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Original article

Rescue effects of ginger extract on dose dependent radiation-induced histological and biochemical changes in the kidneys of male Wistar rats

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ABSTRACT

Radiation is an essential modality in the management of cancer therapy, but its acute and chronic side effects on the normal organs limit the helpfulness of radiotherapy. The deleterious effects of radiation begin with oxidative stress and inflammatory reaction to radiolytic hydrolysis and formation of free radicals. The aim of the current study was to investigate the effect of dose dependent whole body radiation exposure on histological and biochemical alterations in rat kidney. It was also planned to find out whether ginger extract mitigated the deleterious effects of different doses of radiation in rat kidney. Male Wistar rats were exposed to three doses (2, 4, and 8 Gy) of γ- ray with or without a 10 day pretreatment with ginger extract. After 10 days of whole body γ- ray exposure, the results revealed proliferation of glomerular and tubular cells, fibrosis in glomerular and peritubular and a significant increase in 8-OHdG, CRP, cystatin C (in 8 Gy), plasma urea and creatinine levels, as well as a significant decrease in total antioxidant capacity of radiation groups compared to those of the control group. Ginger extract administration once daily for 10 consecutive days before exposure to 2–4–8 Gy radiotherapy, which ameliorated histological and biochemical alterations in kidneys of the rats entirely or partially compared to those in the ethanol group rats. These findings indicate that whole body exposure to radiation induces kidney damage through oxidative DNA damage and inflammatory reactions, and that these effects can be alleviated using ginger pretreatment as an antioxidant and anti-inflammatory agent.

1. Introduction

Radiotherapy is an effective protocol in destroying cancer cells. Moreover, radiation is increasingly applied for diagnostic purposes in medical sciences. However, beside killing cancer cells, the radiotherapy-induced deleterious effects in normal tissues, especially in the marked radiosensitive organs such as the kidney, are limiting issues in treating cancers of the abdominal area [1–3]. A growing body of evidence indicated that hazardous effects of radiation on normal organs during radiotherapy are caused mainly by the generation of reactive oxygen species (ROS) and other toxic substances [3,4]. Radiation-induced ROS generation interacts with biological macromolecules, such as DNA, lipids, and proteins located in cell membrane leading to oxidative DNA damage, lipid peroxidation, and cell injury or death [5,6]. Moreover, radiation-induced ROS generation causes cell injury and death through alteration to the balance of endogenous antioxidant enzymes, such as super oxide dismutase, catalase, and glutathione [7]. Furthermore, previous studies have shown that antioxidant treatment during exposure to radiation were ameliorated or protected against damage induced by radiation in normal organs [8–10]. Oxidative nature of radiation-induced injury on the one hand, and the protective effect of antioxidant therapy, on the other, tempted us to design the current work with considering some precise underlying molecular mechanisms that may help radiation to exert its harmful effects on the kidney during whole abdomen exposure to different doses of γ- ray. In addition, due to the very well documented antioxidant and anti-inflammatory properties of ginger, a second aim of this work was to determine the possible protective effects of ginger extract pretreatment against dose

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dependent radiation-induced histopathological alterations and biochemical changes in the kidneys of rats. In the current study, radiation were applied by gamma-ray. X-ray and gamma ray both are high energy electromagnetic radiation and could transfer energy to matter with analogous physical processes [11]. Denomination of gamma ray or x-ray only depends on the way of production. X-ray are produced by x-ray machines (or other systems such as linear accelerators), and gamma ray emitted by nucleus of atoms in radioactive isotopes. So, for example in X-ray absorptiometry, both x-ray and gamma ray can be used for measuring bone density in the same process [12].

2. Materials and methods

The animal care and handling herein were done according to the Principles of Laboratory Animal Care (NIH publication, no. 85–23, revised 1985) and were approved by the Urmia University of medical sciences Animal Care Committee. In the current study, 56 male Wistar rats weighing 220 ± 20 g were divided into the following seven groups of eight animals each. The group I served as the normal control. The groups II, III, and IV were exposed to the whole body single dose γ-radiation at the three doses of 2, 4, and 8 Gy respectively. The groups V, VI, and VII were pretreated for 10 days by hydro-alcoholic extract of ginger with a dose of 50 mg/kg body weight intragastrically by gavage and were then exposed to the whole body γ-radiation once at doses of 2, 4, and 8 Gy respectively. Wistar rats were exposed to single dose total of body of gamma rays 2, 4 and 8 Gy. According to the output of gamma ray machine (28.336 CGy/min), the exposure time for 2, 4 and 8 Gy radiation were 7.06, 14.12 and 28.24 min respectively.

Ginger extract was prepared according to our previous protocol. Briefly, a dried ginger rhizome (originally Chinese) was purchased from a local market. Sufficient quantity of rhizome was powdered in an electric grinder. Hydro-alcoholic extract of ginger was prepared by mixing three kg of powder with six liters of ethanol 70% in a suitable container. It was then left for 72 h in room temperature. Next, the extract was filtered through filter paper and was then concentrated using a rotary evaporator. The yield of the extract was kept in a refrigerator until the time of use [13]. After a post-radiation of 10 days, the rats were anesthetized by 10% chloral hydrate (0.5 mL/100 g body weight, IP). Next, after weighing the animals, the thoracic cavity was opened and the blood samples were collected directly from the heart and mixed with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Blood samples were then centrifuged at 4000 × *g* for 20 min within 30 min of collection. Furthermore, the yielded plasma was stored at −80 °C without repeated freeze-thaw cycles. Then, the abdominal cavities were opened and both kidneys were dissected. Excised kidneys were freed from adventitial tissues, fat, and blood clots and were subsequently weighed. The right kidney was divided into two parts. For the purpose of histopathological investigations, a part of the kidney was immediately fixed in 10% buffered formalin and then, after standard dehydration steps, it was embedded in paraffin. To perform biochemical analysis, other parts of the kidneys were washed with ice-cold physiological saline and then dried on filter papers. Subsequently, an ice-cold extraction buffer (10% wt/vol), containing a 50 mM phosphate buffer (pH 7.4) was added. It was then homogenized using Ultra Turrax (T10B, IKA, Germany). Next, the homogenates were centrifuged at 10,000 × *g* at 4 °C for 20 min. As the last step, the supernatant sample was collected and stored at −80 °C until the time of analysis [13].

2.1. Histopathological examinations

To evaluate general histological changes, after tissue processing steps, 5 μm sections from paraffin-embedded kidneys were cut and then stained with Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Proliferating cells were implemented, in accordance with our published protocol, by performing immunohistochemistry using an antibody against the proliferation cell nuclear antigen (PCNA). In brief, after taking tissue processing steps, such as deparaffinization, rehydration, and gradual ethanol passage, sections from the kidney tissue with a thickness of 5-μm were stained using the Monoclonal anti-PCNA antibody (Dako Denmark A/S, Denmark). Optimal results were achieved with the EnVision™ visualization system (Dako Denmark A/S, Denmark). Furthermore, Hematoxylin was used as a counterstain. The assessment included proper negative controls. Moreover, all the slides were inspected by two expert pathologists, independently. PCNA-positive indices were considered as indicators of kidney cell proliferation. In order to assess percentages of PCNA-positive indices, four non-overlapping fields of view per section from two to three sections per animal were analyzed. The number of positively stained cells and the total number of cells were counted for each field of view. In addition, for each animal, the number of positively stained cells was then presented as a percentage of the total number of counted cells. The criteria applied in scoring the quality of PCNA-positive indices were as follows: normal (i.e. PCNA-positive indices are present in less than 5% of the kidney cells), mild (i.e. PCNA-positive indices are present in less than 25% of the kidney cells), mild to moderate (i.e. PCNA-positive indices are present in 25–50% of the kidney cells), moderate to severe (i.e. PCNA-positive indices are present in 50–75% of the kidney cells), and severe (i.e. PCNA-positive indices are present in 75–100% of the kidney cells) [14]. In order to evaluate the kidney tissue fibrosis, 5 μm kidney tissue sections were stained using Masson Trichrome, in accordance with the manufacturer's instructions (Asiapajohesh, Amol, Iran). The severity of tissue fibrosis was estimated maintaining a semi-quantitative method explained by Ashcroft et al. and our published protocol [14,15]. A score ranging from zero (normal kidney) to eight (total fibrosis) was set. The criteria appointed in scoring kidney fibrosis were as follows: grade 0 = normal kidney; grade 1 = minimal fibrosis thickening of kidney tissue, grade 2 and 3 = moderate thickening of kidney tissue without obvious damage to the structure of kidney tissue; grade 4 and 5 = increased fibrosis with definite damage to architecture of the kidney and formation of fibrosis bands or small fibrosis masses; grade 6 and 7 = severe distortion of structure and large fibrosis areas; and finally grade 8 = total fibrotic obliteration [15].

2.2. Biochemical assays

Plasma creatinine and urea levels were estimated using urea and a creatinine commercial kit (Pars Azemoon, Karaj, IRAN). Levels of 8-OHdG in kidney tissue homogenates were measured by the quantitative sandwich enzyme immunoassay method using a commercial rat 8-hydroxy-deoxyguanosine Elisa kit (Cusabio, China), following the manufacture guidelines. The total antioxidant capacity (TAC) was measured in kidney homogenate using antioxidant assay kit (Cayman Chemical, USA), in accordance with the manufacture guidelines. The plasma cystatin C levels were determined employing the quantitative sandwich enzyme immunoassay method using a commercial rat cystatin C Elisa kit (Cusabio, China).

2.3. Statistical analysis

To verify normal distribution of data within each group, a Kolmogorov-Smirnov test was carried out. Furthermore, a one-way ANOVA and then the Tukey's post hoc test were conducted to test the statistical differences between the groups. The data obtained