Effect of edaravone on lungs and small intestine in rats with induced radiotherapy

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Radiotherapy is a frequently used method for treatment of cancer which is regarded as one of the top two diseases causing premature death worldwide. However, radiotherapy is known to have many side effects. In this study, we evaluated biologically and histologically the possible protective effects of edaravone, the free radical scavenger and neuroprotective agent used to treat amyotrophic lateral sclerosis, on lung and small intestine against radiation-induced early side effects of 15 Gy total body irradiation in single fraction. Thirty-two rats were divided randomly into four equal groups. Groups were administered 15 Gy of external ionizing radiation to the whole body after 30 minutes of EDA administration (a dose of 500 and 50 mg/kg). Rats were sacrificed at 72 h of the experiment. Tissues were separated to investigate levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPX) and malondialdehyde (MDA); and evaluate histopathological changings. The protective effect of EDA showed statistically significant in MDA, SOD and GSHPX values of lungs except CAT and statistically significant effect was observed in MDA in small intestine. Also, we showed statistically significant values with histopathological changings. Pediatric cancer patients who have a longer life expectancy are as important as their recovery from their normal life. We are expecting that EDA as a modulator of free radical scavenging pathways in many organs can reduce the side effects of radiation damage on lungs and small intestine.

Keywords: Cancer therapy, Free radical scavenging, Pediatric cancer, Tumor

Worldwide, cancer is reported to be the primary cause of premature death in every country\textsuperscript{1}. According to the World Health Organization (WHO), cancer is either first or second cause of death in majority of the countries. With an estimated 19.3 million new cases, cancer causes 10 million deaths across the globe\textsuperscript{2}. The global cancer burden by 2040 is estimated to be 28.4 million cases, 47\% rise from 2020\textsuperscript{2}. Currently, the average childhood cancer burden (<15 years of age) is reported to be 140 new cases per million children\textsuperscript{1}. In USA alone, approximately, one in 285 children are diagnosed with cancer each year, and at any one time, thousands of children are living with cancer\textsuperscript{3,4}.

Radiation therapy using ionizing radiation is one of the treatment approaches for cancer. It is a local treatment and has the advantage of being non-invasive and potentially organ preserving. It is an important therapy in the multimodal treatment for mostly seen childhood solid tumours in abdomen like neuroblastoma or Wilm’s tumor\textsuperscript{5,6} and thorax metastasis of Ewing sarcoma or Hodgkin lymphoma\textsuperscript{7}. Nevertheless, like other treatments, radiation therapy causes some side effects especially in the commonly used abdominal and thoracic region. The most early side effects of thoracic radiation therapy are difficulty of swallowing, shortness of breath, breast or nipple soreness, cough, fever, fullness of the chest, thyroid dysfunction and radiation fibrosis\textsuperscript{8-10}. Radiation fibrosis can engender permanent scarring of the lungs from untreated radiation pneumonitis. High radiation dose can also cause stenosis, occlusion or fistula formation and rib fractures resulting death\textsuperscript{11-14}. Intestinal epithelium renewal is rapid and hence the small intestine is reported to be radiosensitive\textsuperscript{15}. The most common side effects of radiation therapy to the abdomen are diarrhea, nausea, vomiting, constipation, abdominal pain, colitis, tenesmus, rectal bleeding, proctalgia, incontinence, loss of appetite and weight loss\textsuperscript{16,17}.

In this context, the necessity to improve outcomes, particularly for children with cancer looking at the
increased risk, invites attention of researchers on treatment methods or side effects. Some natural compounds and herbal extracts have also been found effective for radiation injuries. Since it is not always possible to treat early and late effects caused by radiation, it has become important to develop agents that can prevent side effects. Free oxygen radicals that are exposed to ionizing radiation have a negative effect on antioxidant defense mechanisms in the cell. It reduces the activity of especially from the antioxidant enzymes present in the cell which are; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPX). It is possible to prevent the damage by bringing radiotherapy to free radicals in normal cells using antioxidant agents.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a free radical scavenger and neuroprotective agent used to treat dementia, mania and amyotrophic lateral sclerosis, has been reported to exhibit potential antioxidant effects. Therapeutic effect is explained by its strong free radical scavenging property which has been proven in vivo and in vitro experimental studies. Edaravone produces useful effects on oxidative stress markers including SOD, malondialdehyde (MDA), GSHPX and CAT. Edaravone has also been shown to have antiapoptotic, anti-necrotic and anti-inflammatory effects. It is expected that edaravone will have protective effect against radiation damage as it is a substance with free radical scavenging properties.

Hence, in this study, we have made an attempt to evaluate biologically and histologically the possible protective effects of edaravone on lung and small intestine against radiation-induced early side effects of total body irradiation in single fraction.

Materials and Methods

Animals

Thirty-two female Sprague Dawley rats (10-11 weeks, weight range 250 ± 25 g) were maintained under standard conditions. All experimental studies were conducted at Karadeniz Technuel University Surgical Application and Research Center according to the guidelines for the ethical treatment of experimental animals. Local institutional approval for researchers was obtained before initiation of the study. The experimental protocol was approved by the Animal Care and Use Committee of Karadeniz Technuel University (Protocol No: 2016/20).

Experimental design

The rats were divided randomly into four equal groups and there were 8 animals in each group. The study groups were designed as follows. Gr. I (Sham group): Rats were given intraperitoneal (i.p.) 1.0 mL of saline and 30 min after the administration of 90 mg/kg of ketamine i.p., general anesthesia was administered, followed by 15 Gy of external ionizing radiation to the whole body under general anesthesia; Gr. II: Under general anesthesia the solution prepared by dissolving edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one; Sigma catalog no: M70800) in saline was administered i.p. at a dose of 500 mg/kg. After 30 min of EDA administration, the whole body was exposed to 15 Gy of external ionizing radiation; Gr. III: Under general anesthesia the EDA solution prepared at a dose of 50 mg/kg in saline was administered i.p. After 30 min of EDA administration, 15 Gy of external ionizing radiation was administered to the whole body; Gr. IV (Control group): Without any intervention sacrificed at 72 h of the experiment.

After 72 h, the lungs and small intestines were removed and some of them are washed with cold isotonic saline solution and fixed in formalin for histopathological examination. The other part is reserved for biochemical measurements and stored at −80°C.

Biochemical and Histopathologic assays

Some of the tissues were separated with the intention to investigate tissue levels of GSHPX, SOD, CAT and MDA. GSHPX activity were measured by spectrophotometric assay kit (Cayman chemical company, USA) according to the manufacturer’s instructions. SOD activities in tissues were measured by Sun and Oberley method. This method was based on reduction of nitroblue tetrazolium by xanthine-xanthine oxidase system. Formazone formation was assessed spectrophotometrically at 560nm. CAT activity was determined by the method of Aebi. This method is based on the principle that the absorbance at 240 nm decreases because of dismutation of H₂O₂.

Other tissue samples were fixed for 48 h in 10% formol for histopathological evaluation. Tissue fragments were made transparent by passing through graded alcohol series. Paraffin blocks of the tissues were prepared and sections with thickness of 5 μM were taken from the blocks with a fully automated microtome. The sections were stained with hematoxyline-eosin (H&E) after deparafinization, in order to evaluate inflammation, intraalveolar...
thickening, edema and capillary hyperemia in lungs and inflammation, epithelial spillage and necrosis and degenerative changes in the intestines. The evaluation has done that there was or not without grading. All samples were scored by pathologist blinded to experimental protocol and groups.

**Statistical analysis**

All values will be treated as mean ± Standard Deviation. Two-way ANOVA, Independent – Samples T-Test, Chi-Square and Duncan multiple comparison tests was used to analyze the differences between the groups. (5% Type I error, 10% Type II error, and 6% SD admission, the minimum number of rats required to achieve an 18% difference between groups was 8 for each group).

**Results**

The study was designed to evaluate the early radioprotective effects of EDA (high and low doses) on lungs and small intestine damage induced by total body irradiation to the rats as observed within 72 hours of administration.

**Biochemical results**

The protective effect of EDA showed statistically significant in MDA, SOD and GSHPX values of lungs compared to the control group (P <0.05). All the three groups, however, no statistically significant effect was observed in CAT results in irradiated lungs compared to the control (P >0.05).

There was only statistically significant effect was observed in MDA results in irradiated small intestine compared to the control (P <0.05). There was no statistically significant effect observed in the other oxidative stress parameters of irradiated small intestine (P >0.05). However, when we compared the groups both lungs and small intestine in term of doses of EDA (Gr. II with Gr. III), there were statistically significant results in only GSHPX (P <0.05).

**Histopathological damage**

The lungs of the animals in the Gr.I, II and III were affected by irradiation. The analysis of the irradiated lungs of rats with low and high dose EDA stained with H&E showed evident signs of inflammation, intraalveolar thickening and edema. The irradiated lungs of rats with high dose EDA results were a little better than the low dose EDA results and the photomicrographs of histological slides of rat lungs were shown in Figs 1 A-D 2 and 3. However, no statistically significant effect was observed in capillary hyperemia results in irradiated lungs of rats compared to the control (P >0.05). They all showed

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**Fig. 1** — Photomicrographs of histological slides of inflammation (marked with arrows) of rat lungs and small intestine. (A) Any inflammation from Gr. IV; (B) One positive of inflammation from Gr. II; (C) Two positive of inflammation from Gr. III; (D) Three positive of inflammation from Gr. I (sham group) (H&E 400X); (E) Any inflammation from Gr. IV; (F) One positive of inflammation from Gr. II; (G) Two positive of inflammation from Gr. III; and (H) Three positive of inflammation from Gr. I (sham group) (H&E 100X).

**Fig. 2** — Photomicrographs of histological slides of (A) any intraalveolar thickening from Gr. II; and (B) intraalveolar thickening (marked with arrows) of rat lungs from Gr. III (H&E 200X).
The analysis of the irradiated small intestine of rats with low and high dose EDA stained with H&E showed evident signs of epithelial spillage and necrosis from Gr. I and III shown in Fig. 4. Also again high dose EDA results were better than the low dose EDA results. Unfortunately no statistically significant effect was observed in inflammation results in irradiated small intestine of rats compared to the control (P > 0.05). All groups had inflammation and there was any enough response from EDA to the small intestines of rats (Fig. 1 E-H).

**Discussion**

In this study, we evaluated the early (within 72 h of administration) radioprotective effects of EDA (high and low doses) on lungs and small intestine damage induced by total body irradiation to the rats. The effect of EDA (low and high doses) therapy on the activities of antioxidant enzymes of GSHPX, SOD, CAT and MDA in the irradiated rat lungs was investigated. Our results have shown that EDA reduced the increased activities of GSHPX, SOD and MDA on lungs except CAT especially with high doses. However, only MDA results have shown statistically significant difference in irradiated small intestine of rats. MDA is considered to be the index of lipid peroxidation and as expected, the first and early impact was seen in MDA results here in this study. The ionized radiation causes an increase in free radicals in the biological system. These radicals act on the DNA, proteins and membrane lipids of the cell to form cell damage. In addition, our histopathological results of lungs have shown statistically significant effect in lungs and small intestine except capillary hyperemia (in lungs) and inflammation (in small intestine). In our histopathological observation of lung, we also evaluated in terms of alveolar thickness, edema and capillary hyperemia besides inflammation, since inflammation could also occur in normal healthy animals.

We observed that EDA reduced the inflammation, intraalveolar thickening and edema on lungs with high doses. Nevertheless we could not show the same effect on capillary hyperemia. Tajima et al.34 have shown that EDA alleviates the progression of lung injury and fibrosis. Also, Asai et al.35 demonstrated same effect of EDA on rabbit lungs by reflecting its ability to scavenge free radicals, which inhibits apoptosis and overexpression of transforming growth factor (TGF)-β. Our results have also shown statistically significant average on epithelial spillage and necrosis of small intestine with high doses of EDA between all experimental groups. On the other hand, inflammation of small intestine results have not shown any statistically significant average between the groups.

EDA clears various free radicals, and the irradiation produces hydroxyl, peroxyl and superoxide radicals. Free radicals are removed by antioxidant enzymes in living cells and antioxidant defences SOD, GPX,
CAT and glutathione. Many studies have shown that protective effect of EDA was associated with the increased SOD activity and CAT. EDA applied a protective effect in animal models of myocardial, lung, intestinal, liver, bladder, testis, pancreatic and renal injuries. EDA has also anti-apoptotic, anti-necrotic and anti-cytokine effects in many different diseases and reduces the release of adhesion molecules and markers of tumorigenesis. Hassan et al. suggested that EDA showed a potential protective effect in ISO (Isoproterenol)-induced myocardial infarction. Yoshizaki et al. have observed the benefical effects of EDA on fibrosis and evaluated for a potential use as an antifibrotic agent in systemic sclerosis. Ito et al. have found a protective effect of EDA against lung injury induced intestine ischemia-reperfusion injury.

Conclusion

The pediatric cancer patients who have a longer life expectancy are as important as their recovery from their normal life. We found the protective effect of EDA showed statistically significant in MDA, SOD and GSHPX values of lungs except CAT and statistically significant effect was observed in MDA in small intestine of rats. Also we showed improvement in histopathological chagings against radiation-induced in both the lung and the small intestines of rats. Finally edaravone is an effective drug for multiple organs and we expected to be able to reduce the side effects of radiation damage.

Conflict of Interest

Authors declare no conflict of interest.

References

The effects of edaravone in induced by intestinal ischemia/reperfusion in rats. 

Modulation of reactive oxygen species in pancreatic cancer. 


