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Hypotensive and neurometabolic effects of intragastric Reishi (*Ganoderma lucidum*) administration in hypertensive ISIAH rat strain

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ABSTRACT

Background: As the standard clinically used hypotensive medicines have many undesirable side effects, there is a need for new therapeutic agents, especially ones of a natural origin.
Purpose: One possible candidate is extract from the mushroom Reishi (*Ganoderma lucidum*), which is used in the treatment and prevention of many chronic diseases.
Study design and methods: To study the effectiveness of Reishi, which grows in the Altai Mountains, as an antihypertensive agent, we intragastrically administered Reishi water extract to adult male hypertensive ISIAH (inherited stress-induced arterial hypertension) rats.
Results: After seven weeks, Reishi therapy reduced blood pressure in experimental animals at a level comparable to that of losartan. Unlike losartan, intragastric Reishi introduction significantly increases cerebral blood flow and affects cerebral cortex metabolic patterns, shifting the balance of inhibitory and excitatory neurotransmitters toward excitation.
Conclusion: Changes in cerebral blood flow and ratios of neurometabolites suggests Reishi has a potential nootropic effect.

Introduction

Hypertension is widespread in modern society and is as a risk factor for myocardial infarction, stroke and other cardiovascular disorders. Essential hypertension is among the most important causes of death (Fudim and Vemulapalli, 2016). Moreover, chronic hypertension is accompanied by a set of psychosomatic abnormalities, including insomnia, depression, and a decline in mental capacity (Carnevale et al., 2012; Kayano et al., 2015).

Treatment of hypertension with pharmaceutical agents is complicated by side effects such as dizziness, nasal congestion, coughing and nausea. Thus, there is a need for new therapeutic agents. One possible candidate is the mushroom Reishi (*Ganoderma lucidum*) extract, which contains multiple bioactive compounds, such as polysaccharides (including beta-D-glycans, heteropolysaccharides and glycoproteins), triterpenes, germanium, saturated and unsaturated amino acids, sterols, lipids, antioxidants, vitamins B1, B2, B6, iron, calcium, and zinc (Klupp et al., 2015). In Chinese traditional medicine, Reishi is used for the treatment and prevention of many chronic diseases. Oral administration of Reishi produced a hypotensive effect in SHR rats, a model of high blood pressure (Tran et al., 2014). Mohamad Ansor et al. (2013) have discovered four promising antihypertensive-related proteins in Reishi mycelia, which are suggested to have a lesser possibility to exert adverse side effects and hence can be a good alternative to conventional antihypertensive drug treatment. A number of studies have also established neurotrophic effects of Reishi. In particular, the long term use of aqueous Reishi extract is protective against apoptosis/necrosis of brain tissue in hypoxia/ischemia induced by ligation of the right carotid artery (Xuan et al., 2015). Furthermore, the antiepileptic action of extracted polysaccharides from *Ganoderma lucidum* is experimentally

Abbreviations: ISIAH, inherited stress-induced arterial hypertension; NMR, nuclear magnetic resonance; NAA, N-acetylaspartate; GABA, gamma-aminobutyric acid; Ala, alanine; Ast, aspartate; Cho, choline; Cr, creatine; PCr, phosphocreatine; Glu, glutamic acid; Gln, glutamine; mIno, myo-inositol; Tau, taurine; Gly, glycine; Lac, lactate; PEA, phosphorylethanolamine; PLS, partial least square method

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proven (Wang et al., 2014). A positive impact of Reishi on brain metabolism was also demonstrated in rats exposed to moderate doses of alcohol (Shevelev et al., 2015a).

The data cited above suggest dietary supplements of Reishi produced a positive therapeutic effect by reducing arterial hypertension. Experimental or genetic models of human pathologies are important for studying the effectiveness of specific drugs and dietary supplements. In the Institute of Cytology and Genetics, a hypertensive ISIAH rat strain with stress-sensitive arterial hypertension was developed by intentional breeding for a significant increase in blood pressure in response to emotional stress (Markel, 1992). We used the male adult ISIAH rats subjected to gavage with water extract of the *G. lucidum* (Reishi) mushroom, collected in the Altai Mountains in order to study the effectiveness of Reishi as an antihypertensive agent. Losartan – the antagonist of AT1 receptors of angiotensin II (Kang et al., 1994) – was used as a positive therapeutic control.

After seven weeks of therapy, a reduction in the blood pressure comparable to the hypotensive effect of the standard pharmaceutical medicine losartan was observed in experimental animals. In addition, unlike losartan, Reishi caused a twofold increase in cerebral blood flow. Various influences of Reishi and losartan on the blood flow in the carotid arteries were associated with multidirectional changes in neurometabolic patterns in the rat cerebral cortex.

Materials and methods

Medications

Mushrooms were collected from larch in the Altai-Sayan region; all mushrooms used in this experiment were identified as *G. lucidum*.

The results of a comparative analysis of 9 nucleotide sequences of the ITS1 region from the Altai populations of *G.lucidum* showed that they are all identical (the data are prepared for publishing). Three of the studied sequences (HM130563, HM130564, HM130566) were previously analyzed by Zhang et al. and were assigned to Group 1. According to Zhang, the species of *Ganoderma lucidum* could be separated into three groups by phylogenetic analysis of ITS1 sequences Group 1 is composed of 56 strains originally determined as *Ganoderma lucidum*, including strains from Italy, India, China, Russia, Armenia, France, UK, Canada, Poland, Sweden, Slovenia, Czech Republic, the USA, Norway and Finland (Zhang et al., 2017).

Mushrooms were pre-air-dried to constant weight and grinded using a jet mill (MAN-30; MVM, Moscow, Russia) to a very fine powder, which disrupts the fungal hyphae structure that form fruiting bodies. For moisture content determination, shredded mushrooms were dried in an air thermostat (TV-20; Instrumental Factory PZ-K, Kasimov, Russia) at a temperature of 70 to a constant weight. The average moisture value was 11.5%. To prepare the extraction, 400 g of powdered fungi and 2.0 l of distilled water were placed in a 3 l glass container, which was then was placed in an air thermostat at 70 °C. After 5 h, a 1 ml sample was placed on a petri dish and dried to a constant weight in an air oven at 70 °C. The dissolved substances content was 18.0%. After an additional 19 h of extraction, the dissolved substances content was 20.5%. After cooling the mixture to 40 °C, it was filtered through a paper filter, and the sediment was washed twice with 200 ml of distilled water at 40 °C. Combined filtrates were evaporated in a rotary evaporator (Unipan-350P; SovChemPhysTech, Ufa, Russia) to a volume of 40 ml.

To obtain the fingerprint of the lipophilic compounds of Ganoderma lucidum, 1.0 g of air-dry mass of the crushed fruit body of the fungus was boiled with 10 ml of methyl alcohol for 1 h, the extract was filtered and the filtrate was passed through a column with an Al_2O_3 layer 15 mm in height for dehydration, 1 ml was taken for analysis by gas chromatography. The analysis was carried out on an Agilent Technologies 6890 N chromatograph with a quartz capillary column DB-1 and an Agilent Technologies 5973 N quadrupole mass spectrometer. Chromatographic mass spectrometric analysis of the samples was carried out both to measure the total ion current in the scanning regime in the mass range 10–800 amu, and in the mode of selective ion scanning.

The identification of components present in the methanolic extract was based on direct comparison of the retention indices and mass spectral data with those for standard compounds, and by computer matching with the Wiley 229, Nist 107, Nist 21 Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Paresh and Normen, 1998). The Kovacs retention indices were calculated from the retention times of Lucero et al. (2009). The data a presented in Data in Brief article.

Losartan (Gedeon-Richter-Rus, Russia) was used as a positive control.

Experimental animals and husbandry conditions

All animals were handled according to the regulations of the Animal Care and Use Committee of the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences and were maintained under pathogen-free conditions. The experimental protocol was approved by the Bioethical Committee of the Institute of Cytology and Genetics. The study was conducted at the Center for Genetic Resources of Laboratory Animals at the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences (RFMEFI61914X0005 and RFMEFI62114X0010). At the beginning of the experiment, 18 ISIAH rats aged from 12 to 13 weeks were used. The animals were housed in individually ventilated cages, with 1 animal per cage. The cages had a height of 20.5 cm and an area of 929 cm^2 (OptiRAT cage; Charles River Laboratories, Chatillon-sur-Chalaronne, France). Water and Chara SPF granulated forage for laboratory rodents (Assortiment-Agro; Puschino, Russia) were provided ad libitum. Rats were maintained in an artificial day-night regime (14-h light/10-h darkness), at a temperature of 22 to 24 °C, and 40 to 50% humidity. Dry, de-dusted wood shavings (Al'bion; Novosibirsk, Russia) were used as litter. The food and litter were autoclaved to 121 °C before use. Drinking water was deionized with a Millipore NF-C8674 (Merck Millipore, Billerica, MA USA) and supplemented with Severyanka mineral additive (Ekoproect; St. Petersburg, Russia).

Experimental scheme

All treatments for the seven weeks were performed daily immediately after lights off (15:00 local time). Reishi extract or losartan solution and water (placebo) were administered by gavage (1 ml) using a metal esophageal probe (FTSS-18S-76, Instech Laboratories, Inc., PA, USA). In accordance with doses used in published experiments (Kwon and Kim, 2011, Shevelev et al., 2015a), Reishi was administered at a dose of 100 mg/kg of body weight. Losartan was used at a dose of 10 mg/kg of body weight. All animals were divided into 3 experimental groups of approximately the same size:

1. Experimental group R (Reishi) ($N = 6$).
2. Experimental group L (losartan) ($N = 6$).
3. Control group C (water) ($N = 6$).

For two days before the treatment and once a week during the experiment, systolic and diastolic blood pressure were measured indirectly by the tail cuff method (CODA, Kent Scientific Corporation, USA).

Magnetic resonance imaging

Blood parameters and neurometabolites were measured on a horizontal tomograph with a magnetic field of 11.7 T (Bruker, Biospec 117/16 USR, Germany). The volumetric flow rate and diameter of the