Original Study

Ameliorative Effects of Resveratrol on Acute Ovarian Toxicity Induced by Total Body Irradiation in Young Adult Rats

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A B S T R A C T

Objective: The purpose of this study was to investigate the ovarian protective effects of resveratrol in rats exposed to total body irradiation.
Design: Experimental study.
Settings: University hospital.
Participants and Interventions: Thirty female rats were randomized into four groups: (1) control group (n = 7); (2) low-dose (10 mg/kg) resveratrol group (n = 8); (3) high-dose (100 mg/kg) resveratrol group (n =7); and (4) sham irradiation group (n = 8). The drugs were administered intraperitoneally as single doses, and the rats were exposed to total body radiation 24 h after the treatment. The animals were sacrificed the following day, and their ovaries were excised for histopathological and biochemical analysis.
Main Outcome Measures: The ovarian follicle counts were calculated, and irradiation-dependent ovarian damage and tissue levels of antioxidant enzymes were evaluated.
Results: Group 2 and Group 3 showed significantly higher numbers of total follicle counts compared with Group 1 ($P < 0.01$). The low-dose resveratrol treatment was associated with significantly higher numbers of primary follicles than the high-dose group. The tissue activities of glutathione peroxidase (GsH-Px) and catalase (CAT) were significantly elevated in the resveratrol-treated animals. Evaluation of ovarian histology revealed no remarkable changes in fibrosis and leucocyte infiltration among the resveratrol-treated and control rats; however, vascularity was significantly reduced in the high-dose group ($P = 0.014$).
Conclusion: Resveratrol attenuated irradiation-dependent ovarian damage, suggesting that this natural antioxidant is effective in reducing the follicle loss induced by ionizing radiation.
Key Words: Radiation, Ovarian follicle, Resveratrol, Oxidative stress

Introduction

Radiation therapy has been used effectively in cancer treatment for many decades; it is used to cure cancer and also to alleviate cancer-associated symptoms.[1] In general, the cells have the capacity to repair damage caused by single-strand deoxyribonucleic acid (DNA), while double-stranded DNA breaks appear to be the most important cause of cell death.[2] Therefore, the primary focus in radiotherapy is to increase double-strand breaks in DNA to kill or to control cancer cells.[3] Radiation damages cells by direct ionization of the atoms that make up the DNA and other cellular targets and by indirect effects through free oxygen radicals.[4,5] The ionization occurs as a result of the ionization of water, forming reactive oxygen species (ROS), notably hydroxyl radicals, and increasing oxidative stress, which then damages the double-strand DNA of the targeted cells.[4,5] Radiation therapy has well-documented toxic effects on the ovarian function and the fertility of women of a young age and children diagnosed with some types of cancer, including malignant tumors of the uterine cervix, rectum, and central nervous system, and hematological malignancies.[6–10] Exposure to ionizing radiation and the increased ROS induces rapid primordial follicle loss in the ovaries; however, the mechanistic role of ROS in radiation-dependent ovarian toxicity and the use of antioxidant compounds to protect the gonadal functions of patients who are candidates for radiotherapy have received limited attention.[11]

Resveratrol (trans-3,40,5-trihydroxystilbene), a natural polyphenolic, nonflavonoid antioxidant, is a phytoalexin found in various food products, with particularly high levels present in grape skin (50–100 μg/g) and red wine (1.52 mg/l).[12] Resveratrol has been shown to have significant anti-inflammatory, antioxidant, and immunomodulatory properties.[13] The anti-oxidant properties of resveratrol are mainly dependent upon the upregulation of endogenous cellular antioxidant systems, but the compound also shows direct ROS-scavenging properties.[14]

On the basis of this background, by examining the oxidant/antioxidant status and the lipid peroxidation and histopathological status of ovarian follicles, we aimed to investigate the potential protective effects of resveratrol on

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acute ovarian damage of female rats exposed to gamma radiation in an experimental model.

Materials and Methods

Care of Animals and Treatment

This study was carried out in the Experimental Research Laboratory of the Faculty of Medicine. It had the approval of the ethic committee and complied with the guidelines for the care and use of experimental animals.[15] Thirty-two young adult female Wistar rats, each weighing between 150 and 200 g and aged between 8–10 weeks, were purchased from an animal laboratory. All of the rats were examined by a veterinarian and determined to be in good health. The rats were housed in plastic cages and maintained under standard conditions: 12-h light and 12-h dark periods, 20°C constant temperatures, and a humidity range between 40 and 60%. The rats had free access to standard dry pellets ad libitum and tap water until the end of the study. Before total body irradiation (TBI), all of the animals were hormonally synchronized in their four-day estrus phase to exclude differences in ovarian morphology, steroid synthesis, and follicle growth. Synchronization was performed by administering two injections of estradiol (55 mg/kg body weight) with a 24-h intermission, followed by one injection of progesterone (7 mg/kg body weight).[16] Daily vaginal smears of the rats were taken to establish the estrus cycle of each animal. Behavioral estrus occurred 4 h after the injection of progesterone. Vaginal smears were taken by cotton swab: the swab was inserted into the vagina and rotated 360° clockwise; then the swab was smeared onto a glass slide and fixed with ethanol. The smears were stained with the usual Papanicolaou method and then evaluated by light microscopy by a cytopathologist who was unaware of which group the animals belonged to. The estrus cycle was determined as follows: proestrus period (many centrally nucleolated epithelial cells), estrus period (cornified epithelial cells without nuclei), metestrus period (leukocytes, mucus, and a few cornified cells), and diestrus period (various epithelial cells, mucus, and leukocytes).[17] The rats were observed for at least two successive four-day estrus cycles.

Drug Preparation and Treatment

The 32 rats were randomized (using random number tables) into four groups: (1) a control (vehicle) group (n = 8), (2) a low-dose resveratrol group (n = 8), (3) a high-dose resveratrol group (n = 8), and (4) a sham-irradiation group.

In the first group, a daily dose of 0.2 ml dimethyl sulfoxide (Sigma Aldrich, Saint-Quentin Fallavier, France), the vehicle of resveratrol, was injected intraperitoneally (i.p.), and this group served as the control. The rats in Group 2 were then treated with resveratrol (Sigma Aldrich) i.p. with a single dose of 10 mg/kg (low-dose group). A single dose 100 mg/kg resveratrol was administered i.p. to the rats in Group 3 (high-dose group). The rats in Group 1, 2, and 3 underwent TBI 24 h after the administration of resveratrol. The rats in Group 4 received no treatment and underwent sham irradiation. The drug doses were chosen based on previous biological studies related to the antioxidant effects of resveratrol in experimental rat models.[18,19]

Total Body Irradiation

All of the rats were anesthetized i.p. with 20–30 mg/kg ketamine hydrochloride (Rotex, Germany) prior to irradiation. Each rat was then restrained and taped by the tail and legs on an acryl plate at a supine position. Each rat in Group 1, 2, and 3 was exposed to a total dose of 720 cGy in one fraction, with a dose rate of 230 mgy/s according to previous studies of linear accelerator 6 MV photon rays.[20] The dosage was calculated at 3 cm depth and 100 cm source-skin distance. Twenty-four hours after radiation exposure, the rats were euthanized by cervical dislocation method, and the right ovaries were carefully dissected from the surrounding tissues, quickly removed, and kept at −80°C until used for biochemical analysis. The left ovaries of each animal were also removed for histopathological evaluation of the ovarian follicles.

Tissue Preparation for Biochemical Analyses

To evaluate the oxidant–antioxidant balance, we determined the free radicals production by measuring lipid peroxidation (malondialdehyde [MDA]) and the activity of some enzymatic antioxidants (catalase and glutathione peroxidase [GSH-Px]).[21,22]

All of the tissues were homogenized in ice-cold 140 mM KCI at 16,000 rpm for 2 min using a homogenizer (Ultra-Turrax T 25 basic homogenizer, IKA, Germany). The MDA levels were measured at this homogenate stage. The homogenate was then centrifuged at 5000 × g for 60 min to remove debris. The clear upper supernatant fluid was removed, and the activity of CAT and GSH-Px and the protein concentration were determined. All preparation procedures were performed at 4°C.

Determination of the Antioxidant's Enzyme Activities

CAT (EC 1.11.1.6) activity was determined according to Aebi's method.[23] Tissue CAT activity was expressed as K/g protein. GSH-Px activity was measured by the method of Pagia and Valentine.[24] The activity of GSH-Px was also expressed as units per gram of protein in the tissue.

Determination of MDA Levels

The tissue and serum MDA were determined by a method which is based on the reaction with thiobarbituric acid (TBA) at 90–100°C.[25] In the TBA test reaction, MDA or MDA-like substances and TBA react and produce a pink pigment, with an absorption maximum at 532 nmol. The reaction was performed at pH 2–3 at 90°C for 15 min. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nmol. The results were expressed as nmol per g wet tissue according to a standard