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Hypobaric-hypoxia-induced pulmonary damage in rats ameliorated by antioxidant erdosteine

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Summary

Free radical-mediated injury to lung and pulmonary vasculature is an important mechanism in hypoxia-induced lung damage. In this study, we aimed to investigate the potential protective effects of erdosteine as an antioxidant agent on hypobaric hypoxia-induced pulmonary hypertension. Adult male rats were assigned randomly to three groups. The first group of rats was exposed to hypobaric–hypoxia and the second group was treated with erdosteine (20 mg/kg, daily) for 2 weeks, during which time they were in a hypoxic chamber. These groups were compared with normoxic controls. All rats were sacrificed after 2 weeks. The hypoxia-induced increase in right ventricle to left ventricle plus septum weight ratio (from 0.20 ± 0.01 to 0.26 ± 0.01) was reduced significantly in the erdosteine-treated group (0.23 ± 0.01). Malondialdehyde levels were elevated (from 0.33 ± 0.11 to 0.59 ± 0.02) and total antioxidant status was not changed significantly (from 1.77 ± 0.42 to 2.61 ± 0.23) by hypoxia. In contrast to the hypoxia-exposed group, malondialdehyde levels were significantly decreased in the erdosteine-treated group (0.37 ± 0.02). Total antioxidant status (4.03 ± 0.22) was significantly higher in erdosteine-treated rats when compared to non-treated rats. Histopathological examination demonstrated that erdosteine prevented inflammation and protected lung parenchyma and pulmonary endothelium of hypoxia-exposed rats.

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1. Introduction

Chronic hypoxia can cause hypoxic pulmonary hypertension (HPH) and right ventricular hypertrophy. The pathogenesis of HPH involves a complex and multifactorial process. The main etiological problem is pulmonary vascular smooth muscle cell proliferation and endothelial dysfunction due to sustained increase in pulmonary arterial pressure (Rabinovitch et al., 1979; Budhiraja et al., 2004; Lal et al., 1999). Production of endothelial vasoactive mediators, such as prostacycline, nitric oxide (NO), endothelin-1 and tromboxane have been found to be altered in patients with HPH (Block and Patel, 1989; Chang et al., 1989). In addition to these mediators, hypoxia may generate oxidative stress in vitro (Chang et al., 1989) and in vivo (Herget et al., 1978a). Reactive oxygen species (ROS) may lead to pulmonary vascular wall injury, and, as such, may initiate the process of vascular proliferation and structural remodeling.

In rats exposed to hypoxia, HPH develops within 10–14 days, then it stabilizes and does not increase in severity (Herget and Palecek, 1978b). It has been shown that the development of HPH proceeded in two phases (Lachmanova et al., 2005). ROS participate in the pathogenesis of HPH, especially at the beginning of hypoxic exposure. Then, the release of oxygen radicals declines to normal values (Wilhelm et al., 1996, 1999).

Erdosteine is a thiol-derived drug that has been introduced into clinical practice as a mucolytic agent (Dechant and Noble, 1996). It is metabolized in the liver and active metabolites with an –SH group are generated. These –SH groups account for free radical scavenging and the anti-oxidant properties of erdosteine (Braga et al., 2000; Inglesi et al., 1994). In vivo and in vitro studies demonstrate that erdosteine has a potent free radical scavenging efficacy (Terzi et al., 2004; Fadillioglu et al., 2003; Vagliasindi and Fregnan, 1989).

In the present study, we investigated whether antioxidant treatment with erdosteine during the early phases of hypoxic exposure could prevent hypoxia-induced right ventricular hypertrophy, lung damage and pulmonary endothelial injury.

2. Material and methods

2.1. Animals and hypoxic exposure

A hypobaric chamber (COMMAT, Ankara, Turkey) has been used to form HPH in this study. This chamber has been designed to study small laboratory animals at low barometric pressures for long periods of exposure. The pressure within the chamber is regulated by a vacuum pump and controlled with a manometer. Rats were randomly assigned to three groups. The first group of rats ($n = 9$) was placed in this chamber and exposed to 560 mmHg pressure for 5 min to allow adjustment. After 5 min, the vacuum was increased and the rats exposed to 380 mmHg (0.5 atm). The second group ($n = 15$) was exposed to the same conditions, but they were also treated orally with the antioxidant erdosteine (Ilsan, Istanbul, Turkey), 20 mg/kg daily, from the first day of hypoxic exposure.

2.2. Specimen collection for histopathological studies

At the time of sacrifice, lungs were collected for histopathological studies. The hearts were used for the assessment of right ventricular hypertrophy. The right ventricle was separated uniformly from the left ventricle plus septal wall and both parts were weighed. The ratio of right ventricle weight and the sum of left ventricle weight and septal wall weight was used as a measure for right ventricular hypertrophy.

2.3. Determination of malondialdehyide (MDA) levels, total antioxidant status (TAS) and hematocrit

Aortic blood samples were centrifuged immediately upon sampling, at 1000 g for 10 minutes. Serum was separated from blood as soon as possible and stored at −20 °C. Serum samples were used for the determination of malondialdehyide (MDA) levels and total antioxidant status (TAS). Lipid peroxidation was used as an indirect measure of oxidative damage induced by ROS. Lipid peroxidation in serum was determined by the thiobarbiturate reaction measuring the formation of MDA (Ohkawa et al., 1979). Briefly, 0.5 ml of 0.5% butylated hydroxytoluene was added to 2 ml of serum to prevent lipid auto-oxidation. For protein precipitation, 2 ml of 20% trichloroacetic acid was added to 2 ml of serum. After mixing and centrifuging, 1 ml of 0.67% thiobarbiturate in water was added to the supernatant and boiled for 60 min. After cooling, the optical density at 530 nm was assayed. 1,1,3,3-tetraethoxypropane was used as standard. MDA levels are expressed as nmol/ml.

The antioxidant system has many components and deficiency in any of the components can cause a reduction in the overall antioxidant status of an individual. The relative efficacy of the contribution