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The protective effect of erdosteine on short-term global brain ischemia/reperfusion injury in rats

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ABSTRACT

Experimental studies have demonstrated that free radicals play a major role on neuronal injury during ischemia/reperfusion (I/R) in rats. Erdosteine is a thioderivative endowed with mucokinetic, mucolytic and free-radical-scavenging properties. The aim of the present study was to investigate the effect of erdosteine treatment against short-term global brain ischemia/reperfusion injury in rats. The study was carried out on Wistar rats divided into four groups. (i) Control group, (ii) ischemia/reperfusion group, (iii) ischemia/reperfusion + erdosteine group, and (iv) erdosteine group. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities as well as thiobarbituric acid reactive substances (TBARSs) and nitric oxide (NO) levels were analysed in erythrocyte and plasma of rats. Plasma NO levels were significantly higher in the ischemia/reperfusion group than the other groups. The activities of SOD and GSH-Px were decreased, while TBARS levels increased in the ischemia/reperfusion group compared to other groups in both plasma and erythrocyte. The erythrocyte CAT activity was higher in erdosteine group and there was a statistically significant increase, when compared with the erdosteine plus ischemia/reperfusion group. By treating the rats with erdosteine, the depletion of endogenous antioxidant enzymes (SOD, CAT, GSH-Px) and increase of TBARS and NO levels were prevented. This study, therefore, suggests that erdosteine reduces parameters of oxidative stress is well supported by the data.

1. Introduction

Cerebral ischemia is caused by a deficiency in blood supply to a part of the brain, which in turn triggers various pathophysiological changes. When the brain is deprived of blood supply (ischemia) its injury is not only by the temporary loss of oxygen and energy supply, but also by the reactive oxygen species (ROSs) that are generated by reactions with the oxygen that is reintroduced during reperfusion.

The brain is very susceptible to energy depriving injuries and is particularly sensitive to oxygen radical-mediated injury due to its characteristics to low fuel reserves, high aerobic metabolism, and low concentrations of oxygen radicals scavenging enzymes (Chen et al., 2000). There are substantial experimental evidences that, ROSs are produced in the brain during ischemia and reperfusion injury (Rodrigo et al., 2005). These reactive species often are divided into two groups, ROSs and reactive nitrogen species (RNSs). ROS usually refers to superoxide, hydrogen peroxide (H_2O_2), hydroxyl radical, singlet oxygen, etc., and RNSs typically include nitric oxide (NO) and peroxynitrite ($ONOO^-$). ROS and RNS, as a rule, are short-lived in biological environments and/or exist in low concentrations, making their direct detection very difficult (Jian Liu and Rosenberg, 2005).

ROSs are potent oxidizing and reducing agents that directly damage cellular membranes by lipid peroxidations (LPOs) (Toyokuni, 1999). Alternative approaches to demonstrate their involvement in cerebral ischemic damage have concentrated on measuring the rate of consumption of endogenous protective molecules or the formation of by-products of LPOs, such as malondialdehyde (MDA) (Schmidley, 1990).

The antioxidant enzyme capacity of the tissue affected by ischemia/reperfusion (I/R) is particularly important for the primary endogenous defence against the free radical (FR) induced injury. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) are endogenous antioxidants which play a role in the prevention of oxidative injury (Akyol et al., 2002). Therefore, an enhancement of antioxidant activities in brain tissues may be potentially beneficial for neuronal recovery from I/R injury.

Endogenous antioxidative defences are likely to be effective due to the excess of oxygen species, inactivation of detoxification systems, and

Abbreviations: CAT, catalase; FR, free radical; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; H_2O_2, hydrogen peroxide; I/R, ischemia/reperfusion; LPO, lipid peroxidation; MDA, malondialdehyde; NBT, nitro blue tetrazolium; NO, Nitric oxide; NO_2^-, nitrite; NO_3^-, nitrate; NOS, nitric oxide synthase; O_2^-, superoxide anion radical; $ONOO^-$, peroksinitrit; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; RNS, reactive nitrogen species; SEM, standard error of the mean; SOD, Superoxide dismutase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances.

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degradation of antioxidants (Chan, 1996). Numerous antioxidants and scavengers of FRs have been tested, and many have shown neuroprotective effects (Aabdallah and Eid, 2004; Chen et al., 2000; Ergun et al., 2002; Gupta and Sharma, 2006; Irmak et al., 2003; Shah et al., 2005; Wei et al., 2005).

Erdosteine [*N*-(carboxymethylthioacetyl)-homocysteine thiolactone] was introduced in the market as a mucolytic agent for chronic pulmonary diseases more than 10 years ago. The drug contains two blocked sulfhydryl groups one of which, after hepatic metabolization and opening of the thiolactone ring, becomes available both for the mucolytic and FR scavenging and antioxidant activity too. Erdosteine prevents the accumulation of ROS when their production is accelerated and increases antioxidant cellular protective mechanisms (Moretti and Marchioni, 2007). However, its neuroprotective effects have not been investigated on global cerebral ischemia in rats. Therefore, the aim of the present study was to investigate the effect of erdosteine treatment against to short-term global brain I/R injury in rats.

2. Materials and methods

2.1. Animals

The animals involved in this study were procured, maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Inonu University, Animal Ethical Committee. All experiments were performed on male Wistar rats (each weighing 200–250 g at the time of the experiment). Rats were housed in polycarbonate cages with wire lids and given the standard laboratory chow and water. The housing room was maintained at 24 °C with 42±5% relative humidity and had a 12–12-h light–dark cycle (lights on 06:00–18:00 h). Body temperature was maintained around 37±5 °C throughout the surgical procedure.

2.2. Experimental design

The study was carried out on the Wistar rats divided into four groups. (i) Control group (n=6), (ii) I/R group (n=8, ischemia was induced by bilateral occlusion of the carotid arteries for 10 min and reperfusion was achieved by releasing the occlusion to restore the circulation for 30 min), (iii) I/R+erdosteine group (n=9, oral 50 mg/kg/day for 3 days before I/R), and (iv) erdosteine group (n=6, oral 50 mg/kg/day for 3 days without I/R procedure).

2.3. Administration of erdosteine

The erdosteine as a suspension of 175 mg per 5 ml was obtained from the drug company ILSAN-ILTAS (Turkey), and applied orally once a day (for 3 days before experiments) at a dose of 50 mg/kg body weight via plastic disposable syringes (Ozyurt et al., 2004).

2.4. Induction of cerebral ischemia

Rats were anesthetized with an i.p. injection of urethane (i.p. 1.2–1.4 g/kg) (Sigma Chemical Company, St Louis, MO, USA) on the day of the experiment. Animals in I and IV groups received only the vehicle and served as sham-operated control, and underwent a surgical procedure similar to the other groups but the arteries were not occluded. While groups II and III were all subjected to ischemia, and two common carotid arteries were exposed through lateral incisions and were separated from the vagus. Ischemia was induced by bilateral clamping of the common carotid arteries for 10 min. Following cerebral ischemia, reperfusion was achieved by declamping the arteries, after which the circulation was restored for 30 min.

Venous blood samples were taken into heparinized tube. After centrifugation of the blood, plasma and erythrocyte were separated. Erythrocyte was washed three times with 0.9% NaCl solution and packed. Erythrocyte and plasma were then stored at −70 °C until study.

2.5. Biochemical determination

All biochemical determinations were performed on the erythrocyte and plasma obtained after centrifugation using spectrophotometric methods. In order to evaluate the prooxidant–antioxidant balance, we determined the free radicals production by measuring of lipid peroxidation, NO levels, and activity of some enzymatic antioxidants (SOD, CAT, and GSH-Px).

2.5.1. Determination of catalase activity in erythrocyte

Catalase (CAT, EC 1.11.1.6) activity was determined according to the method of Aebi (1974). The principle of the assay is based on the determination of the rate constant (s^{-1}, k) or the H_2O_2 decomposition rate at 240 nm.

2.5.2. Determination of superoxide dismutase activity in erythrocyte and plasma

Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al. (1988) with a slight modification (Durak et al., 1993). The principle of the method is based on the inhibition of NBT reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the sample after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate.

2.5.3. Determination of glutathione peroxidase activity in erythrocyte and plasma

Glutathione peroxidase (GSH-Px, EC 1.6.4.2) activity was measured by the method of Paglia and Valentine (1967). The enzymatic reaction in the tube, which is containing the following items: NADPH, reduced glutathione (GSH), sodium azide, and glutathione reductase, was initiated by the addition of H_2O_2 and the change in absorbance at 340 nm was monitored by a spectrophotometer.

2.5.4. Determination of thiobarbituric acid reactive substances level in erythrocyte and plasma

The erythrocyte and plasma thiobarbituric acid reactive substances (TBARSs) level was determined by a method (Esterbauer and Cheeseman, 1990) based on the reaction with thiobarbituric acid (TBA) at 90–100 °C. In the TBA test reaction, malondialdehyde (MDA) or MDA-like substances and TBA react with the production of a pink pigment having an absorption maximum at 532 nm. The reaction was performed at pH 2–3 at 90 °C for 15 min. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm. The results were expressed according to a standard graphic which was prepared from a standard solution (1, 1, 3, 3-tetramethoxypropane).

2.5.5. Determination of nitric oxide levels in erythrocyte and plasma

Nitric oxide has a half-life of only a few seconds, because it is readily oxidized to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-) which serves as index parameters of NO production. The method for plasma nitrite and nitrate levels was based on the Griess reaction (Cortas and Wakid, 1990). Samples were initially deproteinized with Somogy reagent. Total nitrite (nitrite+nitrate) was measured by spectrophotometry at 545 nm after conversion of nitrate to nitrite by copperized cadmium granules. A standard curve was established with a set of serial dilutions (10^{-8}–10^{-3} mol l^{-1}) of sodium nitrite.