EGYPTIAN DENTAL JOURNAL
VOL. 63, 3279:3285, OCTOBER, 2017
I.S.S.N 0070-9484
ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY
www.eda-egypt.org • Codex : 154/1710

EFFICIENCY OF FISH OIL IN PROTECTION OF PERIODONTAL LIGAMENT AND MANDIBULAR BONE OF RATS TREATED WITH CISPLATIN AND GAMMA RADIATION

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ABSTRACT

Head and neck cancer is commonly treated with surgery, chemotherapy, radiotherapy either alone or in combination. The purpose of this study is to evaluate the efficiency of fish oil (FO) to preserve periodontal ligament and mandible in gamma irradiated albino rats treated with cisplatin (CP). Thirty male Albino rats were randomly divided into five groups; group C; served as control, group CP; rats were given a single dose of CP (10 mg/ kg body weight i.p), group R; rats were exposed to a single dose of 0.7 Gy γ-rays, group CP + R; rats were given CP and after 24 hours exposed to γ-rays, and group FO+ CP + R; similar to the previous group except for administration of FO (2 ml/ kg body weight) orally and daily for 12 consecutive days before CP injection. The mandibles were collected one day post irradiation and stained with hematoxylin and eosin for histological and histomorphometric analysis. CP and radiation alone or in combination induced bone resorption, degeneration of periodontal ligament fibers. Histomorphometric analysis revealed significant decrease of osteocyte number, fibroblastic and collagen area % and increase of periodontal ligament width. FO protected group showed normal histological appearance of both periodontal ligament and bone. In addition, histomorphometric data revealed normal values of all studied parameters. CP and/ or gamma radiation damage alveolar process and periodontal ligament probably due to osteocyte apoptosis, and supplementary treatment with FO may be potentially useful in protecting alveolar process and periodontal ligament during combined CP chemotherapy and radiotherapy.

KEY WORDS: Cisplatin; Gamma Radiation; Fish oil; Mandible; Periodontal ligamen

INTRODUCTION

Head and neck cancer is a wide range of neoplasms that develop within the upper digestive tract or the different glands in this region. Their treatments include surgery, chemotherapy, radiotherapy administered either alone or in combination.[1] Anticancer chemotherapy currently involves the use of drugs that inhibit proliferation of the tumor cells and/or cause their destruction,

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however the problem is the lack of selectivity of most chemotherapeutic drugs, since they also act upon normal cells.[2] Cisplatin (CP), a chemotherapeutic agent, is widely used for treatment of head and neck cancers.[3] However, its use is limited because of induced cytotoxicity that attributed to reactive oxygen species generation, cell cycle arrest and induction cell death via apoptosis.[4, 5] Radiotherapy kills cancer cells but also damages normal cells and affects their function, and bone is no exception.[6] The effects are mediated by the formation of free radicals, increased lipid peroxidation and cell death.[7]

Bone is a metabolically active tissue, whose activity is essential to keep tissue integrity and body homeostasis. Maintenance of this tissue controlled by a process called bone turnover.[8] Periodontal ligament (PL), a vascularized, richly enervated loose connective tissue, has a characteristic localization and structure to accommodate its function. It is responsible for assimilation of shock and trauma in addition to perception of proprioceptive stimuli. PL consists primarily of fibroblastic cells and collagen fibers mainly type I and III. The collagen fibers crossed throughout PL as thick bundles that insert in the cementum or in the alveolar process.[9] Fibroblasts are responsible for the renewal of teeth support structures and play a fundamental role in teeth adaptability towards mechanical loads.[10]

The goal of chemo-preventive agents is to increase the efficiency of chemotherapy while decrease the toxicity to normal tissues.[11] Fish oil (FO) mainly omega-3 polyunsaturated fatty acids such as docosahexaenoic acid and eicosapentaenoic acid were shown to exhibit chemopreventive and anti-apoptotic effect.[12, 13] In addition, FO reduced osteoclasts and pre-osteoclasts following pulp exposure,[14] reduced osteoclastic activity and alveolar bone resorption,[15] and reduced the gingival tissue levels of lipid inflammatory mediators in induced experimental periodontitis.[16]

So, this study aimed to investigate the efficacy of FO to reduce CP and gamma rays side effects on bone and periodontal ligament.

MATERIALS AND METHODS

Experimental animals

This study was approved to be carried out by the Committee of Scientific Ethics at Faculty of pharmacy, Al-Azhar University, Egypt, following the guidelines for animal use. Thirty adult male Sprague-Dawley rats weighing 200-250 gm were used in this study. They were attained from Nile Pharmaceutical Co, Cairo, Egypt. They were maintained in stainless steel cages in a well-ventilated animal house at temperature of about 22°C ± 5°C under a 12:12 h light–dark cycle for two weeks for adaptation. The rats were maintained on a standard diet and provided water *ad libitum*.

Irradiation

Rats exposed to whole body γ- irradiation with a single dose of 0.7 Gy, using cesium 137 irradiation units at the National Center for Radiation Research and Technology (NCRRT) with a dose rate of 0.43 Gy/min. The dose of 0.7 Gy was not so high to enhance the synergistic action of both chemo-radiotherapy and avoid radiation risk hazards.

Chemicals

CP was obtained from Pfizer Co. Egypt, and was injected intraperitoneally at dose of 10 mg/ kg body weight.[17] FO was purchased from the Arab Co. for Gelatin and Pharmaceutical Products, and was administrated orally at dose of 2 ml/kg body weight.[18]

Experimental design

Rats were randomly divided into five equal groups (6 rats each). Group C; control untreated group. Group CP; rats were given a single dose of CP (10 mg/ kg body weight i.p). Group R; rats were exposed to a single dose of 0.7 Gy γ-rays. Group CP + R; rats were given CP i.p. and after 24 hours exposed to a single dose of 0.7 Gy γ-rays. Group FO+ CP + R; rats were administered FO (2 ml/ kg

body weight) orally and daily for 12 consecutive days and then injected with CP i.p. after half an hour of the last dose of FO. Twenty-four hours after the CP injection, the animals were irradiated with 0.7 Gy γ-rays.

Histopathological and histomorphometric analysis

Animals were euthanasially decapitated 1 day post radiation exposure then the mandibles were collected and fixed in 10% formalin for 48 hours. Fixed specimens were decalcified in 10% formic acid. After processing the tissues in alcohol, they were embedded into paraffin wax and 5μ thick sections were cut and stained with hematoxylin and eosin.

The mandibular bone and related PL were examined by pathologists for histopathological alteration under light microscope with magnification × 400. Osteocyte number, fibroblast Cell Area%, collagen area% and PL width (μm) were measured in 5 histological fields (x400) in each slide randomly captured with a digitized image analysis system using the software Leica Qwin 500.

Statistical analysis

The data was analyzed using statgraphics X64 and expressed as mean ± SD. Statistical analysis was done using ANOVA analysis and comparison of results between groups was made using multiple range test. $P < 0.05$ was considered statistically significant.

RESULTS

Examination of alveolar bone of control mandible (group C) revealed normal bundle bone thickness with well-formed Haversian system. The osteocytes were normal in number, size and morphology. The nuclei were of normal size and stain. On the bone surface osteoblasts could be detected. The periodontal ligament was of normal width and consisted of well-arranged collagen fibers obliquely inserted in bone (Fig. 1, A).

The alveolar process of CP treated group showed marked decreased cellularity with areas of osteoblastic disruption. The osteocytes showed decreased number and size. Some lacunae were empty. The periodontal ligament was wide and showed vacuolization, fiber disarrangement and areas of fiber detachment (Fig. 1, B). Histomorphometric analysis revealed significant decrease of osteocyte number, fibroblast cell and collagen area % in addition to periodontal ligament widening as compared to control group (Table, 1).

The irradiated mandible (group R) showed almost normal thickness and morphology. The bundle bone showed normal Haversian system. The osteocytes were normal in size and morphology with decreased number. The nuclei were of normal size and stain. The PL showed normal thickness, mild vacuolization and well-arranged collagen fibers (Fig. 1, C). Data revealed significant decrease in osteocyte number, fibroblast cell and collagen area % but to lesser degree than CP group (Table, 1).

Combination of CP and irradiation (group CP+R) resulted in thinning of alveolar process. The bundle bone showed multiple areas of osteoclastic resorption and osteoblastic disruption. There were many decreased sized osteocytes in addition to many empty lacunae. The PL showed vacuolization, hyalinized fibers and area of degeneration (Fig. 1, D). Significant decrease of osteocyte number, fibroblast cell and collagen area % in addition to periodontal ligament widening was detected in comparison to control group (Table, 1).

The alveolar process of protected group (FO+CP+R) showed normal bone thickness and architecture. There was normal Haversian system. The osteocytes were normal in number, size and

morphology. The nuclei were uniform and of normal size and stain. The PL was of normal width and revealed normally arranged collagen fibers (Fig. 1,E). All studied parameters of bone and periodontal ligament were almost normal and more or less similar to the control group (Table, 1).

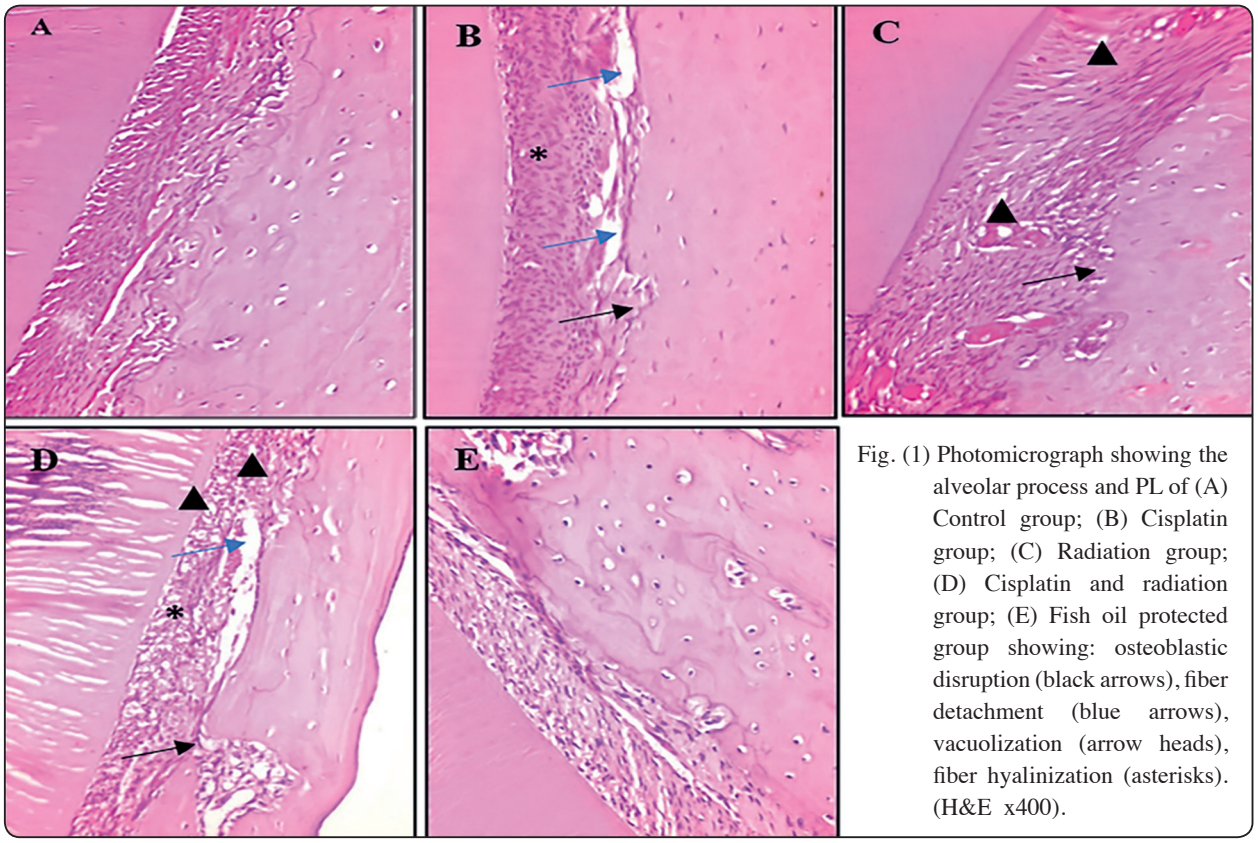

Fig. (1) Photomicrograph showing the alveolar process and PL of (A) Control group; (B) Cisplatin group; (C) Radiation group; (D) Cisplatin and radiation group; (E) Fish oil protected group showing: osteoblastic disruption (black arrows), fiber detachment (blue arrows), vacuolization (arrow heads), fiber hyalinization (asterisks). (H&E x400).

TABLE (1) Periodontal ligament and mandibular bone parameters of different studied groups.

Groups	Osteocyte No	Fibroblast Cell Area%	Collagen Area%	PL Width (μm)
C	43.7±2.5	3.59±0.25	32.38±1.3	215.28±12.8
CP	35.0±3.0*	2.15±0.35*	22.24±1.4*	283.67± 8.5*
R	39.3±1.5*	2.95±0.27*	27.53±1.5*	236.40±16.9
CP+R	27.7±2.5*	1.45±0.14*	15.91±1.2*	339.62±28.4*
FO+CP+R	47.3±1.5	3.14±0.29	34.27±1.4	222.04±36.9

**** Denotes a statistically significant difference ($P < 0.05$).***

DISCUSSION

The rat model was used because it is the most utilized for evaluating secondary effects of radiotherapy[19]. Several reports indicate that anticancer agents induce bone mineral loss in patients.[20, 21] CP is a potent chemotherapeutic antitumor agents against a variety of neoplasms, particularly for head and neck cancers.[22] CP produce hepatoxicity, nephrotoxicity but also the impairment of bone morphology and healing is reported as main side-effect. Ionizing radiation is an effective modality for the treatment of malignancies however; more effective treatment has meant experience of more long-term side effects caused by radiation damage to normal tissues near the tumor. Bones located within the irradiation field can absorb dose and have an increased fracture risk.[23] Many studies suggest that using protective agents in combination with chemotherapy and radiation can enhance their therapeutic efficacy and lower their toxicity to normal tissues.[24, 25]

Rats that received CP alone or in combination with radiation showed decreased thickness of alveolar bone, diminished cellularity, areas of osteoblastic disruption and small sized osteocytes in addition to some empty lacunae. Histomorphometric analysis revealed significant decrease of osteocyte number. These results were in accordance with those obtained by **van Leeuwen et al.,**[26] where CP decreased height of the proliferating layer and trabecular volume and **Xian et al.,**[27] who found that chemotherapy induced chondrocyte and osteocyte apoptosis, suppressed chondrocyte, osteoblasts and preosteoblasts proliferation and reduced total thickness and width of the growth plate. In addition, chemotherapy reduced bone volume, which was associated with an increased osteoclast formation and their bone surface density as well as a decreased osteoblast bone surface density.[28] The reduced number of osteocytes could be related to disruption of normal bone cell division by chemotherapeutic agents.[29]

Examination of gamma-irradiated mandible showed decreased number of osteocyte. Similar result was obtained by **Regezi and Sciubba,**[30] who found destroyed osteocytes in irradiated bone tissues. This may be attributed to damaged terminal vascular bed, inducing injury to the local microcirculation causes a decrease in the vascularization ending with osteocytes necrosis.[31]

In this study, treatment of rats with FO prevented the pathological changes in the form of increased number of normal sized osteocyte, restoration of osteoblastic layer compared with rats treated with CP and exposed to gamma radiation. This improvement could be attributed to reduction of TNF-a and bone resorption.[32] According to **Watkins et al.,**[33, 34] FO, which is rich in n-3 polyunsaturated fatty acids, stimulates bone formation and reduces bone resorption by up regulating osteoblastogenesis and reducing prostaglandin E2 synthesis in bone that stimulate osteoclast activity, resulting in secondary osteoporosis. In addition, omega-3 fatty acids modulate cyclooxygenase-2 protein expression and increase osteoblastic bone formation markers.[35] **Sun et al.,**[36] postulated that fish oil inhibit calcium excretion, and increase its absorption, causing inhibition of osteoporosis. FO intake increased bone mineral content, bone volume, trabecular thickness, mineral apposition rate at the endocortical surface and decreased serum concentration of tumor necrosis factor alpha and the rate of bone resorption after ovariectomy.[37]

CONCLUSION

These results suggest that use of CP alone or in combination with gamma radiation damage alveolar process and PL probably due to osteocyte apoptosis, and supplementary treatment with FO may be potentially useful in protecting alveolar process and PL during CP combined radiotherapy.

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