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Preventive effect of selenium pretreatment against cisplatin-induced oral mucositis in rats

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ABSTRACT

Objectives. The aim of the present study was to investigate the protective role of selenium against cisplatin-induced oral mucositis in rats. ***Methods.*** Healthy wistar albino rats (n=21) were randomly divided into three groups: Cisplatin (Cis), cisplatin and selenium (Cis+Se) and control (C). Cisplatin was administered for 3 days to Cis and Cis+Se groups. Cis+Se group received selenium 5 days before cisplatin injection and continued for 11 consecutive days. Malondialdehyde (MDA) levels of the rats were determined before and at the end of experimental protocol. The tongues of rats were harvested for immunuhistochemical examinations. ***Results.*** In biochemichal analysis, MDA levels significantly increased in the Cis group (p=0.018). On the contrary, there was no significant difference between pretreatment and posttreatment MDA levels in Cis+Se group (p=0.128). In the immunohistochemical examinations of lingual tissues stained with anti-caspase-3, when the Cis and Cis+Se groups were compared, there were significantly more immunopositivity in the Cis group and significantly less immunopositivity in the Cis+Se group (p<0.05). ***Conclusions.*** Selenium might have played a protective role against cisplatin-induced oral mucositis in rats. Further studies are necessary before its clinical use in patients.

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Keywords: Cisplatin; oral mucositis; selenium; toxicity

Introduction

Oral mucositis is a common side effect of chemotherapy and radiotherapy regimens and has negative effects on patient's quality of life. It may cause swallowing and nutrition difficulty on patients; accordingly it may require parenteral nutrition by leading to anorexia, cachexia, dehydration and malnutrition [1]. Oral mucositis results in chewing, swallowing and speech difficulties due to edema and inflammatory lesions in the mouth. In some patients these side effects may necessitate termination or reduction of the chemotherapy dose [2]. Due to increased preference of chemotherapy and radiotherapy especially in treatment of patients with head and neck malignancy, mucositis is a serious

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problem in these patients and needs for the solution of this problem increased in recent years [3]. Cisplatin is an effective chemotherapeutic drug for a variety of malignant solid tumors including head and neck cancers, lung cancers, esophageal cancers, osteosarcoma, urogenital system cancers, central nervous system tumors, and neuroblastoma [4, 5]. The well-known toxicities of cisplatin are nephrotoxicity, myelotoxicity, neurotoxicity, ototoxicity, and gastrointestinal toxicity. Oral mucositis is one of the toxic effects of cisplatin. The rate of oral mucositis is increased when combined with radiotherapy. Generation of oxidative stress and reactive oxygen species by cisplatin is the primary accepted event in most pathways leading to mucositis and other side effects [6, 7]. Therefore, the effects of various antioxidants were evaluated for the prevention of cisplatin-induced oral mucositis [8]. But any accepted treatment modality entered into clinical use has not been developed yet.

Selenium plays an important role in cellular redox state regulation and essential to the function of glutathione peroxidase, because it is a structural component of the active site of selenoenzyme which has critical role in protecting cellular components from oxidative damage [6-8]. Although protective effect of selenium against some side effects of cisplatin has been shown previously, as much as we know, this is the first study investigating the efficacy of Se against cisplatin-induced oral mucositis.

Methods

Animals and experimental design

The study's protocol was approved by the institutional animal care and use committee, and the animals were treated in accordance with protocols approved by this committee. Twenty-one adult male Wistar albino rats weighing 280-300 g were used. All of the rats were maintained under conditions of 12 h light/dark cycle environment (lights on 07:00 AM-07:00 PM) at a temperature of 22±1°C and 50% humidity. Rats had free access to food and water ad libitum. The rats were randomly divided into the following three groups (n=7 in each group): Cisplatin (Cis), cisplatin and selenium (Cis+Se) and control (C). Cisplatin (Sigma–Aldrich Co, Germany) was administered to rats in the Cis and Cis+Se groups at a dose of 12 mg/kg body weight/day, intraperitoneally for 3 consecutive days. Moreover, 3 mg/kg body weight/day selenium (sodium selenite, Sigma–Aldrich Co, Germany) was given by oral gavage to rats in the Cis+Se group twice a day as 1.5 mg/kg for 11 consecutive days, starting 5 days before cisplatin administration. On the other hand, the C group received only saline intraperitoneally and orally at the same volume and the same time. Before and after treatment, blood samples of animals were collected for biochemical analysis. At the end of the experimental protocol, the animals in all groups were sacrificed under anesthesia using 30 mg/kg of ketamine (Ketalar®, Eczacibasi, Istanbul, Turkey) and 4 mg/kg xylazine (Rompun®, Bayer, Istanbul, Turkey). Their tongues were excised for pathological and immunohistochemical examinations. All surgical procedures were performed with sterilized instruments.

Lipid peroxidation assay

Malondialdehyde (MDA) values were measured for the evaluation of lipid peroxidation. Thiobarbituric acid (TBA) reaction was used as described previously in the literature (16). The principle of the method depends on the evaluation of the pink color appeared by the TBA and MDA interaction. The pink color absorbance was measured at 532 and 520 nm. MDA levels were measured as nmol/mg protein.

Histological and immunohistochemical analysis

The tongues of the rats were fixed in 10% neutral buffered formalin for 24 h at +4C temperature. Afterwards, the specimens were embedded in paraffin and then mounted in order to obtain multiplane cuts. Sections of 4 μm of thickness were collected on glass slides and stained with haematoxylin and eosin. Immunohistochemical staining was performed by using the DAKO Autostainer Universal Staining System (Autostainer Link 48 DAKO, Glostrup, Denmark). Firstly, the sections with the thickness of 4 μm were taken onto positively charged lames. Then, the sections, which were de-paraffinised with xylol, were exposed to an alcohol series and dehydrated. Afterwards, antigen retrieval was conducted in the thermostatic bath at 96°C (10 mM/L citrate buffer, pH: 6) for 40 minutes (PT Link). Sections were incubated with Caspase-3 (CPP 32-Novocastra™ Lyophilised Mouse Monoclonal Antibody Product Code; NCL-(CPP 32): by using the tonsil tissue recommended by the Premier antibody manufacturer as a positive

control tissue) for 60 minutes. An automation system was used with the Streptavidin-biotin immunoperoxidase technique (K8000 Envision Flex, DAKO, Glostrup, Denmark). Immunoreactions were shown with Diaminobenzidin tetrachloride (DAB) in order to obtain the view that would give the color. Negative staining was applied to the sections with haematoxylin for ground staining. Sections were respectively exposed to an alcohol series at increasing proportions for dehydration and covered with balsam in the automatic tissue closing device after the transparentation in xylol. Following the staining step, the sections were examined by only one pathologist, blind to clinical information, under the light microscope (Olympus BX51, Tokyo, Japan) and at the magnifications 4, 10, 20 and 40. Considering the number of the cells stained without taking the staining density into account, the following assessment was made in the histopathologic examination: 0=no staining (less than 5% of the cells stained, +=slight (5-10% of the cells stained), ++=medium (11-20% of the cell stained), and +++=severe (staining of more than 20%).

Statistical Analysis

Data analysis was performed using the statistical package for the social sciences (Version 16; SPSS, Chicago, IL). Values of $p<0.05$ were considered statistically significant. Mann-Whitney U and Kruskall-Wallis tests were used in the comparison of inter group measurements. Comparisons of pretreatment and posttreatment measurements were performed by Wilcoxon signed-rank test.

Results

The MDA levels measured before and after treatment are shown in Table 1. In biochemichal analysis, MDA levels significantly increased in the Cis group rats ($p=0.018$). On the contrary, the treatment with selenium decreased the elevation of MDA levels in Cis+Se group rats and there was no statistically significant difference between pretreatment and posttreatment MDA levels in this group ($p=0.128$) (see Table 1).

In the immunohistochemical examinations of lingual tissues stained with anti-caspase-3 antibody, there were no immunopositive cells in the lingual tissues of the control group, however, a significantly higher degree of immunopositivity was found in tissues of the Cis group. In the Cis group, 14.2% of specimens showed no staining, 42.9% of specimens showed medium staining (+), and 42.9% of specimens showed medium staining (++). Fewer immunopositive cochlear cells were found in the Cis+Se group compared to the Cis group. In the Cis+Se group, 71.4% of specimens showed no staining (0), 28.6% of specimens showed slight staining (+). When the Cis+Se group and Cis group were compared, significantly less immunopositivity occurred in the Cis+Se group ($p<0.05$). Semi-quantitative scores and evaluation for the immunopositive cochlear cells are presented in Table 2.

Discussion

Oral mucositis frequency reported in the literature varies between 30-40% and 100% in different series. Since there are no standardized scoring systems, there are no sufficient data about prevalence and incidence of oral mucositis based on chemotherapy and/or radiotherapy [9, 10]. In patients undergoing hematopoietic system transplantation, this rate is about 65-85%, in conventional chemotherapy patients 20-40%, and in head and neck radiotherapy patients it is about 100% [11, 12]. Severity and duration of

Table 1. Pre- and posttreatment malondialdehyde values of groups

	Cis group (n=7)	Cis+Se group (n=7)
Mean MDA value, nmol/mg (Pretreatment)	12.60	15.40
Mean MDA value, nmol/mg (Posttreatment)	20.67	16.00
p	0.018*	0.128

*Wilcoxon signale-ranked test. Cis=cisplatin, Cis+Se=cisplatin+selenium

Table 2. Evaluations and semi-quantitatively scoring of immunopositive cells in the lingual tissues of all groups

	Cis group		Cis+Se group		C group		Total	
	n	%	n	%	n	%	n	%
0	1	14.2	5	71.4	7	100.0	13	61.9
+	3	42.9	2	28.6	0	0.0	5	23.9
++	3	42.9	0	0.0	0	0.0	3	14.2
+++	0	0.0	0	0.0	0	0.0	0	0.0
Total	7	100.0	7	100.0	7	100.0	21	100.0

Cis=cisplatin, Cis+Se=cisplatin+selenium, C=control, n=the number of rats, 0=no staining, +=slight staining, ++=medium staining, +++=severe staining

mucositis varies depending on the received chemotherapy and concomitant radiotherapy. The risk factors in development of oral mucositis are based on chemotherapy drug and dosage and mode of therapy (radiation, chemotherapy, or combined chemo-radiotherapy) applied to patient. Due to morbidity caused by oral mucositis in patients having cancer therapy, many agents have been tried for prevention of this toxicity. One of the drugs used to prevent mucositis development is a free-radical scavenger amifostine. It is an agent showing mucoprotective effect by reducing salivary gland damage and oral dryness. However results of studies conducted with amifostine are conflicting [13, 14]. Other agent studied for prevention of mucositis is N-acetyl cysteine having antioxidant activity. It is shown that N-acetyl cysteine reduces the incidence and duration of severe mucositis [15]. Palifermin (keratinocytes growth factor 1) is an agent used to prevent from formation of oral mucositis. Ability of palifermin to inhibit mucosal damage capability depends on its effects on glutathione activity also it reduces oxygen-free radical damage by upregulating palifermin NRF 2 and exhibit anti apoptotic effect [16]. In addition to palifermin, studies for prevention of mucositis continue on growth factors such as sargramostim, filgrastim, and velafermin. Although there are studies conducted on drugs that increase TNF production and inflammatory cytokines such as benzydamine HCL and pentoxifylline, results are controversial [17]. Despite all these studies oral mucositis continues to exhibit serious toxicity that may increase morbidity during treatment of cancer patients and lead to disruptions in the treatment process. Therefore there is a need for new studies on the subject and the development of new treatment modalities.

The mechanism of cisplatin-induced oral mucositis is not completely understood, however many studies have suggested that toxic effects of cisplatin are related to the depletion of antioxidant enzymes (glutathione reductase, superoxide dismutase and glutathione peroxidase) with an increase in lipid peroxidation (MDA levels) [18]. Increased MDA levels may activate caspases pathway resulting in breakdown of DNA and apoptosis [19]. Caspase-8 and caspase-9 activates caspase-3, resulting in chromosomal DNA fragmentation and the cellular morphological changes of apoptosis. Activated caspase-3 is important for morphological changes in apoptotic cells and plays a pivotal role in the terminal phase of apoptosis [20, 21]. The results of this study showed that, the administration of cisplatin resulted in an increase in MDA levels, which is partially inhibited by selenium pretreatment. Moreover cisplatin treatment increased caspase-3 expression, which was significantly inhibited by selenium treatment, suggesting that selenium decreased cisplatin-induced lipid peroxidation and cell death in the oral mucosal tissues of rats.

Conclusions

To our knowledge, this is the first study that investigates the preventive effect of selenium against cisplatin-induced oral mucositis. It is our opinion that selenium might have played a protective role against cisplatin-induced oral mucositis in rats, as shown with biochemical and immunohistochemical analyses. Although the results confirmed that selenium reduced cisplatin-induced apoptosis in the oral mucosal tissues of rats and prevented oral mucositis, its clinical utility in patients remains uncertain. Finally, further studies before its clinical use against cisplatin-induced oral mucositis in patients are required.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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