Biomedicine & Pharmacotherapy 102 (2018) 517–530

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Zingerone ameliorates cisplatin-induced ovarian and uterine toxicity via suppression of sex hormone imbalances, oxidative stress, inflammation and apoptosis in female wistar rats

Erdal kaygusuzoglu[a], Cuneyt Caglayan[b,*], Fatih Mehmet Kandemir[c], Serkan Yıldırım[d], Sefa Kucukler[c], Mehmet Akif Kılınc[a], Yavuz Selim Saglam[d]

[a] Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Bingol University Bingol, Turkey
[b] Department of Biochemistry, Faculty of Veterinary Medicine, Bingol University, Bingol, Turkey
[c] Department of Biochemistry, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Turkey
[d] Department of Pathology, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Turkey

ARTICLE INFO

Keywords:
Apoptosis
Cisplatin
Inflammation
Ovarian toxicity
Uterine toxicity
Zingerone

ABSTRACT

Cisplatin (CP) is a widely used chemotherapeutic drug, effective against a variety of solid tumours, though its utility is limited due to its multiple organ toxicity. Zingerone (ZO), one of the most important components of dry ginger root, has several pharmacological activities, such as antioxidant, anti-inflammatory and anti-apoptotic properties. This study aimed to investigate the ameliorative effect of ZO on CP-induced ovarian and uterine toxicity in female rats. The rats were subjected to a prophylactic oral treatment of ZO (25 and 50 mg/kg body weight) for seven days to measure the protective effect against ovarian and uterine toxicity induced by a single (i.p.) of CP (7 mg/kg body weight) on the first day whereas the rats were sacrificed on the eighth day. The results showed that ZO decreased the serum FSH hormone level, increased the serum E2 hormone level, and also maintained the ovarian and uterine histological architecture and integrity. In addition, ZO obviously increased the measured activity of antioxidant enzymes (SOD, CAT and GPx) and the GSH content, and significantly reduced MDA levels. ZO was able to reduce the levels of the inflammatory markers NF-κB, TNF-α, IL-1β, IL-6, COX-2 and iNOS in CP-induced ovarian and uterine damage. It also inhibited apoptosis and reduced oxidative DNA damage markers by the downregulation of caspase-3 and 8-OHdG expression coupled with an upregulated Bcl-2 level. The results indicate that ZO may be beneficial in ameliorating CP-induced oxidative stress, sex hormone imbalances, inflammation and apoptosis in ovarian and uterine tissues of female rats.

1. Introduction

With the advance of early disease detection and therapeutic treatments, the five-year cancer survival rates have increased from 49% to 68% over the past 30 years [1,2]. Despite the increasing number of cancer survivors, however, there are still problems associated with the side effects of treatment during and after chemotherapy [3]. Ovarian toxicity due to chemotherapy is one of the main concerns for women of reproductive age (15–44 years) [4], the main issue being the limited amount of oocytes found in pre-birth ovarian follicles. This population of primordial follicles represents a woman's total ovarian reserve, and a woman's reproductive life ends when this number of follicles decreases to usually less than a thousand [5]. Until now, many chemotherapeutic agents have been reported to damage these ovarian follicles, as well as increase the risk of premature ovarian failure, premature menopause and infertility. This in turn reduces both the quality of life of patients and increases medical costs [1,4,6,7]. Developments in cancer have undoubtedly improved survival, but efforts to preserve the quality of life still need to be improved, even after chemotherapy has already been administered.

Cisplatin [cis-diamminedichloroplatinum II, (CP)] is one of the most effective chemotherapeutic drugs commonly used in the treatment of various solid tumours, such as head, colon, neck, lung, breast, testis, bladder, ovary and uterine cervical carcinomas [8,9]. Unfortunately,

Abbreviations: 8OHdG, 8-hydroxy-2'-deoxyguanosine; B.wt, body weight; Caspase-3, cysteine aspartate specific protease-3; CAT, catalase; COX-2, cyclooxygenase-2; Cp, cisplatin; Bcl-2, B-cell lymphoma-2; E2, Estradiol; ELISA, enzyme-linked immunosorbent assay; FSH, follicle stimulating hormone; GPx, glutathione peroxidase; GSH, glutathione; IL-1β, interleukin-1β; IL-6, interleukin-6; i.p., intraperitoneal injection; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; ZO, zingerone

* Corresponding author at: Department of Biochemistry, Faculty of Veterinary Medicine, Bingol University, 12000-Bingol, Turkey.
E-mail address: ccaglayan@bingol.edu.tr (C. Caglayan).

https://doi.org/10.1016/j.biopha.2018.03.119
Received 3 January 2018; Received in revised form 20 March 2018; Accepted 20 March 2018

the clinical use of CP is limited by adverse side effects, such as ototoxicity, hepatotoxicity, nephrotoxicity, neurotoxicity, gastrointestinal toxicity, myelosuppression, ovarian and uterine toxicity [10–13]. Moreover, CP-induced infertility has been reported to be caused by toxic effects to the primordial follicles. Because these follicles cannot be regenerated, this damage can cause permanent ovarian failure and infertility [14]. Although the exact mechanism of CP toxicity is not fully understood, it is thought to be caused by either a reactive oxygen species (ROS) [11], inflammation through the activation of pro-inflammatory cytokines [15] or as a result of cell apoptosis [16], with the most likely explanation being that CP toxicity occurs as a result of the formation of ROS. ROS, such as the superoxide anion (O_2·-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (·OH), are thought to cause toxicity by interacting with DNA, lipids and proteins [17]. CP has been reported to be cytotoxic to normal cells due to the production of ROS that are not specific to tumours, resulting in overall oxidative stress in the body [17,18]. There is also conclusive evidence that natural compounds with antioxidant properties can reduce CP-induced organ toxicity [19,20]. This is especially useful because there are very few options available for the protection of the fertility, the ovaries and the uterus of female patients during chemotherapy [21–24]. For this reason, there is a need to discover natural compounds that can effectively reduce the toxicity caused by CP in order to increase the chemotherapeutic activity.

In recent years, the use of phytochemicals as free-radical scavengers and oxidative-stress inhibitors has received considerable attention. For example, it has been suggested that antioxidants play an important role in reducing the tissue damage caused by metabolic disorders (metabolic syndrome, diabetes, etc.) [25,26], cardiovascular diseases [27] and cancer [28]. Zingerone [4-(4-hydroxy-3-methoxyphenyl)-2-butanone, (ZO)] is a cheap and non-toxic compound with various pharmacological activities [29]. It is a phenolic alkanone found in many plant species and is one of the most important components of dry ginger root [30]. It has been reported to have several biological properties, such as antioxidant [30], anti-inflammatory [31], anticancer [32], anti-apoptotic [30] and antimicrobial [33] effects.

After an extensive literature review, there were no studies related to the ameliorative effects of ZO against CP-induced ovarian and uterine damage. Therefore, the antioxidant, anti-inflammatory and anti-apoptotic effects of ZO against CP-induced ovarian and uterine toxicity were investigated in female rats in this study.

2. Materials and methods

2.1. Chemicals and reagents

CP (25 mg/50 mL injectable solution) was obtained from Koçak Farma (Istanbul, Turkey). ZO (Vanillylacetone ≥ 96%, CAS Number: 122-48-5) and other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The selection of CP and ZO doses was based on the previously published studies by Pani et al. [34] and Rehman et al. [31]. In this study, distilled water was used to dissolve the ZO. To do this, it was gently heated to 40 °C and allowed to cool slowly to room temperature.

2.2. Animals

The female Wistar rats (12 weeks old; 180–200 g) were obtained from the Animal Experiment Research Center at Bingol University. The rats were maintained in a 12 h light/dark cycle with a humidity of 45 ± 5% at a constant temperature (24 ± 1 °C). The animals were selected a week before the experiment and adapted to their new environment. Food and water were provided ad libitum. The rat experiments were conducted under an ethics approval from the Animal Experimentation Ethics Committee of the Bingol University (Permit Number: 2016-4/04).

2.3. Treatment regimen

The animals were divided into five groups of eight rats each and were treated as follows. Group I (control) received normal saline orally for seven days; Group II (CP) received a single dose of CP (7 mg/kg of body weight; i.p.) on day 1; Group III (ZO 50) received ZO (50 mg/kg body weight) orally for seven days and Group IV (CP + ZO 25) and Group V (CP + ZO 50) received a 25 or 50 mg/kg body weight dose of ZO once a day for seven days, respectively, along with the single dose of CP (7 mg/kg body weight; i.p.) on day 1.

At the end of the treatment, animals were sacrificed under mild sevoflurane anesthesia (Sevorane liquid 100%, Abbott Laboratory, Istanbul, Turkey). Blood samples were collected and centrifuged for 10 min at 3000 rpm to obtain a clear serum that was stored at −20 °C for Follicle stimulating hormone (FSH) measurements and Estradiol (E2) hormone analyses. One of the ovaries and a part of uterus were immediately washed with ice-cold physiological saline, blotted dry and then stored at −20 °C for biochemical analysis. The other ovary and the remaining part of the uterus were transferred into 10% buffered formaldehyde for histopathological and immunohistochemical examinations.

2.4. Analysis of serum FSH and E2 hormone levels

Serum FSH and E2 hormone levels were measured using a rat enzyme-linked immunosorbent assay (ELISA) kit. (Yehua Biological Technology, Shanghai, China). The analysis was performed using an ELISA Plate Reader (Bio-Tek, Winooski, VT, USA) according to the manufacturer's instructions.

2.5. Analysis of oxidative stress markers

The ovarian and uterine tissues were homogenized in a Teflon-glass homogenizer using a buffer of 1.15% potassium chloride (KCl) to obtain a 1/10 (w/v) homogenate. The superoxide dismutase (SOD) activity of the ovarian and uterine tissues was determined according to the method of Sun et al. [35], and it was expressed as units (U)/g of protein. The catalase (CAT) activity was performed according to the method of Aebi [36], and it was expressed as katal/g of protein. The glutathione peroxidase (GPx) activity was determined according to the method of Lawrence and Burk [37], and it was also expressed as U/g of protein. The glutathione (GSH) level was measured according to the method of Sedlak and Lindsay [38], and it was expressed as nmol/g of tissue. The malondialdehyde (MDA) levels (as a marker of lipid peroxidation) in ovarian and uterine tissue homogenates were measured according to the method of Placer et al. [39], and its levels were expressed as nmol/g of tissue. The protein content was measured according to the method of Lowry et al. [40] using bovine serum albumin as the standard.

2.6. Analysis of NF-κB, TNF-α, IL-1β and IL-6 levels

Pro-inflammatory cytokines in the ovarian and uterine tissues were measured by ELISA using commercial kits. Nuclear Factor Kappa B (NF-κB), Tumour Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β) and Interleukin-6 (IL-6) levels were determined using a rat ELISA kit (Yehua, Shanghai, China).

2.7. Analysis of COX-2 and iNOS activities

Cyclooxygenase-2 (COX-2) and inducible Nitric Oxide Synthase (iNOS) activities in the ovarian and uterine tissues were determined by a rat ELISA kit. (Yehua, Shanghai, China).

2.8. Analysis of Bcl-2 levels

The B-cell lymphoma 2 (Bcl-2) levels in the ovarian and uterine