Biomarkers and Genomic Medicine (2015) 7, 154–158

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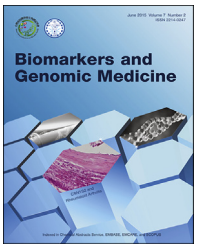

ORIGINAL ARTICLE

Protective effect of combined vitamin C and E against ovarian and endometrial toxicity in rats that receiving oral rhodamine B

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Received 23 January 2015; received in revised form 27 August 2015; accepted 8 November 2015
Available online 12 December 2015

KEYWORDS
dye;
endometrium;
oxidative stress;
ovarium;
xenobiotic

Abstract This study aimed to investigate whether combined supplementation with vitamin C and vitamin E was able to modify the superoxide dismutase (SOD) and malondialdehyde (MDA) levels in the ovarium of rats exposed to rhodamine B. Twenty-five female Wistar albino rats were divided into five groups ($n = 5$ each), including control (untreated group); rhodamine B group; rhodamine B group which received vitamin C (0.2 mg) + vitamin E (0.04 IU/g body weight); rhodamine B group which received vitamin C (0.4 mg) + vitamin E (0.04 IU/g body weight); and the rhodamine B group which received vitamin C (0.8 mg) + vitamin E (0.04 IU/g body weight). Analysis of MDA levels as a marker of lipid peroxidation was done spectrophotometrically. Analysis of SOD levels was done by enzyme-linked immunosorbent assay technically. Endometrial histology was analyzed in hematoxylin eosin staining. This increase in ovarian MDA was significantly ($p < 0.05$) attenuated by the two highest dose treatments of combined vitamin C and vitamin E. Rhodamine B significantly decreased SOD levels compared

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http://dx.doi.org/10.1016/j.bgm.2015.11.001

to the untreated group. This decrease in ovarian SOD level was significantly attenuated by the second and third doses of the combined vitamin C and vitamin E. The vascular number and gland density were significantly lower in the rhodamine B group compared to the untreated control group ($p > 0.05$). All doses also significantly prevented rhodamine B-induced decrease in the vascular number and gland density. In conclusion, the protective effect of combined vitamin C and vitamin E against ovarian and endometrial toxicity in rats receiving oral rhodamine B is due to inhibition of the lipid peroxidation, modulation of SOD levels, and the endometrium repairing effect.

Introduction

In the reproductive system, reactive oxygen compounds play a role in physiological processes such as oocyte maturation. Excessive reactive oxygen compounds without adequate antioxidant defenses will trigger oxidative stress. Ovarian oxidative stress will lead to damage the structure of the oocyte and granulosa cells in the follicle. Reactive oxygen species can affect the quantity and quality of the ovaries and so have an impact on the capacity of the ovary. Women with impaired capacity will experience failure and high stimulation pregnancy failure.[1–3] The endometrium is a complex tissue that lines the inside of the endometrial cavity. It is morphologically divided into functional and basal layers. The functional layer forms two thirds of the endometrial thickness and it presents different compartments, including the luminal epithelium, the glandular epithelium, stroma, and the vascular compartments.[4–6]

One of the chemical compounds that can trigger oxidative stress is rhodamine B. This compound is a synthetic dye of green or red purple crystals. Utilization of rhodamine B dye includes paper, textile dyes, dyes of histology specimens, and cosmetics.[7] When exposed to light, rhodamine B can form reactive oxygen compounds. Reaction formation of reactive oxygen compounds is divided into two types. The first reaction will increase the energy of rhodamine B and transfer to biomolecules to form reactive oxygen compounds. The second reaction is the reaction of the energy transfer to molecular oxygen to form singlet oxygen.[8–10] Various studies have shown that exposure to rhodamine B triggers oxidative stress on ovarian follicles and a decrease in the number of primary, secondary, and Graafian follicles.[11] To inhibit oxidative stress requires intake of antioxidants.

The α-tocopherol is an antioxidant in the lipid compartment to protect against lipid peroxidation, changing gene expression, modulation of cell signaling, and proliferation. This compound is found in significant amounts in the ovaries and follicular fluid.[12–14] Combined supplementation of vitamin C and vitamin E is the best choice for antioxidant treatment. As a result of continuous oxidative stress, there is an increase in the concentration of ascorbate radical that shows as a peak followed by a steady decline. After disappearance of the ascorbate radical, the tocopheroxyl radical appears.[15] This study aimed to investigate whether combined supplementation of vitamin C and vitamin E was able to modify the superoxide dismutase (SOD) and malondialdehyde (MDA) levels in the ovarium of rats exposed to rhodamine B. In addition, the effects on endometrial histology were also explored.

Material and Methods

Animals

Twenty-five female Wistar albino rats, aged 8–12 weeks, weighing 160–250 g were used for the present investigation. The animals were divided into five groups ($n = 5$ each), including control (untreated group); rhodamine B group; rhodamine B group receiving vitamin C (0.2 mg) + vitamin E (0.04 IU/g body weight); rhodamine B group receiving vitamin C (0.4 mg) + vitamin E (0.04 IU/g body weight); and the rhodamine B group receiving vitamin C (0.8 mg) + vitamin E (0.04 IU/g body weight). They were housed in a clean wire cage and maintained under standard laboratory conditions (temperature $25 \pm 2^{\circ}$C with dark/light cycle 12/12 hours). They were fed a standard pellet diet and received water *ad libitum*. The animals were acclimatized to laboratory conditions for 1 week prior to the experiment.

Tissue sampling

At the end of the treatment, the animals in all groups were anesthetized. The ovarium and endometrium were collected, weighed, and later rinsed with physiological saline. All samples were stored at -80°C until analyzed.

Rhodamine B

Rhodamine B was dissolved with double-distilled water and administered orally using a probe. The duration of administration of rhodamine B in the treatment group refers to the previous study related to subchronic toxicity tests of rhodamine B administered for 36 days.[11]

Vitamin C and vitamin E

Vitamin C was dissolved with *aqua dest* 0.5 mL, but vitamin E was dissolved with sesame oil 0.5 mL. All of these