on the ISS impaired dynamic of cerebrovascular autoregulation and reduced cerebrovascular CO₂ reactivity [9].

3. Earth-based models studying the effect of microgravity on the autoregulation of cerebral circulation
Research on humans in space is expensive and complicated. Also only a few participants can be involved in such studies, which leads to reduced study results. Ground-based models have the potential of providing greater understanding of the mechanisms of physiological changes during spaceflight and there correction [10].

Currently, earth-based analogs are used in scientific research to investigate potential cerebral circulation disorders associated perturbations that might occur during space missions. Antiorthostatic position is one of the most commonly used pathologic models [11, 12, 13].

3.1. Clinical trials
In a study by Marshall-Goebel K et al., nine healthy male subjects were measured at baseline (supine, 0°) and after 4.5 h of antiorthostatic position at -6°, -12° (with and without 1% CO₂), and -18°. A decrease in total arterial blood flow from the initial level was found at all angles of the antiorthostatic position. The venous outflow decreased during -12° antiorthostatic position (P = 0.039). Moreover, the
addition of 1% CO\textsubscript{2} to -12° antiorthostatic position caused an increase in total arterial blood flow (P = 0.016) and jugular venous outflow (P < 0.001) compared with -12° antiorthostatic position with ambient atmosphere. Overall, results of this study indicate a decreasing of cerebral blood flow during antiorthostatic position, which may have implications for microgravity-induced changes in cerebral hemodynamics [14].

3.2. Preclinical studies
Antiorthostatic hypokinesia with a tilt 45° angle was originally proposed as a ground-based model of cerebrovascular disorders arising in space. This model has been widely used in preclinical studies in the search for drugs that correct cerebral circulation [15, 16].

With antiorthostatic hypokinesia with a tilt 45° angle in a laboratory animal, the movement of liquid media in the cranial direction initially occurs. This leads to an increase in hydrostatic pressure in the vessels of the brain, and, on the other hand, to venous congestion [17, 18]. As a result, there is a slight swelling of the tissues and a slowdown in blood flow in the brain [13, 19].

One possible effect of this condition is an alteration of the myogenic reactivity of cerebral blood vessels.

4. Effect of new 1,2,4-triazole derivatives on cerebral circulation disorders
A number of scientific experiments were carried out at the Department of Pharmacology of the Medical Academy of the Crimean Federal University. The pharmacological effects of new triazole derivatives on the reactivity of cerebral vessels were studied in the course of these experiments.

4.1. Investigated Substances
In this scientific work the effects of three new 1,2,4-triazole derivatives have been studied: APK-38 (morpholinium 5- (4-nitrophenyl) -1,2,4-triazolyl-3-ythioacetate), YUPK-2 (morpholinium 5- (3-pyridyl) -1,2,4 -triazolyl-3-ythioacetate), thimetrizole (morpholinium 2- (5- (4-pyridyl) -4- (2-methoxyphenyl) -1,2,4-triazol-3-ythio) acetate). The drug Thiotriazoline was used as a reference drug. This drug is also a derivative of 1,2,4-triazole and is registered in some countries. These chemicals and reference drug were synthesized at the Department of Inorganic and Toxicological Chemistry of Zaporozhye State Medical University under the leadership of Professor Y G Knysh.

Doses of the investigated substances were selected based on the average therapeutic doses of thiotriazoline and amounted to 25 mg / kg of body weight. The preference was given to the intravenous route of administration, providing a rapid drug entry into the bloodstream and high bioavailability.

4.2. Methods
The experiments were performed on awake rabbits. Antiorthostatic hypokinesia with a tilt 45° angle was originally proposed as a ground-based model of cerebrovascular disorders arising from a decrease in gravity.

The study of the cerebral vessels reactivity was carried out using needle platinum electrodes implanted into various structures of the rabbit brain (thalamus, hypothalamus, frontal cortex).

The hydrogen clearance method was used in the course of this scientific work [15]. To saturate the tissues with hydrogen, inhalation of a 5% mixture of H\textsubscript{2} with air was used using a mask designed in the laboratory of the department of our university [1]. The ability of vessels to dilate was determined by inhalation of carbon dioxide, the ability to constriction was determined by inhalation of oxygen. The vasodilator (KpCO\textsubscript{2}) and vasoconstrictor (KpO\textsubscript{2}) vascular reactivity in the cerebral cortex, thalamus, and hypothalamus were determined.
Changes in vascular reactivity were expressed as a percentage in relation to the results obtained before pathological exposure. The obtained relative values were subjected to statistical processing using the Student's test. The calculation of absolute and relative data, as well as their statistical processing was carried out on a computer using the Lotus 1-2-3 editor for the OS/2 operating system.

4.3. Results
The antioverse position without the action of drugs caused a significant inhibition of the vasodilator reactivity of blood vessels (KpCO₂) of the brain to inhalation of a mixture of CO₂. This confirms the data obtained in the course of experiments carried out in our scientific laboratory [15]. This phenomenon increased during the entire observation period, and was most pronounced in the cerebral cortex, and reached a decrease of 19% in the cerebral cortex, 15% in the thalamus and hypothalamus after 120 minutes of the experiment (Figure 1).

![Figure 1. Vasodilator reactivity.](image)

In experiments with the use of triazole derivatives, the inhibition of vasodilator reactivity was significantly less pronounced. The action of the investigated substances was most significantly manifested in the cerebral cortex, as evidenced by a decrease in the inhibition of KpCO₂. Thiotriazoline and thiometrizole had the most pronounced pharmacological effects. These chemicals almost equally reduced the inhibition of KpCO₂, approximately 3 times at the end of the experiment. This effect is due to the preservation of the vasodilating effect of the investigated substances.

The decrease in the constrictor coefficient of vascular reactivity (KpO₂) in the antioverse position without the use of drugs was even more significant and also progressive (Figure 2). The most pronounced changes were observed in the hypothalamus, the least in the thalamus. The decrease in vasoconstrictor reactivity in the percentage dimension reached 41% in the cerebral cortex, 31% in the thalamus and 46% in the hypothalamus after 120 minutes of the experiment.
4.4. Discussion

The main findings in our study are that antiorthostatic hypokinesia with an inclination angle of -45° as a pathological model of weightlessness causes inhibition of the cerebral vessels reactions, to a greater extent constrictor reaction.

New derivatives of 1,2,4-triazole prevent the inhibition of vascular reactivity. Thiometrizole has shown the best effect in maintaining vascular reactivity. This chemical compound, equally or better than the reference drug thiotriazoline, prevented the inhibition of vascular reactivity.

5. Conclusions

At present, little is known about the possibility of correcting cerebrovascular accidents in space. However, with interplanetary missions and space tourism in mind, it is important to explore this area of pharmacotherapy. This will ensure the safety and health of the crew, allow the development of new and optimization of existing measures to counter the negative health space factors. Preclinical studies have shown that new derivatives of 1,2,4-triazole, developed by Ukrainian scientists, can neutralize disorders of the cerebral vessels reactivity caused by microgravity. Thiometrizole is potential medicinal products and require further study, because can be useful in reducing the negative impact of space factors on the human health.

6. References

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