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Neuroprotective effects of *p*-tyrosol after the global cerebral ischemia in rats

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ABSTRACT

Background: Salidroside is a biologically active compound derived from *Rhodiola rosea* L. Studies showed that salidroside after i.v. injection is extensively metabolized to *p*-tyrosol and only trace amounts of salidroside are found in the brain tissue.
Objective: The aim of the study was to investigate the neuroprotective effects of *p*-tyrosol in the global cerebral ischemia-reperfusion (GCI) model.
Study design: A total of 103 Wistar rats were assigned to groups of sham-operated (n=10), control (n=42), *p*-tyrosol-treated (n=36), and pentoxifylline-treated (n=15) animals. The rats of control, *p*-tyrosol-treated, and pentoxifylline-treated groups received intravenously 0.9% NaCl solution, 2% solution of *p*-tyrosol in doses of 5 mg/kg, 10 mg/kg, and 20 mg/kg, and pentoxifylline in a dose of 100 mg/kg, respectively, daily for 5 days. Rats were examined at days 1, 3, and 5 after GCI. After evaluation of neurological deficit, animals were euthanized for morphological and biochemical characterization.
Methods: Rats of control, *p*-tyrosol-treated, and pentoxifylline-treated groups were exposed to three-vessel model of GCI. Neurological deficit, numeric density of neurons in hippocampal CA1 region, and percentage of neurons with focal and total chromatolysis were studied. Biochemical study assessed contents of conjugated dienes and fluorescent products in brain homogenate.
Results: In control group, only 50.0% of rats survived by day 5 after the GCI; 38.1% of survived animals had severe neurologic deficit. In brain tissue of PTX-treated rats, the levels of diene conjugates and fluorescent products were 79% and 73%, respectivley, at day 5 compared with control. Differences in diene conjugates were statistically significant compared with control. The survival rate of animals treated with 20 mg/kg p-tyrosol was 82.3% at day 5 after GCI. In *p*-tyrosol-treated GCI rats, the numeric density of neurons in the hippocampal CA1 region was higher by 31% compared with control. The percentage of neurons with focal and total chromatolysis decreased by 27% and 43%, respectively. At day 5 after GCI, the levels of conjugated dienes and fluorescent products were significantly lower (by 37% and 45%, respectively) in group of animals treated with 20 mg/kg p-tyrosol compared with control. Moderate neuroprotective effects of 5 mg/kg *p*-tyrosol administration were documented only at day 5 after GCI. In case of 10 mg/kg *p*-tyrosol administration, neuroprotection was documented sooner: at day 1 or 3 after GCI. However, administration of 5 and 10 mg/kg *p*-tyrosol did not affect animal survival.
Conclusion: Course administration of intravenous *p*-tyrosol in a dose of 20 mg/kg increased survival, reduced neurological deficit after GCI, attenuated neuronal damage in the hippocampus, and attenuated lipid peroxidation in brain tissue in animals subject to GCI with reperfusion.

Abbreviations: GCI, Global cerebral ischemia; PTX, Pentoxifylline.

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Introduction

Salidroside is a biologically active compound derived from *Rhodiola rosea L.* Salidroside has a wide spectrum of pharmacological activity (Saratikov and Krasnov, 2004; Panossian et al., 2010a). The neuroprotective and antioxidant effects represent the important properties of salidroside. Salidroside exerts protective effect against oxidative damage and increases cell viability (Cao et al., 2005; Zhu et al., 2011). Salidroside inhibits oxidative stress and apoptosis induced by H_2O_2 or amiloid β in the hippocampal neurons and neuroblastoma cells (Chen et al., 2009; Zhang et al., 2010). Due to its antioxidant activity, salidroside has a potential to reduce focal and global cerebral ischemia-reperfusion injury in rats (Shi et al., 2012; Zou et al., 2009).

After intravenous injection, salidroside is extensively metabolized to *p*-tyrosol (Panossian et al., 2010b; Guo et al., 2014). Moreover, after intravenous injection, only trace amounts of salidroside are found in the brain tissue where glycosylation metabolite of salidroside, *p*-tyrosol, is present in significant concentrations (Guo et al., 2014). These data suggest that salidroside may be considered a prodrug whose neuroprotective action may be due to the effects of *p*-tyrosol.

The aim of this study was to elucidate the neuroprotective effects of *p*-tyrosol in the model of global cerebral ischemia-reperfusion (GCI).

Materials and methods

Chemicals and drugs

HPLC-grade acetonitrile and ultrapure hexane were purchased from Cryochrom (Russia). Ultrapure orthophosphoric acid was purchased from Chimspecializaciya Co. (Russia). Analytical standard of Tyrosol > 99.5% (GC), sucrose, EDTA disodium salt, and chloral hydrate were purchased from Sigma-Aldrich Chemical Co. Cresyl violet solution was obtained from Fluka (USA). 10%-Neutral formalin was purchased from GEM (Russia). Chemically pure chloroform was purchased from EKOS Co. (Russia). HPLC-grade methanol was purchased from Vecton (Russia). Ether for anesthesia was purchased from Kusbassorgchim (Russia). Water for HPLC was purified by using Cyclon, Fistreem (United Kingdom). Pentoxifylline (Trental) was purchased from Sanofi India Limited. Sterile 2% solution of *p*-tyrosol in ampoules was made NPO Virion (Russia).

Study drugs

Test substance of *p*-tyrosol was synthesized by the original method (RF patent No 2,558,329) in the Institute for Problems of Chemical and Energetic Technologies, Siberian Branch of the Russian Academy of Sciences. The synthesized substance was compared with the analytical Tyrosol standard. HPLC analyses were performed with the Agilent 1200 HPLC system equipped with gradient pump and diode array detector. Separation was achieved by using a Zorbax SB-C18 column (3.0 × 150 mm, 3.5 μm). Elution was performed at a flow rate of 0.35 ml/min at 25 °C. The solvents for eluent were 0.2% (*v*/*v*) orthophosphoric acid (solvent A) and acetonitrile (solvent B). The solvent gradient changed according to the following conditions: 90% A to 75% A over 10 min. The injection volume was 2 μl. The UV detector was set at a wavelength of 225 nm.

Fig. 1A and B present the chromatographic analysis curves for *p*-tyrosol substance and standard *p*-tyrosol sample, respectively.

Retention time of *p*-tyrosol was 8.1 min. Analysis of the *p*-tyrosol sample showed that *p*-tyrosol was comparable with the analytical standard. Chromatograms of the *p*-tyrosol substance and the analytical standard demonstrated the presence of one unidentified impurity in the amount of 0.01%. Retention time of the impurity was 4.237 min. *p*-Tyrosol contents in the substance and the standard sample were 99.99%. HPLC-curves illustrate the sameness of the pharmaceutical substance and the standard *p*-tyrosol sample.

Linearity of the method was evaluated based on a determination of the contents of primary substance in the solutions of standard *p*-tyrosol sample in various concentrations. For evaluation of the linearity, solutions of the standard *p*-tyrosol sample in concentrations of 0.4, 0.6, 0.8, 1.0, and 1.2 mg/ml were analyzed. Based on obtained data, correlation coefficient (r = 0.9999) was calculated. Fig. 1C shows standard *p*-tyrosol curve.

Positive control

A nonspecific blocker of phosphodiesterase 4, pentoxifylline (PTX), was used as a positive control (McCarty et al., 2016). Animal experiments demonstrated the ability of PTX to decrease the severity of ischemic/reperfusion brain injury and spontaneous brain ischemia in spontaneously hypertensive stroke-prone rats (Eun, Liu, 2000; McCarty et al., 2016). Dose of 100 μg/kg used in our study was within the range of PTX doses showing neuroprotective activity in rats (McCarty et al., 2016). *p*-Tyrosol and pentoxifylline have similar spectrums of pharmacological activity: both substances exert antioxidant (McCarty et al., 2016; Chimi et al., 1995; Di Benedetto et al., 2007; Storozhok et al., 2011) and hemorheological effects (McCarty et al., 2016; patent of the Russian Federation No 2,239,423).

Animals

The study was conducted in accordance with internationally accepted principles for the use and care for laboratory animals (European Community-Guidelines) and approved by the Animal Care and Use Committee at the E.D. Goldberg Institute of Pharmacology and Regenerative Medicine (protocol #22032012). The experiments were performed by using a total of 103 adult male Wistar rats (250–300 g) obtained from the Department of Experimental Biological Models of the E.D. Goldberg Institute of Pharmacology and Regenerative Medicine. Rats were housed in groups of five animals per cage (57 × 36 × 20 cm) under standard laboratory conditions (ambient temperature of 22 ± 2 °C, relative humidity of 60%, 12/12 h light/darkness cycle) in cages with sawdust bedding. Animals were provided with standard rodent chow (PK-120-1, Ltd., Laboratorsnab, Russia) and *ad libitum* water access.

Experimental design, dosage, and route of drug administration

Animals were assigned to four groups: sham-operated (n = 10), control (n = 42), *p*-tyrosol-treated (n = 36), and PTX-treated (n = 15). Acute global cerebral ischemia (GCI) in control, *p*-tyrosol-treated, and PTX-treated groups was induced according to the new 3-vessel model (Chernysheva et al., 2014). The rats of control, *p*-tyrosol-treated, and PTX-treated groups received intravenous 0.9% NaCl, 2% of *p*-tyrosol solution in a dose of 5 mg/kg (9 rats), 10 mg/kg (10 rats), 20 mg/kg (17 rats), and PTX in a dose of 100 mg/kg, respectively, daily for 5 days. At day 5 after GCI, 5 animals from control group and 5 animals from 20 mg/kg-*p*-tyrosol-treated group were used for histological examination; 5 animals from control group, 7 animals from 20 mg/kg-*p*-tyrosol-treated group, and 6 animals from PTX-treated group were used for biochemical studies.

Selection of a *p*-tyrosol dose range was based on data showing its antioxidant and neuroprotective effects. Indeed, Bu et al.