Reproductive Toxicology 43 (2014) 85–93

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Sodium selenite treatment restores long-lasting ovarian damage induced by irradiation in rats: Impact on oxidative stress and apoptosis

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ARTICLE INFO

Article history:
Received 18 May 2013
Received in revised form
12 November 2013
Accepted 19 November 2013
Available online 27 November 2013

Keywords:
Selenite
Irradiation
Folliculogenesis
Proliferation
Oxidative stress
Apoptosis

ABSTRACT

The deleterious damage of reproductive function following radiotherapy is of increasing importance. In the present study, we investigated the impact of long-term sodium selenite (SS) treatment on radiotherapy-induced ovarian injury in a rat model. Two-week after radiation exposure vaginal cyclicity was arrested, and serum FSH level was elevated in irradiated female rats. SS significantly ameliorated ovarian and uterine oxidative stress induced by irradiation through decreasing the lipid peroxide level and increasing the glutathione level, and glutathione peroxidase activity. In the presence of SS, ovarian cytochrome c and caspase 3 expressions triggered by radiotherapy were decreased. SS significantly counteracted radiation-induced a widespread loss of ovarian follicles and caused further stimulation of follicular proliferation through enhancing PCNA expression. Despite such alteration in ovarian function, serum estradiol level did not change after irradiation, whereas SS significantly increased it. In conclusion, long-term SS treatment improved reproductive development, which was impaired by radiotherapy.

1. Introduction

During the normal reproductive lifespan of females, 300–500 mature oocytes will be ovulated and the vast majority of follicles (over 99%) are lost by a process of atresia, which occurs throughout folliculogenesis as well as from the primordial pool. The non-renewable nature of the oocyte pool makes it so vulnerable. Therefore, one of the important health problems for young women is exposure to external agents such as radiotherapy that contribute to accelerating oocyte depletion and premature onset of menopause [1]. Over the last decades, great attempts have been made to minimize the toxic effects of radiotherapy on the ovaries and to reduce the well-known risk of premature ovarian failure (POF) and infertility [2]. Wallace et al. [3] estimated the dosage at which half of the follicles are lost in humans (LD_{50}) to be 4 Gy. It is important to mention that gastrointestinal malignancies and anal carcinoma patient received median radiation dose range from 45 to 56 Gy using fraction doses of 1.8–2 Gy, twice daily [4,5]. Recently, rapid ovarian dysfunction has been observed in young females after initiation of cancer radiotherapy treatment [6]. POF is characterized by ovary dysfunction, decreased the number of the growing ovarian follicles, increased concentrations of gonadotropins, and reduced concentrations of ovarian steroids [7]. Besides, it was declared that radiation prior to puberty has been associated with irreversible damage to the uterus, with prepubertal uterine morphology observed in post-pubertal patients [8].

The deleterious effects of ionizing radiation on biological systems are mainly mediated through the generation of reactive oxygen species (ROS) in cells as a result of water radiolysis [9]. Low levels of ROS are important signal in regulation of physiological functions in female reproduction, including folliculogenesis, steroidogenesis, corpus luteum function, and luteolysis [10]. However, increasing ROS production has a key role in pathological processes in female reproduction [11,12]. Additionally, apoptosis is an essential component of ovarian function and development, which is responsible for oocyte loss [1,13], and it can be further induced by radiation [14].

Selenium (Se), an essential trace element, protects cells from oxidative stress by expression of selenoprotein genes and through anti-inflammatory mechanisms [15]. Low Se states have been associated with reproductive dysfunctions [16] leading to degeneration

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http://dx.doi.org/10.1016/j.reprotox.2013.11.005

of the ovaries and atresia of the follicles [17] which is related to an increased prevalence of infertility [18] and idiopathic miscarriage [19]. It was assumed that radiotherapy-induced Se deficiency possibly enhancing radiation side-effects [20]. Further, Se improved *in vitro* development of follicles through enhancement of granulosa cell's proliferation, increasing follicular total antioxidant capacity and glutathione peroxidase (GPx) activity, as well as decreasing ROS [21]. Indeed, the expression of FSH receptor, as well as the estradiol (E2) biosynthetic pathway was apparently influenced *in vitro* by Se [22]. Therefore, whether SS can stimulate the E2 biosynthesis *in vivo*? And by this way, it may stimulate the folliculogenesis process? Moreover, Se radioprotective effects on gastrointestinal tract, liver, spleen, and submandibular gland were previously demonstrated [23,24].

Recently, our previous study examined the effect of Se on ovarian folliculogenesis and fertility *in vivo*, and we found that Se improved folliculogenesis and provided evidence for ovarian and uterine radioprotection [25]. Throughout the study, it was founded that irradiation-induced depletion of certain types of ovarian follicles within 24 h of exposure, but what about the long-lasting effect of radiation on ovarian follicles' population? Additionally, it was stated that irradiation-induced ovarian oxidative stress and apoptosis, and hindered cell proliferation of ovarian follicles within 24 h of exposure. Can these damaging effects of radiation be continued after that? Can SS treatment for another one-week following radiation exposure have any different response, which may alter the ovarian function?

Subsequently, in the current study, we investigated the long-term effect of sodium selenite (SS) treatment on ovarian function impaired by irradiation to determine if SS could elicit a distinct response than its previous radioprotective one. With this objective, in addition to determining hormonal levels, we have followed the evolution of the follicular population by morphological approaches, studied the expression of follicular proliferation marker, investigated follicular atresia, and assessed oxidative stress markers.

2. Materials and methods

2.1. Chemicals

Sodium selenite, reduced glutathione (GSH), Ellman's reagent [5,5-dithio-bis (2-nitrobenzoic acid); DTNB], and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO,USA). N-butanol, dipotassium hydrogen phosphate (K_2HPO_4), potassium di-hydrogen phosphate (KH_2PO_4) and trichloroacetic acid (TCA) were purchased from El-Nasr Chemical Co. (Egypt). Glutathione peroxidase (GPx) kit was purchased from Randox Laboratories (UK). All other chemicals and solvents were of the highest grade commercially available.

2.2. Animals

The study was conducted according to the ethical guidelines (Faculty of Pharmacy, Ain Shams University, Egypt). Immature female Sprague-Dawely rats (23 days of age) were obtained from Nile Co. for Pharmaceutical and Chemical industries (Egypt). Rats were housed in an air-conditioned atmosphere, at a temperature of 25 °C with alternatively 12 h light and dark cycles. Animals were kept on a standard diet and water *ad libitum*. Standard diet pellets (El-Nasr, Egypt) contained not less than 20% protein, 5% fiber, 3.5% fat, 6.5% ash and a vitamin mixture.

2.3. Irradiation

Whole body gamma-irradiation was carried out using a Cesium (^{137}CS) source, Gamma Cell-40 biological irradiator, at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. The animals were exposed to 3.2 Gy with a dose rate of 0.48 Gy/min. The dose of radiation which had been studied by Lee et al. [26] was also tested in our earlier study [25], and we found that 3.2 Gy of gamma-radiation was sufficient to induce ovarian failure in immature female rats according to the decline in the number of ovarian follicles and according to the hormonal disturbance [25]. The plastic boxes containing female rats were positioned in a chamber fixed to the irradiation equipment.

2.4. Experimental design

Animals were divided randomly into four groups (25 animals per group) and treated for two weeks as follows; the first group acting as a control received saline (0.5 ml/100 g B.W., i.p.) once daily. The second irradiated group received saline (0.5 ml/100 g B.W., i.p.) once daily then exposed at the end of the first week to a single dose of 3.2 Gy, whole-body irradiation. The third group was injected with SS (0.5 mg/kg, i.p.) once daily for two weeks. The dose of SS was chosen according to the study of Pontual et al. [24]. The fourth group was administered SS (0.5 mg/kg, i.p.) once daily for two weeks, and after the first week of SS injection, rats were exposed to a single dose of 3.2 Gy, whole-body irradiation. The advantages of using our present model of immature rats because of minimal prior exposure of the ovaries to endogenous gonadotropins. So, variability in follicular development and ovarian steroid secretion in different days of the estrous cycle characteristic of cycling adult rats is avoided [27]. Animal body weight was recorded daily until the sacrificed day. Two weeks after irradiation and one week after the last SS injection, twelve rats for each group were utilized for biochemical assessment of serum E2 and FSH, as well as ovarian and uterine oxidative stress markers. Five animals in each group were utilized for histopathology and immunohistochemistry studies and eight animals within each group were utilized for fertility assessment. Blood samples were collected from the retro-orbital plexus and allowed to clot. Afterwards, rats were sacrificed; ovarian and uterine tissues were dissected, washed with ice-cold saline and then weighed.

2.5. Tissue collection and processing

Serum was separated by centrifugation at 3000 × *g* for 15 min and kept frozen at −80 °C until assayed for E2 and follicle stimulating hormone (FSH). Samples of ovarian and uterine tissues were homogenized at 1:10 (w/v) in saline, (pH 7.4) with an Ultra Turrax homogenizer after that the supernatant was obtained by centrifugation at 10,000 × *g* for 15 min then, stored at −80 °C until analysis of oxidative stress markers, including reduced glutathione (GSH), GPx activity and lipid peroxidation. In addition, further ovarian and uterine tissues were fixed in an appropriate buffer for light microscopical examination as well as ovarian immunohistochemical detection of the proliferation marker; proliferating cell nuclear antigen (PCNA) and apoptotic markers; caspase 3 and cytochrome c.

2.5.1. Assessment of serum E2 and FSH

To determine whether the fall in the follicles' population-induced by irradiation may alter the ovarian function, serum E2 and FSH were measured. An ELISA kit (DRG International, Inc., USA) was used for estimating the circulating level of serum E2. As well, serum FSH measurement was performed by commercially available radioimmunoassay kit (rat FSH IRMA C.T., IBL International GMBH, Germany). The intra- and inter-assay coefficients of variation were found to be less than 9% and 10%, respectively for E2, and less than 3% and 8%, respectively for FSH. The minimum detectable concentration for E2 and FSH was 3.6 pg/ml and 0.2 ng/ml, respectively.