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Original Article

Protective effects of erdosteine against radiation-induced lung injury in rats

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Abstract: The aim of this study was to investigate the protective effects of erdosteine (ERD) against whole-body irradiation-induced lung injury in rats by assessing biochemical parameters. Thirty albino rats were divided into three equal groups, including control, radiation (RT) alone, and RT + ERD. This study began the day before radiation treatment and continued for four days. Erdosteine was administered orally via a gastric tube at a dose of 50 mg/kg/day, one hour prior to radiation and every day at the same time. Radiation was applied with a total body irradiation dose of 800 cGy in one fraction. Catalase (CAT) and superoxide dismutase (SOD) activities and malondialdehyde (MDA) levels in lung tissue of rats were evaluated three days after radiation treatment. CAT and SOD activities were found to be significantly lower in the RT group than control ($p < 0.001$ and $p < 0.001$). They were higher in the RT + ERD group than both RT and control groups ($p < 0.001$, $p < 0.001$ and $p < 0,001$, $p < 0,061$). MDA levels were significantly higher in the RT group compared to control ($p < 0.001$), whereas MDA levels decreased in the RT + ERD group compared to RT group ($p < 0.001$). Erdosteine may prevent radiotherapy-induced lung injury by increasing activities of CAT and SOD and by decreasing levels of MDA.

Keywords: Erdosteine, radiotherapy, lung, rat

Introduction

Ionizing radiation is effective in the treatment of many types of tumors. It is an important part of treatment in approximately 60% of cancer patients [1]. Normal lung tissue is one of the most sensitive tissues to ionizing radiation. Radiation-induced lung toxicity is common [2]. Despite the use of modern radiotherapy devices and techniques (4-dimensional conformal radiation therapy, intensity modulated radiation treatment, volumetric modulated arc therapy), lungs remain the most important dose-limiting organs, especially when radiation therapy is applied over a certain dose [3].

The main causes of increased radiation pneumonitis are high total dose, high fraction number, large volume, large volume of irradiated normal tissue, administration of radioprotectors or chemotherapeutic agents, and cell and tissue properties. Risks are increased especially when chemotherapy is administered concurrently with radiotherapy [4].

The most remarkable group of actual radioprotectors are sulfhydryl compounds. These compounds tend to have some of the most effective structural properties: a free SH group (or potential SH group) at one end of the molecule and a strong basic function, such as amine or guanidine at the other end, seperated by a straight chain of two or three carbon atoms. Sulfhydryl compounds are effective radioprotectors against sparsely ionizing radiations like X- or gamma-rays [5].

The most important mechanisms of SH-mediated cytoprotection include: a) free radical scavenging to protect against oxygen-based free radical generation by ionizing radiation or chemotherapeutic agents such as alkylating agents; b) hydrogen atom donation to facilitate direct chemical repair at DNA damage sites [5].

Erdosteine (ERD) is a mucolytic drug widely used in clinical practice. It has antibacterial, anti-inflammatory, and antioxidant effects [6]. Erdosteine, itself, has no free thiol group. Once

catabolized in the liver, erdosteine generates three active metabolites containing -SH groups which are responsible for its antioxidant activity. Erdosteine can act as a free oxygen radical scavenger due to the presence of two sulphydryl groups in its metabolites [7].

Protective effects of erdostein against tissue damage caused by radiation and chemotherapeutic drugs in various organs and tissues have been demonstrated [6, 8-12]. Although there are very few studies examining the protective effects of erdosteine against radiation-induced lung injury, they are not at the biochemical level [13, 14]. In this study, the radioprotector effects of erdosteine on radiation-induced lung injury in whole-body irradiated rats was investigated by evaluating catalase (CAT) and superoxide dismutase (SOD) activities and malondialdehyde (MDA) levels in lung tissue.

Materials and methods

Animals

Thirty in-bred adult male Swiss Albino rats were obtained from the Test Animals Brooding Center of Erciyes University. Animals were fed with a standard diet and water under sterile hygienic conditions. The rats were 12 weeks-old and weighed 250 ± 30 grams. Light and dark cycles were automatically regulated every 12 hours.

All experiments were performed in accordance with National Institutes of Health guidelines for animal research and were in compliance with protocol approved by the Committee on Animal Research and Ethics at Erciyes University, Kayseri/Turkey.

Treatment groups

Animals were divided into three groups, each containing 10 rats. The first group was control (C), which received an equivalent volume of distilled water (vehicle of erdosteine) orally during the period of erdosteine administration (n = 10). The second group was radiation alone (RT). They were treated with 800 cGy radiation to total body (n = 10) in one fraction and received an equivalent volume of distilled water orally during the period of erdosteine administration, similar to the control group. The third group was RT + Erdosteine (RT + ERD), in which the rats were administered 50 mg/kg/day of erdosteine via gastric tube (n = 10). It should be emphasized that the third group also received the same dose of radiation as the RT group. Rats were sacrificed 72 hours after radiation exposure and lungs were removed under general anesthesia with ketamine (50 mg/kg intraperitoneally).

Radiotherapy

External irradiation to the total body was given in a special 30 × 30 × 5 cm animal-fixing box using gamma rays from the Co60 teletherapy machine (Theratron 780 C) with parallel opposed field at a mean dose rate of 3.13 cGy/MU. Radiation dose for total body was 800 cGy in one fraction. Animal fixing boxes contained five rats for each irradiation. Dosage was calculated as Dmax dose at 2.5 cm depth for SSD 80 cm.

Application of erdosteine

Erdosteine was administered orally via a gastric tube at a dose of 50 mg/kg once a day. The first dose of Erdosteine was administered 24 hours prior to radiation. It was continued the next day, along with radiation, and for 2 more days after radiation treatment. Erdosteine was prepared from dry powder (Sandoz Pharmaceutical Indus. Inc., Istanbul, Turkey) as a 100 mL suspension with a concentration of 175 mg/5 mL.

Determination of CAT activity

CAT activity was determined according to Aebi's method [15]. The principle of this method is based on determination of H_2O_2 decomposition rate at 240 nm. Results are expressed as U/mg protein.

Determination of SOD activity

Total (Cu, Zn, and Mn) SOD (EC 1.15.1.1) activity was determined according to the methods of Sun et al. [16]. The assay was based on inhibition of Nitro Blue Tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lysate following addition of 1.0 mL of ethanol-chloroform mixture (5:3, v/v) to the same volume of sample and centrifugation. One unit of SOD was defined as the amount of enzyme causing 50% inhibition

Table 1. CAT and SOD activities and MDA levels in control, RT, and RT + ERD groups (mean ± SD, n = 10)

	CAT U/mg protein	SOD U/mg protein	MDA nmol/mg wet-tissue
Control	8,492 ± 0,516	0,781 ± 0,021	131,784 ± 0,926
RT	7,260 ± 0,268	0,415 ± 0,066	233,404 ± 1,531
RT + ERD	12,943 ± 0,740	0,853 ± 0,078	137,947 ± 2,627
p values			
Control-RT	0.001	0.001	0.001
Control-RT + ERD	0.001	0.061	0.001
RT-RT + ERD	0.001	0.001	0.001

RT: Radiotherapy, ERD: Erdosteine, CAT: Catalase, SOD: Superoxide dismutase, MDA: Malondialdehyde.

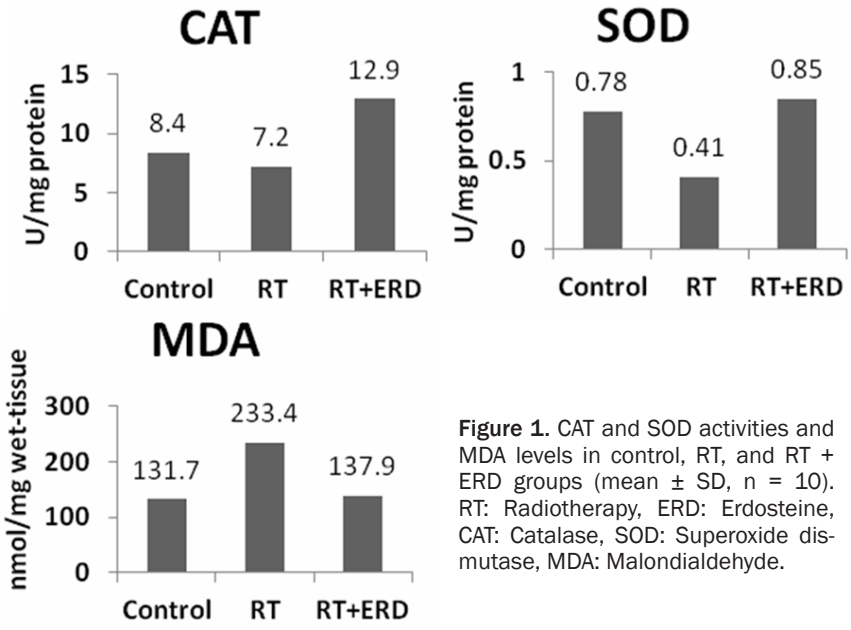

Figure 1. CAT and SOD activities and MDA levels in control, RT, and RT + ERD groups (mean ± SD, n = 10). RT: Radiotherapy, ERD: Erdosteine, CAT: Catalase, SOD: Superoxide dismutase, MDA: Malondialdehyde.

in NBT reduction rate. SOD activity is expressed as U/mg protein.

Analysis of lipid peroxidase levels

Seventy two-hours after the last treatment, rat lungs were promptly removed following decapitation under general anesthesia with intraperitoneal ketamin (50 mg/kg). Lungs were weighed and chilled in ice-cold 0.9% NaCl. After washing with 0.9% NaCl, tissue homogenates were prepared in 6 mL of 1.15% KCl buffer solution by using Edmund Buhler 7400 Tubingen HO4 homogenizer.

Kohn and Liversedge described the colorimetric reaction of thiobarbutiric acid (TBA) with an unknown substance during aerobic incubation of tissue homogenates. This was later identified by Patton and Kurtz as MDA, a secondary product of lipid peroxidation. The reaction of lipid peroxides with TBA has been widely adopted as a sensitive assay method for measuring lipid peroxidation in animal tissue [17].

The reaction mixture contained 0.2 mL of sample, 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 20% acetic acid solution (pH:5.5), and 1.5 mL of 0.8% aqueous solution of TBA. This mixture was made up to 4.0 mL with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 mL of distilled water and 5.0 mL of n-butanol mixture and pyridine (15:1 v/v) was added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the absorbency of the upper layer was measured at 532 nm by spectrophotometer. Protein concentration was determined by the Biuret method. Levels of lipid peroxides were expressed in terms of nmol MDA/mg wet-tissue, calculated from the absorbance at 532 nm.

Determination of protein content

Protein measurements were performed at all stages, according to Lowry's method [18].

Statistical analysis

Data were analysed using a commercially available statistics software package (SPSS 20.0). All groups showed normal distribution. There-

fore, parametric statistical methods were used to analyze the data. One-way ANOVA test was performed and post hoc Tukey HSD multiple comparisons were made using least-squares differences. Comparison to the control group was performed with Dunnett's test. Results are presented as mean ± standard deviation (SD) and $P < 0.05$ is regarded as statistically significant.

Results

Tissue CAT and SOD activities and MDA levels for each group are given in **Table 1**. *P* values between groups are shown in **Table 1**. Tissue CAT and SOD activities and MDA levels for each group are also shown in **Figure 1**.

CAT and SOD activities in the RT group were significantly lower than the control group ($p < 0.001$ and $p < 0.001$). There were significant increases in CAT and SOD activities in the RT + ERD group compared to RT group ($p < 0.001$ and $p < 0.001$). There were significant increases in CAT levels in the RT + ERD group, compared to control, while increases in SOD levels did not reach statistical significance ($p < 0.001$ and $p < 0.061$).

MDA levels in the RT group were significantly higher than the control group ($p < 0.001$) and significantly lower in the RT + ERD group compared to RT group ($p < 0.001$). However, decreases did not reach statistical significance in the control group ($p < 0.001$).

Discussion

Part of the altered alveolar environment in lung fibrosis involves oxidative stress induced by an imbalance between oxidant production and antioxidant defenses [19]. Reactive oxygen species (ROS) are normal byproducts of cellular metabolism that are continuously produced at low levels under basal conditions. Ionized radiation produces fibrotic responses and forms hydrogen atom radicals (H), OH, and hydrated electrons from ionization of water in tissues. All three of these radicals are highly reactive and can cause different ROS cascades to activate and amplify. Whole body radiation lowers endogenous antioxidant (SOD, catalase, and glutathione peroxidase mimics) levels in animals and humans and increases lipid peroxidation markers [19, 20]. Elevated oxidative stress has been reported in radiation pneumonitis in humans and radiation-induced lung injury in rats [21]. It has been shown that both catalytic antioxidants (SOD, catalase, manganese-metaloporphyrins) and antioxidant scavengers (Vitamin E, NAC, amifostine, curcumin, ginkgo biloba) alleviate radiation-induced lung injury and fibrosis in animals [19]. In this present study, decreases in SOD and CAT activities, the most important endogenous antioxidant enzymes, and increases in MDA, a lipid peroxidation product, were detected in lung tissues of rats subjected to whole body irradiation.

Erdosteine is a thiol derivative used in the treatment of chronic bronchitis [22]. It contains a bifunctional sulfhydryl group released after metabolic transformation in the liver. Liberated sulfhydryl groups can trap mucus glycoprotein fibers and sweep away free radicals. It has been shown to inhibit oxidative damage in rats by increasing antioxidant enzymes, such as SOD, CAT, and GSH-Px [7].

Based on free radical scavenging activity, erdosteine has been reported to have beneficial effects in studies with chemotherapeutic agents, bleomycin-induced pulmonary fibrosis, cisplatin-induced renal failure, and doxorubicin-induced cardiomyopathy and hepatotoxicity [8-11].

A study conducted in rats demonstrated that erdosteine lowered the extent of radiation-induced cochlear damage but did not prevent progression [23].

Recently, a study investigating the protective effects of erdosteine against radiation-induced renal damage in rats was published. Erdosteine was shown to act as a strong scavenger of free radicals in order to prevent or ameliorate radiation-induced toxicity in kidneys of whole-body gamma irradiated rats with 5 Gy and provide considerable protection against radiation-induced inflammation [12].

In studies conducted with patients undergoing pulmonary radiotherapy, it has been reported that erdosteine may inhibit overexpression of plasma TNF-α after radiotherapy and may reduce the severity of lung injuries. Erdosteine can prevent and treat radiation-induced lung injuries [13].

In a study in which both lungs of rats were irradiated on the 30th day, at a single dose of 20 Gy with 6 MV photon using a linear accelerator, and 60 days later, the ratio of macrophages, lymphocytes, and neutrophils in bronchialveolar lavage and radiation-induced histopathologic changes in lungs were evaluated. Erdosteine was reported to decrease neutrophil accumulation in the lungs after radiation but did not have an effect on histological changes [14].

In the present study, when erdosteine was co-administered with radiation, there was a decrease in MDA levels compared to the radiation group. SOD and CAT activities, however, were found to increase. In addition, when erdosteine was co-administered with radiation, the increase in CAT activity compared to the control was found to be statistically significant (12.9 vs. 8.4 U/mg protein, respectively). However, SOD activity was found to be higher than the control group (0.85 vs. 0.78 U/mg protein, respectively), although not statistically significant. Moreover, MDA levels were low (137.9 vs. 131.7 nmol/mg protein, respectively), although statistically significant, compared to the control group. Results indicate that erdosteine exhibits strong protective effects against radiation-induced lung injuries through free radical scavenging activity, increasing activity of the most important endogenous antioxidants, CAT and SOD, and decreasing levels of MDA, a lipid peroxidation product.

Conclusion

In conclusion, the pathogenesis of radiation-induced lung injury may involve a reduction in antioxidant enzymes and an increase in free radicals and lipid peroxidation products. Erdosteine may prevent radiation-induced lung injury by acting as an antioxidant that inhibits free oxygen radicals in the region due to its thiol groups.

Disclosure of conflict of interest

None.

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