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Atorvastatin mitigates testicular injuries induced by ionizing radiation in mice

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ABSTRACT

Background: Radiotherapy in patients with pelvis malignancy causes testes irradiation and resulted in testicular damages. Atorvastatin (ATV) in the low-dose is considered as antioxidant and anti-inflammatory properties.
Objective: This experimental study was investigated protective effects of ATV on irradiation-induced testicular injury.
Material and methods: Sixty male balb/c mice were randomly divided into 6 groups: 1: control, 2: irradiated (IR), 3, 4 and 5: IR plus ATV (10, 20 and 50 mg/kg), 6: only ATV (50 mg/kg). The ATV treated groups were received ATV for 7 days via oral gavage before IR. Irradiated groups exposed to 2 Gy whole body X-ray on day 8. Biochemical, histological and immunohistological parameters were evaluated for radioprotective effect of ATV.
Results: In the ATV pretreatment in irradiated mice, MDA levels were significantly decreased compared with the IR group. The effect of all three doses of ATV caused reduced MDA level, but ATV to dose of 50 mg/kg had more effect than other doses of ATV. Significant decrease in the concentration of testosterone was observed in only irradiated mice compared with the ATV plus irradiated. In addition, the histological examination showed Johnsen Score in the IR group was lower compared to ATV pretreated groups. ATV significantly reduced caspase-3 immunoreactivity induced by irradiation.
Conclusion: The results from this study suggest that ATV at low dose has a protective effect against irradiation-induced testicular damage. This result provides a new indication of ATV for protection of testis during radiation therapy in treatment of cancer patients.

1. Introduction

Ionizing iradiation (IR) therapy is one of the most important strategy for treatment of various cancers [1]. Pelvic irradiation for the treatment of pelvic cancers can cause side effects on normal cells that have rapidly proliferating rate such as spermatogenic cells in testis [2]. So infertility is a common complication of cancer treatment with chemotherapies and radiation therapy in men and boys [3]. On the other hand, ionizing radiation generates oxidative stress that is a carcinogen [4]. Testis is a radiosensitive organ that having variety of cells is different in the degree of radiosensitivity. Spermatogonia cells are radiosensitive cells and are completely killed with IR at dose of 3 Gy [5]. IR induces apoptosis [6], reduced sperm count [7] and loss of testis weight [8] that lead to infertility. Radioprotective compounds are able to protect normal tissue against irradiation-induced damage through free radical scavenging and anti-inflammatory properties [9,10].

Statins are the first choice for lowering cholesterol [11]. Atorvastatin (ATV), the most common statin, with inhibitor of 3-hydroxy-3-methyl-glutarylcoenzyme A (HMG Co-A) reductase inhibits cholesterol [12,13]. ATV, with doses of 10, 20, 40, and 80 mg/day has been prescribed for the treatment of dyslipidemias

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[14]. In addition to the cholesterol-lowering activity, atorvastatin has therapeutic beneficial pleiotropic effects [15] that occurs at low doses. Several biological and pharmacological properties have been reported for ATV are including anti-inflammatory [16] and antioxidant properties [17–19], cardioprotection, nephroprotection and antitumoral activity, immunosuppressive [14,18,20–24]. On the other hand, high-dose atorvastatin has adverse effects such as nephrotoxicity [25], testicular pain [26], erectile dysfunction [27], modify of sperm parameters (total number of sperm, vitality and motility) and seminal fluid composition [28], Inhibit the production of testosterone [29]. But, atorvastatin with low dose does not have side effects on fertility and reproduction [30].

The aim of this study was to evaluate the protective effect of ATV in ionizing irradiation –induced testicular toxicity. Based on benefit effects of ATV in several diseases, the potential radioprotective effect of ATV at different doses was evaluated using biochemical, immunohistological and histopathological evaluations in mice.

2. Materials and methods

2.1. Animals

Sixty male Balb/c mice (25–30 g) were prepared from Animal Research Center of Mazandaran University of Medical Sciences, Sari, Iran. Animals kept in standard conditions of light/dark cycle (12-h), humidity (55 ± 5%) and temperature (23 ± 2 °C). Mice had free access to food and water. Animals in each group were kept in polypropylene cages. All the experimental procedures were approved by the Institutional Animal Ethics Committee of the Mazandaran University Medical Sciences (ID: 2244).

2.2. Study design

In experimental study, animals were randomly divided into 6 groups (10/group):

Group I; Mice were received phosphate buffered saline (PBS) (same volume with other groups) as a vehicle of atorvastatin for 7 days (no-treated drug or radiation).

Group II; Mice were received 50 mg/kg ATV daily for 7 days without radiation exposure.

Group III; Mice received 10 mg/kg ATV daily for 7 days and on the 8 day received 2 Gy whole-body X-ray irradiation.

Group IV; Mice received 25 mg/kg ATV daily for 7 days and on the 8 day received 2 Gy whole-body γ-irradiation.

Group V; Mice received 50 mg/kg atorvastatin daily for 7 days and on the 8 day received 2 Gy whole-body irradiation. Animals in all groups were killed a week after irradiation.

Group VI; Mice only received 2 Gy whole-body irradiation.

2.3. Preparation and administration of atorvastatine and whole-body X-ray irradiation

Atorvastatin (from Sobhan Darou Pharmaceutical Company, Rasht, Iran) was freshly prepared in PBS and was administered daily with three doses in a volume of 0.2 mL by oral gavage using sterile 26-gauge needle a week prior to whole-body irradiation. On the 8 day, mice were placed in a well-ventilated perspex box and were irradiated with 6 MV X-ray beam produced by a radiotherapy machine (Linear accelerator, Siemens, Primus, Germany) at a single dose of 2 Gy. Animals were not anesthetized prior to irradiation. One day after radiotherapy, biochemical assay and 7 days after irradiation, histological and immunohistilogical assay was evaluated.

2.4. Specimen collections

One day after radiation exposure, half of the animals were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg). Testis were immediately removed and after washing in PBS weighed. Samples stored at −80 °C for biochemical study. After 7 days, the other half of the mice were anesthetized with ketamine and xylazine. Later, blood samples were collected from the heart. After 15 min of centrifugation at 3000 × g, the serum samples were separated and stored at −20 °C until evaluate testosterone analysis. Then animals were sacrificed, testis were immediately removed and fixed in 10% buffer formalin for histological and immunohistochemistry assay.

2.5. Biochemical analysis

The samples were homogenized in ice-cold Tris-HCl buffer solution for 2 min at 11.200 × g. The homogenate was centrifuged at 3500 × g (4 °C) for 60 min. The malondialdehyde (MDA) levels in testicular tissues were measured based on the method that reacts with thiobarbituric acid at 95 °C [31]. In the thiobarbituric acid test reaction, MDA and thiobarbituric acid react together and produce a pink pigment with an absorption maximum of 532 nm. The results were expressed as nmol/g tissue.

2.6. Testosterone assay

Levels of testosterone was determined by radioimmunoassay in serum according to manufacturer's instructions (Mouse/Rat Testosterone ELISA Kit, BioVendor) and expressed as ng/ml. All samples were carried in duplicate.

2.7. Histological assay

For microscopic evaluation and to determine the effect of whole-body irradiation in testes, testis was fixed in 10% buffer formalin. After processing and embedding, sections with 5 μM thickness were cut, slides stained with hematoxylin and eosin (H & E). Spermatogenesis was investigated with Johnsen scoring system, which five sections per slide and 10 seminiferous tubules per field were assessed using a score of 1–10 [32].

2.8. Histomorphometric assay

For quantitative evaluation, the average diameter of seminiferous tubule (ST) and thickness of the epithelium of the seminiferous tubules (from the basement membrane to lumen) in 10 tubule per testicular section and 10 section per groups were measured at ×400 magnifications by using calibrated OLYSIA Soft Imaging System GmbH, version 3.2 (Japan). All specimens were evaluated by a pathologist as blind [33].

2.9. Immunohistochemical assay

Immunohistochemical technique was performed according to the instructions kit manufacturer (Abcam Company, USA). After deparaffinization with xylene and rehydration in alcohol series, endogenous peroxidase activity was blocked by 0.3% H_2O_2 in methanol by incubating the sections for 30 min. Then, tissue sections were incubated at 4 °C overnight with primary antibodies (anti-caspase 3 rabbit polyclonal antibody, 1:100 in PBS, v/v, Abcam, lat: GR224831-2). After intubated with secondary antibody conjugated with horseradish peroxidase (Mouse and Rabbit Specific HRP/DAB, Abcam, Lat: GR2623314-4) for 2 h, sections were incubated with diaminobenzidine tetrahydrochloride for 5 min [34]. Then, the samples were dehydrated and mounted. The