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Nordihydroguaiaretic acid ameliorates cisplatin induced nephrotoxicity and potentiates its anti-tumor activity in DMBA induced breast cancer in female Sprague–Dawley rats

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ABSTRACT

Cisplatin is a widely used antineoplastic drug, but its clinical usefulness is limited due to dose dependent nephrotoxicity. Nordihydroguaiaretic acid (NDGA) is a natural compound with broad pharmacological properties like antioxidant, anti-inflammatory and anticancer activity. The present study was undertaken to evaluate the possible beneficial effects of NDGA on cisplatin induced nephrotoxicity as well as its anticancer activity in rats bearing DMBA induced mammary tumors. The effect of NDGA on cisplatin induced nephrotoxicity was evaluated by checking serum nephrotoxicity markers, antioxidant enzymes and inflammatory markers level and kidney histopathology. NDGA induced amelioration of cisplatin nephrotoxicity was clearly visible from significant reductions in serum blood urea nitrogen (86.51 g/dl) and creatinine (5.30 g/dl) levels and significant improvement in body weight change (−10.34 g) and kidney weight (728 mg/kg). The protective effect of NDGA against cisplatin induced nephrotoxicity in the rats was further confirmed by significant restoration of antioxidant enzymes like SOD (86.28% inhibition), inflammatory markers like TNF-α (34.6 pg/ml) and histopathological examination. Moreover, our results showed that NDGA potentiated anti-breast cancer activity of cisplatin through an increment in the expression of antioxidant enzymes like SOD (85.35% inhibition) in breast cancer tissue. These results indicated that NDGA potentiated the anti-breast cancer activity of cisplatin, which was clearly evident from the tumor volume and % tumor inhibition in breast cancer rats. The current study demonstrated that NDGA may modify the therapeutic effect of cisplatin in DMBA induced breast cancer in female Sprague–Dawley rats.

1. Introduction

Despite recent advances in screening and treatment, breast cancer is the one of the most serious problems in oncology [1]. The continuous rising graph of breast cancer is a real threat, challenging all efforts to screening, prevention and management prospects to reduce this cancer [2,3]. According to the American Cancer Society, in 2013, 232,340 cases of invasive breast cancer were diagnosed among adult females [4]. Although in 2013, approximately 39,620 women died from breast cancer. Only lung cancer accounts for more cancer deaths in women than breast cancer [4]. Although conventional medicine has witnessed significant progress in the treatment of breast cancer, pharmacological interferences are uptight with their own endogenous complications, including many known and unknown legal and ethical issues [5]. Besides, breast cancer cells acquire resistance to chemotherapeutic drugs by means of different cell signaling mechanisms [6,7].

Currently, paclitaxel, cyclophosphamide, carboplatin, and cisplatin are commonly employed drugs in breast cancer chemotherapy. Cisplatin is a platinum derivative and widely used as an anti-cancer drug and effectively used in the management of several solid tumors like head and neck, mammary tumor, etc. [8]. Unfortunately, even after cisplatin having a great knock in chemotherapy, dose dependent side effects make it an unsafe drug [9]. In the body, cisplatin gradually accumulates in the kidney, activates NF-κB, and promotes the formation of inflammatory mediators such as TNF-α, IL and macrophases [10,11]. NF-κB and TNF-α are the main inflammatory factors which

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mediate cisplatin induced nephrotoxicity [12]. Several other studies have suggested that oxidative stress is also clinical in cisplatin linked nephrotoxicity. Oxidative stress occurs due to imbalance between the production and removal of reactive oxygen species (ROS) [13]. Cisplatin causes generation of ROS such as superoxide anions and hydroxyl radicals, which depletes glutathione levels and gradually hampers the protective anti-oxidant cascade in renal tissues. These ROS may produce cellular injury and necrosis via peroxidation of lipid membrane, denaturation of proteins and DNA damage [14]. Kratsovnik et al. demonstrated that generation of ROS showed potent activation of NF-κB [15].

Nordihydroguaiaretic acid (NDGA) is a phenolic lignan present in the evergreen shrubs *Larrea divaricata* and *Guaiacum officinal* [16]. NDGA is a main metabolite of creosote bush, has been shown to have promising applications in the treatment of several diseases such as cancer, diabetes, arthritis, kidney injury and neurological disorders [17]. NDGA has been shown to selectively inhibit the arachidonic acid pathway, which reduces leukotriene and prostaglandin synthesis, thus leading to the suppression of inflammatory pathway [17,18]. The structural diversification of NDGA possesses its scavenging activity against free oxygen radicals [19]. It has one o-dihydroxy catechol moiety along with four hydroxyl groups. The presence of four hydroxyl groups reacts with free oxygen radical and prevents damage from free oxygen radicals [20].

In this study, we analyzed the anti-inflammatory, anti-oxidant and also anti-cancer potential of NDGA in 7,12-dimethyl benz{a}anthracene (DMBA) induced breast cancer in female Sprague–Dawley rats.

2. Materials and methods

2.1. Drug and chemicals

NDGA was purchased from LKT Laboratory. DMBA and olive oil were purchased from Sigma (St. Louis, MO, USA). Proteinase K, agarose, NADPH (nicotinamide adenine dinucleotide 3-phosphate), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), glutathione reductase, 2-thiobarbituric acid (TBA), reduced glutathione (GSH), 2,7-dichlorofluorescein diacetate (DCFH-DA), superoxide dismutase assay kit, Lowry reagent, hydrogen peroxide and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Steinheim, Germany). Diagnostic kits for blood urea nitrogen (BUN) and creatinine were purchased from Accurex Biomedical Pvt. Ltd. (Gujarat, India). Interleukin (IL)-1β, IL-6, IL-10 and tumor necrosis alpha (TNF-α) ELISA kits were purchased from Invitrogen (Invitrogen Corporation, Frederick, USA).

2.2. Animals

Female Sprague–Dawley rats were purchased from the College of Veterinary Sciences Khanapara (Registration no. 770/03/ac/CPCSEA/FVSC,AAU/IAEC/06/21). The animals were housed three/cage in standard polycarbonate cages with room maintained at constant temperature, humidity and 12-h light and dark cycle. A complete health status was determined. None of the rats exhibited major lesions, and all were pathogen free. Before initiating the experiment, we acclimatized all rats on pulverized diet for 4 days. Experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the Gauhati Medical College & Hospital (CPCSEA Regd. No. 351; 3/1/2001).

2.3. Induction of mammary carcinogenesis

Female rats at the age of 8 weeks weighing 160–180 g were gavaged with DMBA 80 mg/kg body weight, a dose sufficient to cause 100% tumor incidence in the control group over the course of the study as described by Tikoo et al. [21]. The DMBA was dissolved in olive oil in a stock solution of 30 mg/ml. Animals were sacrificed when the tumor diameter reached 3 cm or after the completion of the experiment.

2.4. Experimental design

Female rats were divided initially into two different groups, named, normal control (Group I) received olive oil and DMBA treated group received DMBA (80 mg/kg). After twelve weeks, mammary carcinoma was confirmed by breast palpation and animals were regrouped into four different groups on the basis of tumor volume and body weight. DMBA treated rats received normal saline (Group II). Mammary carcinoma induced rats treated with NDGA (10 mg/kg) dissolved in 0.5% DMSO by intraperitoneal route (Group III). Mammary carcinoma induced rats treated with cisplatin (7.5 mg/kg) dissolved in normal saline (0.9% w/v) by intraperitoneal route (Group IV). Mammary carcinoma induced rats first pre-treated with NDGA for 5 days and then on the 5th day a single dose of cisplatin (7.5 mg/kg) dissolved in normal saline (0.9% w/v) was administered by the intraperitoneal route (Group V). These animals were maintained on a standard diet and water for 3 months prior to drug ingestion (Fig. 1).

2.5. Measurement of nephrotoxicity markers

The development of nephrotoxicity was assessed in rats after 5 days of cisplatin administration by estimating creatinine (Cr), and BUN in the serum samples by using auto blood analyzer (Siemens, Dimension Xpand Plus). Furthermore, body weight reduction and kidney/body weight ratios were calculated as rates of kidney injury.

2.6. Measurement of NF-κBp65 and inflammatory markers

Measurement of NF-κBp65 and inflammatory markers, like TNF-α, IL-6 and IL-10 levels was done by using enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen Corporation, Frederick, USA), according to the manufacturer's instructions. In all the cases, a standard curve was constructed from standards provided by the manufacturer.

2.7. Measurement of biochemical parameters

The biochemical parameters were estimated in rat kidney as well as in breast cancer tissues. The animals were sacrificed and tissues were quickly removed from the experimental groups for the estimation of antioxidant enzymes. Tissues were washed thoroughly with ice-cold normal phosphate buffer saline, pH 7.2 and cut into small pieces. Tissue were homogenized by a glass homogenizer tube in ice cold PBS and centrifuged at 20,000 rpm for 10 min (4 °C). The effect of NDGA on the activities of MDA, GSH, SOD, and catalase was studied in the kidney as well as in breast cancer tissues in all experimental groups.

2.7.1. Measurement of lipid peroxidation

Lipid peroxidation (LPO) was measured in terms of malondialdehyde (MDA) formation which is the major product of membrane lipid peroxidation and performed by using the method of Wright et al. [22] with slight modification. The reaction mixture in a total volume of 3 ml contained mainly TCA 1 ml (10%) and TBA 1.0 ml. Test tubes having reaction mixture were kept in boiling water for about 45 min and transferred into ice cold water and then centrifuged at 2500 *g* for 10 min. The MDA formation in each sample was detected as optical density observed at 532 nm. The results were expressed as nmol of MDA formed per minute per gram of tissue using molar extension coefficient $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ [22].

2.7.2. Measurement of glutathione reductase

For reduced GSH, 1.0 ml of 10% PMS was mixed with 1.0 ml of 4% sulphosalicylic acid, and then incubated at 4 °C for a minimum time period of 1 h and then centrifuged at 4 °C at 1200 *g* for 15 min. Briefly