The Effect of Zinc Sulphate in The Prevention of Radiation-induced Dermatitis

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Radiotherapy/Dermatitis/Zinc sulphate/Radioprotection.

There is currently substantial clinical interest in zinc (Zn) as a protective agent against radiation-related normal tissue injury. To further assess this drug’s potential, the effect of Zn was studied in rats using a radiation-induced skin injury model. Sprague-Dawley rats were divided into four groups. Group 1 received neither Zn nor irradiation (control group). Group 2 received 30 Gy of gamma irradiation as a single dose to the right hind legs of the rats (RT Group). Groups 3 and 4 received the same irradiation plus 5 mg/kg/day Zn (RT+5 Zn group) or 10 mg/kg/day Zn orally (RT+10 Zn group), respectively. The rats were irradiated using a cobalt-60 teletherapy unit. Acute skin reactions were assessed every three days by two independent radiation oncology experts. At the endpoint of the study, light-microscopic findings were assessed by two independent expert pathology physicians. Clinically and histopathologically, irradiation increased dermatitis when compared with the control group (p < 0.05). The severity of radiodermatitis of the rats in the RT+5 Zn and RT+10 Zn groups was significantly lower than in the RT group (p < 0.05); radiodermatitis was seen earlier in the RT group than in the other groups (p < 0.05). Zn was found to be efficacious in preventing epidermal atrophy, dermal degeneration such as edema and collagen fiber loss, and hair follicle atrophy. The most protection for radiation dermatitis was observed in the RT+10 Zn group. It would be worthwhile studying the effects of zinc sulphate supplements in radiation-treated cancer patients, in the hope of reducing radiation-induced toxicity.

INTRODUCTION

Since X-ray was discovered by Wilhelm Roentgen in 1895, radiation-induced dermatitis has been well-known.1) Though numerous tissues and organs are affected by radiotherapy, the skin covering all of the body is primarily affected.2) Injurious effects of radiation on the skin are a common side effect of radiotherapy and can be one of the dose limiting factors of radiotherapy.3) After exposure of the skin to relatively high doses of ionizing radiation, acute reactions such as, erythema, epilation, dry desquamation, with or without hyperpigmentation, moist desquamation, and erosions develop depending on radiation quality, the total dose, the dose rate, dose fractionation, the area or volume irradiated, anatomic site and its vascularity, and the age and general condition of the subject. Acute skin reactions develop depending on radiation damage to the germinative cells of the epidermis and its appendages.4)

Numerous researchers have determined the pivotal role of zinc (Zn) such as growth and development, maintenance and priming of the immune system, and tissue repair.5) In previous studies, zinc has been shown to have an antioxidant role.6–9) It is known that Zn has many biochemical functions including the maintenance of membrane structure and function and a special role in skin and connective tissue metabolism and in wound healing.7,10,11) Zn plays a part in the maintenance of epithelial and tissue integrity through promoting cell growth and suppressing apoptosis, and through its under-appreciated role as an antioxidant, protecting against free radical damage during inflammatory responses.11) For many years, zinc salts have been used both topically and orally to treat burns as well as to enhance wound repair in men and animals.12–14) From all these investigations, one can conclude that Zn has a number of beneficial effects on intact skin and wound healing. Zn also has a role as an antioxidant and an anti-inflammatory agent.7,8,11) At the same time, there is currently

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substantial clinical interest in Zn as a protective agent against radiation-related normal tissue injury.\textsuperscript{15–18} To further assess this drug’s potential radiation injury-preventive effects, these effects of Zn were studied in rats by using a radiation-induced skin injury model.

\textbf{MATERIALS AND METHODS}

\textit{Rats, Drugs and Irradiation}

Thirty-seven male Sprague-Dawley rats, 10–12 weeks old and weighing 180 \pm 20 g at the time of radiation, bred at Atat\text{"u}rk University Medical School, Department of Pharmacology Experimental Animal Laboratory, were used for the experiment. The local Ethical Committee approved the study protocol. The rats were quarantined for at least three days before irradiation, housed seven for control group and ten for the other group to a cage in a windowless laboratory room with automatic temperature (22 \pm 1°C) and lighting controls (14 hr light / 10 hr dark), and fed standard laboratory chow and water ad libitum.

The rats were divided into four equal groups. Group 1 received neither Zn nor irradiation (control group) but received both 1 ml saline orally and sham-irradiation. Group 2 received 30 Gray (Gy) of gamma irradiation as a single dose to the right hind legs of the rats plus 1 ml saline orally (RT Group). Group 3 received 30 Gy of gamma irradiation as a single dose to the right hind legs of the rats plus 5 mg/kg/day Zn orally (RT+5 Zn group). Group 4 received 30 Gy of gamma irradiation as a single dose to the right hind legs of rats plus 10 mg/kg/day Zn orally (RT+10 Zn group). The rats in the RT+5 Zn and RT+10 Zn group received 5 mg/kg/day and 10 mg/kg/day Zinc Sulphate (containing 50 mg zinc, Zinco 220 capsule, Berko İlaç, İstanbul) as diluted in 1-ml physiological saline through orogastric tube orally, respectively, starting from 2 days before irradiation and during 3 days after irradiation (total 5 days). Excess oral ingestion of zinc to the point of toxicity (100 to 300 mg/day) is rare. Zinc sulphate in amounts of 2g/day or more can cause gastrointestinal irritation and vomiting. 19) In our study, 5 and 10 mg/kg/day doses of Zinc Sulphate were calculated non-toxic doses for gastrointestinal tract in 70 kg (150 pound) of mankind. Prior to radiotherapy, the rats were anesthetized with 50 mg/kg ketamin HCl (Pfizer İlaç, İstanbul, Turkey) and placed on Plexiglas tray with the prone position. While the rats in the control group received sham-irradiation, the rats in the RT, RT+5 Zn, and RT+10 Zn group were irradiated using a cobalt-60 teletherapy unit (Picker-C 9, USA) from a source-to-surface distance of 80 cm, by 5 \times 5 cm anterior fields with 30 Gy of gamma irradiation as a single dose to the right hind legs. The dose was calculated at the depth of 1 cm on the right hind leg. The dose rate was 0.68 Gy/min.

\textit{Determination of Clinical Findings of Radiation-Induced Dermatitis}

Acute skin reactions were assessed every three days by two independent physicians, experts of Radiation Oncology, using the skin score system proposed by Abe et al.\textsuperscript{20} as follows: Grade 0= normal, Grade 0.5= slight epilation, Grade 1= epilation in an about 50% area, Grade 1.5= epilation in a more than 50% area, Grade 2= complete epilation, Grade 2.5= complete epilation with definite edema or dry desquamation in a more than 50% area, Grade 3= moist desquamation in a small area, and Grade 3.5= moist desquamation in most of the area.

\textit{Determination of Histopathological Findings of Radiation-Induced Dermatitis}

For the histopathologic study, at the endpoint of the study, the rats were anesthetized again with 50 mg/kg ketamin HCl (Pfizer İlaç, Istanbul, Turkey) and specimens of right hind legs were obtained from the irradiated field by doing a biopsy deeply and widely 1 cm. The tissue samples were fixed in 10% formalin. After routine processing, the tissues were imbedded in paraffin wax. Four-µm-thick slices were prepared and stained with hematoxylin and eosin for evaluation with light microscopy. Light-microscopic findings were assessed by two independent physicians, experts of Pathology. Damaged areas were evaluated using damage (epidermal atrophy, the findings of dermal degeneration such as edema and collagen fiber loss, and hair follicle atrophy) in terms of percentages, which were scored on a 5-points ordinal scale as follows; Grade 0= normal, Grade 1= minimal, Grade 2= mild, Grade 3= moderate, Grade 4= marked, and Grade 5= severe. The semiquantitative scores reflect the population examined as follows; Grade 1= < 5%, Grade 2= 6–20%, Grade 3= 21–50%, Grade 4= 51–75%, and Grade 5= 76–100%. This method is modified to assess the acute skin reactions from the light microscopic changes for radiation-induced cerebral tissue damage proposed by Erol et al.\textsuperscript{21}

\textit{Statistics}

In the study, we planned to evaluate the duration, severity and onset of dermatitis depending on radiotherapy. After necessary data had been collected, statistical analyses were made by using SPSS 11.0 packet programme (Statistical Package for Social Science; Windows version 11.0). The results were given as mean \pm standard deviation. In histopathological and clinical grading, the potential difference among groups was evaluated using Anova test, and statistical significance of differences between the groups was tested with the Mann Whitney-U test. p < 0.05 was accepted as statistically significant.
RESULTS

The drug was well-tolerated without toxic effects. There wasn’t any significant distinction among groups in the measurement of body weight carried out weekly.

Clinically and histopathologically, skin reactions of the two zinc sulphate groups were less clearly than those of the RT group.

Clinically, the finding of radiodermatitis (Grade 0.5) began in seven of 10 rats in the RT group and in three of 10 rats in the RT+5 Zn group on the 3rd day of postirradiation, whereas none of the rats in the RT+10 Zn group exhibited this finding on the 3rd day. This finding began in two of 10 rats in the RT+10 Zn group on the 9th day of postirradiation. There was a statistically significant difference in the starting days of radiodermatitis between the groups of RT+5 Zn and RT+10 Zn when compared with the RT group (p < 0.05). Complete epilation (Grade 2) became to appear in the RT group on the 12th day. While it began in the RT+5 Zn group on the 24th day, it appeared in the RT+10 Zn group on the 30th day. Moist desquamation (Grade 3) developed in two of 10 rats in the RT group on the 30th day. On the 36th day, that was the endpoint of the study, grade 3 and 3.5 radiodermatitis scores developed in six and in one of 10 rats in the RT group, respectively, whereas none of the rats in the RT+5 Zn group and RT+10 Zn group exhibited moist desquamation. In the analysis carried out among groups for radiodermatitis scores, in the RT+5 Zn and RT+10 Zn groups, there were significant decrease in severity of radiodermatitis when compared with the RT group, and continued until the moist desquamation was spotted. However, there was also a statistically significant difference in severity of radiodermatitis between the RT+5 Zn and the RT+10 Zn groups. This statistically significant difference between the RT+5 Zn and the RT+10 Zn groups started on the 15th day of postirradiation (Fig. 1).

In the histopathologic examination after the endpoint of the study, there were statistically significant differences between the control and the RT groups in terms of epidermal atrophy, dermal degeneration such as edema and collagen fiber loss, and hair follicle atrophy (p < 0.05). There were also statistically significant differences between the control and RT+5 Zn groups for all parameters (p < 0.05). While the differences in the RT+5 Zn and the RT+10 Zn groups were significant for all of these parameters when compared with the RT group, the differences between the RT+5 Zn and the RT+10 Zn groups were insignificant for dermal degeneration such as edema and collagen fiber loss (p > 0.05) but significant for epidermal atrophy and hair follicle atrophy (p < 0.05, p < 0.05 respectively). There was no significant difference between the control group and the RT+10 Zn group for all parameters (p > 0.05). In the RT+10 Zn group a significant protection was observed when compared with the RT and RT+5 Zn groups (p < 0.05, p < 0.05 respectively). When compared with the RT group, the histopathological examination results in the RT+5 Zn group were not better than in the control group, but in the RT+10 Zn group, these results were observed similar to those of the control group (Table 1; Fig. 2).

Table 1. Histopathological values in all groups (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Histopathological values</th>
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<tr>
<td></td>
<td>Epidermal atrophy</td>
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<tr>
<td>Radiotherapy</td>
<td>4.50 ± 0.70&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Radiotherapy plus 5 mg/kg/day zinc sulphate</td>
<td>2.20 ± 1.22&lt;sup&gt;b,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Radiotherapy plus 10 mg/kg/day zinc sulphate</td>
<td>0.80 ± 0.78&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>n</sup> = 7 for control group; 10 for the other groups
<sup>a</sup> p < 0.05 vs control group
<sup>b</sup> p < 0.05 vs radiotherapy group
<sup>c</sup> p < 0.05 vs radiotherapy plus 5 mg/kg/day zinc sulphate group
<sup>d</sup> p < 0.05 vs radiotherapy plus 10 mg/kg/day zinc sulphate group

Fig. 1. The time courses of the mean clinical skin score after 30 Gy irradiation. Each data point (±SE) represents an average of ten animals.
DISCUSSION

The goal of radiation treatment is to deliver completely measured doses of ionizing radiation to a defined tumor volume with the minimum accepted injurious effects of ionizing radiation to surrounding healthy tissue by eliminating tumor cells, giving a high quality of life and prolongation of survival at reasonable cost to cancer patients. Though numerous tissues and organs are affected by radiotherapy, the skin covering the whole body is primarily affected. Acute skin reactions such as, erythema, epilation, dry desquamation with or without hyperpigmentation, moist desquamation develop depending on radiation damage to the germinative cells of the epidermis and its appendages. Hebbar et al. reported that acute skin reactions started developing on the 4th day of post-irradiation after single dose of 35 Gy and increased with time in treatment groups. While Chen et al. reported that, at a single dose of 40 Gy, radiodermatitis scores progressed to moist desquamation in the rats in the radiation group; Murakami et al. reported that at 40 Gy dose of radiation developed complete epilation in all rats in the radiation group, without moist desquamation. In our study, the finding of radiodermatitis (Grade 0.5) began in seven of 10 rats in the RT group on the 3rd day of post-irradiation. Complete epilation (Grade 2) became to appear in the RT group on the 12th day of post-irradiation. Moist desquamation (Grade 3) developed in two of 10 rats in the RT group on the 30th day of post-irradiation. On the 36th day which was the endpoint of study, grade 3 and 3.5 radiodermatitis scores developed in six and in one of 10 rats in the RT group, respectively.

Fig. 2. The histopathological images of all groups in the present study. A: Normal histopathological image of the skin in the control group. Epidermal, dermal structures and hair follicles were intact; B: Radiation damage of the skin in the radiotherapy group. Radiotherapy group clearly reflected epidermal atrophy, dermal degeneration as edema and collagen fiber loss, and hair follicle atrophy. C and D: The groups, received radiotherapy plus sub-cutaneously either 5 mg/kg/day zinc sulphate (RT+5 Zn group) or 10 mg/kg/day zinc sulphate (RT+10 Zn group) respectively, reflected a radioprotection against radiation-induced skin damage in terms of epidermal atrophy, dermal degeneration such as edema and collagen fiber loss, and hair follicle atrophy. In the RT+10 Zn group was observed a perfect radioprotection as well as in the control group (H&E x 40).
Injurious effects of ionizing radiation on the skin are secondary to the production of free radicals, release of inflammatory mediators/cytokines. Therefore, previous experimental and clinical studies were carried out by setting off from base on that anti-inflammatory, antioxidant, and cytotoxic drugs were able to reduce injurious effects of irradiation on the skin. Consequently, abundant evidence has demonstrated the antioxidant and anti-inflammatory roles of Zn on the tissues, including the skin. It is known that Zn has several biochemical functions including the maintenance of membrane structure and function and a special role in skin and connective tissue metabolism and in wound healing.

According to the results of their experimental study, authors reported that Zn exerted a direct selective action on cancer cells, determining their death through apoptosis. In the present study, we demonstrated that Zinc Sulphate supplementation in patients with head and neck cancer protected against radiation-induced oropharyngeal mucositis. It has been reported that the use of topical zinc stimulated leg ulcer healing by enhancing re-epithelialization, decreasing inflammation and bacterial growth. For many years, zinc salts have been used both topically and orally to treat burns as well as to enhance wound repair in men and animals, because of their anti-inflammatory effects.

Currently, there are increasing evidences, from human and experimental studies, suggesting that Zn could be a beneficial agent in the protection against radiation-related normal tissue injury. Zinc salts are a new class of radioprotectors against total body irradiation lethality. In a recent study, we demonstrated that Zinc Sulphate supplementation in patients with head and neck cancer protected against radiation-induced oropharyngeal mucositis. It was reported that metallothionein induction by Zn was a highly effective approach in preventing cardiotoxicity and hepatotoxicity caused by daunorubicin in the rats. According to the results of their experimental study, authors suggested that the use of Zn in the chemotherapy of cancer patients was able to reduce daunorubicin-induced cardiotoxicity and hepatotoxicity. In addition, Provincialis et al. reported that Zn exerted a direct selective action on cancer cells, determining their death through apoptosis. At the same time, Zn protects against the apoptosis induced by diverse physical, chemical, or immunologic stimuli. It means that Zn may not only protect intact tissues by decreasing apoptosis induced by injurious conditions, but also increases apoptosis with a direct selective action on cancer cells. Briefly, Zn as a radioprotector agent may protect intact tissues against injurious effects of cancer treatments, as chemotherapy or radiotherapy, without an inhibitor effect against their therapeutic effects. These are some of other reasons why we used Zinc Sulphate as a potent radioprotector agent in the present study.

In conclusion, Zinc Sulphate seems to have beneficial effects on postponing the start of radiodermatitis and decreasing the severity of radiation-induced dermatitis. These results are pioneer to studies that will be performed with Zinc Sulphate to protect from radiation toxicity. It would be worthwhile studying the effects of zinc sulphate supplements in radiation-treated cancer patients, in the hope of reducing radiation-induced toxicity.

REFERENCES


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