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Original Research Article

The effects of astaxanthin on salivary gland damage caused by cisplatin in the rat

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ABSTRACT

Background: Cisplatin is a potent antineoplastic agent widely used for a variety of malignancies. However, it has many dose-limiting side effects such as neurotoxicity, cytotoxicity and ototoxicity. The aim of our study was to determine the effectiveness of astaxanthin (ASX) as a cytoprotective agent against cisplatin-induced cytotoxicity in the submandibular glands of rats.
Methods: Thirty-six adult male Wistar albino rats were divided into six groups as follows: group I: saline control; group II: 75 mg/kg/day ASX; group III: 16 mg/kg cisplatin; group IV: 25 mg/kg/day ASX + cisplatin; group V: 75 mg/kg/day ASX + cisplatin; group VI: olive oil + cisplatin. In all groups, submandibular gland histopathological and histochemical investigations were done using a light microscope. Every rat section was semi-quantitatively scored. Neutrophil infiltration density, myoepithelial cell density in the degeneration area, degenerative granular duct cell density, degenerative seromucous acinus cell density, and changes in the content of the secretory granules of seromucous acini and granular ducts of the parenchyma and stroma were calculated.
Results: The results of the analysis of the mean acinus area of the submandibular gland revealed that there was a significant decrease in cisplatin group rats when compared to control rats ($p<0.05$, $p=0.00$). However, there was no significant difference between-group IV, V and control group in terms of mean acinus area. ($p=0.541$, $p=0.773$). The results of the analysis of the mean ducts area of the submandibular gland also showed that there were significant increased group III compared to control rats ($p<0.05$, $p=0.031$). There was no significant difference between-group IV, V and control group in terms of mean ducts area ($p>0.05$, $p=0.921$). Similarities were observed in the mean ducts area with the group IV and the group V ($p>0.05$, $p=0.571$).
Conclusions: These results suggest the possibility that the clinical use of ASX could reduce or prevent damage to the salivary gland of patients receiving cisplatin chemotherapy.

Keywords: Astaxanthin, Cisplatin, Protective effect, Submandibular gland

INTRODUCTION

Chemotherapy has been indicated for the treatment of various tumours or prior to radiotherapy and surgery. However, chemotherapeutic agents can lead to morphologic damage in salivary gland tissue. These therapeutics may lead to directly or indirectly changes in oral mucosa and minor salivary glands.[1,2] The mucositis status secondary to the stomatotoxic effects of chemotherapeutic agents increases the morbidity and cost of treatment in cancer patients. Swallowing dysfunctions and altered saliva production are common sequelae of

radiation with or without chemotherapy in the management of head and neck cancers. This complication also significantly affects the duration of hospitalisation and survival rates.[3,4]

Cisplatin is a potent anticancer agent widely used for a variety of human neoplasms. However, it has many dose-limiting side effects such as neurotoxicity, cytotoxicity and ototoxicity.[2,5-7] Astaxanthin (ASX) is a red carotenoid pigment, which is mainly found in certain marine animals such as microalgae, fishes and shrimps. ASX has certain pharmacological properties, including antioxidative, antitumor, anti-inflammatory, antidiabetic, hepatoprotective and immunomodulatory.[8-13] The aim of our study was to determine the effectiveness of ASX as a cytoprotective agent against cisplatin-induced cytotoxicity in the submandibular glands of rats.

METHODS

The study was approved by the University Ethics Committee on Animal Research (2014/66). The study was conducted at the Experimental Animal Study Laboratory of Recep Tayyip Erdogan University. In this study, Thirty-six adult male Wistar albino rats weighing approximately 250-280 g and 3-3.5 months of age were used. The animals were kept in 12h light/dark cycles and controlled conditions (22±3ºC temperature, 55-60% relative humidity). All had free access to pulverised standard rat pellet food and tap water. Cisplatin (cisplatinum, Ebewe, 1 mg/ml) was obtained from Liba Drug Company, (Istanbul, Turkey), while AST (CAS 472-61-7), purity 98%, was purchased from Sigma Chemicals (St. Louis, MO, USA). ASX was dissolved in olive oil just before use (5 mg/ml).

Experimental design

The animals were randomly divided into six groups of six rats per group. Group I: received no cisplatin or ASX; group II: received 75mg/kg/day ASX ASX for ten days; group III : administered only cisplatin at 16mg/kg/day intraperitoneally single dose; group IV: administered intraperitoneal cisplatin at 16mg/kg/day, and 25 mg/kg/day ASX (5mg/ml, dissolved olive oil) intraperitoneally for 7 days prior to cisplatin injection and for three days after cisplatin injection; group V: administered intraperitoneal cisplatin at 16mg/kg/day for one day, and 75mg/kg/day ASX (5mg/ml, dissolved olive oil) intraperitoneally for 7 days prior to cisplatin injection and for 3 days after cisplatin injection; group VI: received olive oil + cisplatin.

Histological analysis

Submandibular gland excision was then performed; the submandibular gland tissues of rats were given code numbers and put into bottles containing 10% neutral formaldehyde. After 72 hours, the following tissue process was applied to these tissues: dehydration in an increased alcohol series, clearing through a xylene series, immersion in liquid paraffin, and embedding in paraffin blocks. From the paraffin blocks of each rat, four 5-µm serial sections with intervals of 50 µm were taken using a microtome (Leica RM2125RT, Nussloch, Germany).

The obtained sections were brought from deparaffinisation to the water and stained with haematoxylin and eosin (H&E). Later the cover-slipped sections were photographed with a camera attached to a light microscope (Nikon Eclipse E600, Japan). Management of the same light settings was performed for photographing, especially in the histochemical analysis, in order to permit unbiased evaluation.

Semi-quantitative analysis

Histopathological and histochemical investigations were done using a light microscope. Every rat section was semi-quantitatively scored. For each section, five microscopic areas, nearly 100 µm2, were selected randomly. Neutrophil infiltration density, myoepithelial cell density in the degeneration area, degenerative granular duct cell density, degenerative seromucous acinus cell density, and changes in the content of the secretory granules of seromucous acini and granular ducts of the parenchyma and stroma were calculated at X10 objective. The arithmetic mean of the histopathological evaluation was scored semi-quantitatively. The scoring was labelled as follows: none= –, mild= +, moderate= ++, severe= +++.

Stereological analysis

In this study, the mean granular duct and seromucous acinus areas were calculated using the nucleator method, one of the stereological methods with an unbiased counting frame. The Stereo Investigator (MicroBrightField 9.0, Colchester, VT, USA) software system was used. This system consists of a camera attached to a light microscope, a motorised system that carries a microscope tray, and a computer with a software system. H&E-stained sections were put on the microscope tray, and their sectional boundaries were deter¬mined using this program. After determining the area, frames separated from each other were determined by systematic random sampling of the sections, per the rules of space fragmentation with the step interval of the x- and y-axis. Then, in 20 different selected areas, the mean areas of the seromucous acini and granular ducts of all groups were measured.

Statistical analysis

Statistical analysis of the mean area of the seromucous acini and granular ducts of all groups was performed using SPSS (IBM SPSS Statistics 21.0, IBM Corporation, Somers, NY, USA). Because the data showed a normal distribution with the coefficient variables which was more than 20%, differences between the groups were

tested using one-way analysis of variance (ANOVA) followed by a Duncan test. The numerical data of groups was also analyzed (a p-value <0.05 was selected as significant). The values were determined as means±standard deviation.

RESULTS

Histological results

In the submandibular gland tissues of the control group, the structure of the seromucous acini, ducts and connective were observed to be normal (Figures 1A-B). In the cisplatin group, the presence of intense substitution of the parenchyma by fibrous tissue was detected. Moreover, there were excessive neutrophil cell infiltrations into degenerative connective tissue (Figures 1C-D). Light microscopic sections obtained from submandibular gland tissue of subjects belonging to the sham group, in which the submandibular gland was limited irregularly, were similar to the cisplatin group. Remarkably, intense acinar atrophy and neutrophil cell infiltrations were detected (Figures 1 E-F). Submandibular gland histology in the ASX 25 mg and ASX 75 mg groups was normal in structure; seromucous acini, ducts and connective tissue were normal (Figures 1 G-J).

Regular normal submandibular gland histology was seen in the cisplatin+asta 25 mg treated group, but there were minimal neutrophil cell infiltrations into the connective tissue (Figures 1 K-L). In the cisplatin+asta 75 mg treated group, normal submandibular gland histology was seen (Figures 1 M-N).

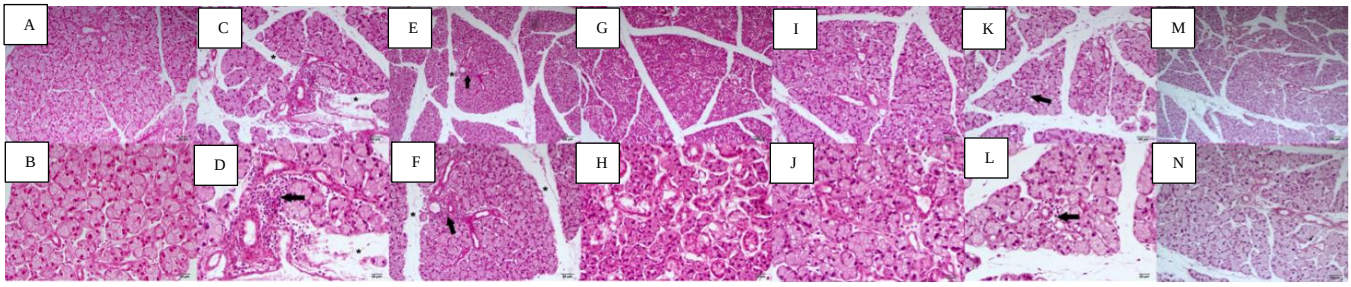

Figure 1: Micrograph of parotid gland in the lower and higher magnification for all groups; A-B: control group; normal lobule and acinar structure; C-D: cisplatin group; excessive neutrophil cell infiltrations to connective tissue degenerative (arrow).

Stereological results

Table 1: Assessments of mean seromucous acinus area and mean granular duct area of all groups.

Groups	Mean seromucous acinus area (Mean±SD)	Mean granular duct area (Mean±Std. Deviation)
Control	707.73±70.17[a]	225.67±49,29[a]
Cisplatin	479.54±86.75[a,b]	317.48±71.41[a,b]
Sham	647.84±49.01[a]	252.02±41.71[a]
Asta 75 mg.	649.70±37.21[a]	201.14±17.35[a]
Cis+ASX 25mg.	641.56±75,01[a,b,c]	248.15±45.87[a,b,c]
Cis+ASX75mg.	636.27±79.12[a,c]	196.68±21.13[a,b,c]

Control: healthy group; Sham: Cis+Oil group; aP <0.05 versus control group; bP <0.05 versus Cisplatin group; bP <0.05 versus Cisplatin+Asta treatment group

The results of the analysis of the mean acinus area of the submandibular gland revealed that there was a significant decrease in cisplatin group rats when compared to control rats (p<0.05, p=0.00). However, there was no significant difference between-group IV, V and control group in terms of mean acinus area. (p=0.541, p=0.773). The results of the analysis of the mean ducts area of the submandibular gland also showed that there were significant increased group III compared to control rats (p<0.05, p=0.031). There was no significant difference between-group IV, V and control group in terms of mean ducts area (p>0.05, p=0.921). Similarities were observed in the mean ducts area with the group IV and the group V (p>0.05, p=0.571) (Table 1).

DISCUSSION

Mucositis is a serious problem that increases morbidity and the cost of treatment of cancer patients with chemotherapy or radiotherapy. Reduced saliva secretion is the main factor increasing patient susceptibility to mucotoxic effects. Most chemotherapeutic agents, such as cisplatin, are assumed to worsen salivary gland functioning and increase the incidence of mucositis.[1] Both radiotherapy and chemotherapeutic agents may lead to morphologic damage in salivary gland tissue.[15,17] Cisplatin is a highly effective chemotherapeutic agent that is widely used to treat head and neck malignancies.

Despite its potent antitumour effects, cisplatin is known to cause serious side effects. Kitashima reported that an earlier experimental study described the structural damage caused by cisplatin in the submandibular gland cells. He showed that cisplatin-induced morphological changes in the submandibular gland of rats were observed in both acinar cells and the ductal system. On the fifth day after cisplatin infusion, a series of protein synthesis processes were damaged, resulting in a decrease in secretory granules. On the seventh day after infusion, the shape of the acinar cells remained irregular, but the number of secretory granules increased.[2] Hey et al reported that prospective non-randomized study the influence of concomitant radio-chemotherapy with cisplatin tends to cause a higher probability of complication compared to radiotherapy alone.[15] Yamamoto et al reported that, according to their in vitro experimental study, one can assume that cisplatin has enough potential to considerably damage the salivary gland tissue.[16] In the present study, we examined changes in rat submandibular glands after cisplatin infusion, and in the cisplatin group we detected the presence of intense substitution of the parenchyma by fibrous tissue was detected. Moreover, there were excessive neutrophil cell infiltrations into degenerative connective. Per these studies, cisplatin can reduce saliva production in at least two ways: initially, through blocking of aquaporine expression, or conventionally, by stabilizing DNA strands. The latter especially prevents the regeneration of glandular tissue when progenitor cells are damaged.[15] Another study, by Özel et al., determined that the first sign of the effect of 5-FU on acinar cells in the submandibular gland was changes in cytoplasmic secretory granules, according to histopathological examination with a light microscope. The damage caused by these cytoplasmic secretory granules should be considered among the mechanisms that lead to cell apoptosis.[3] In the present study, we examined the protective effect of ASX as a cytoprotective agent against cisplatin-induced cytotoxicity in the rat submandibular gland of rats. ASX is known as a powerful antioxidant and an inhibitor of oxygen radical-mediated lipid peroxidation. The activity of ASX has been reported to be approximately 10 times stronger than that of other carotenoids including zeaxanthin, lutein and canthaxanthin, and 100 times greater than that of alpha–tocopherol.[9-12] Our results indicated that regular normal submandibular gland histology was seen in the cisplatin+ASX 25 mg treated group, but there were minimal neutrophil cell infiltrations in the connective tissue. In the cisplatin+ASX 75 mg treated group, normal submandibular gland histology was seen.

CONCLUSION

These results suggest the possibility that the clinical use of ASX could reduce or prevent damage to the salivary glands of patients receiving cisplatin chemotherapy. However, additional research and prolonged observation are needed.

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