Toxicology and Applied Pharmacology 251 (2011) 191–200

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Tannic acid ameliorates doxorubicin-induced cardiotoxicity and potentiates its anti-cancer activity: Potential role of tannins in cancer chemotherapy

Kulbhushan Tikoo *, Mukta Subhash Sane, Chanchal Gupta

Laboratory of Chromatin Biology, Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Mohali-160062, Punjab, India

ARTICLE INFO

Article history:
Received 30 October 2010
Revised 17 December 2010
Accepted 20 December 2010
Available online 29 December 2010

Keywords:
Doxorubicin
Cardiotoxicity
Tannic acid
PARP-1
Bax
Bcl-2

ABSTRACT

Doxorubicin, an anthracycline antibiotic, is widely used in the treatment of various solid tumors including breast cancer. However, its use is limited due to a variety of toxicities including cardiotoxicity. The present study aimed to evaluate the effect of tannic acid, a PARG/PARP inhibitor and an antioxidant, on doxorubicin-induced cardiotoxicity in H9c2 embryonic rat heart myoblasts and its anti-cancer activity in MDA-MB-231 human breast cancer cells as well as in DMBA-induced mammary tumor animals. Doxorubicin-induced cardiotoxicity was assessed by measurement of heart weight, plasma LDH level and histopathology. Bcl-2, Bax, PARP-1 and *p53* expression were examined by western blotting. Our results show that tannic acid prevents activation of PARP-1, reduces Bax and increases Bcl-2 expression in H9c2 cells, thus, preventing doxorubicin-induced cell death. Further, it reduces the cell viability of MDA-MB-231 breast cancer cells, increases *p53* expression in mammary tumors and shows maximum tumor volume reduction, suggesting that tannic acid potentiates the anti-cancer activity of doxorubicin. To the best of our knowledge, this is the first report which shows that tannic acid ameliorates doxorubicin-induced cardiotoxicity and potentiates its anti-cancer activity both *in vitro* (H9c2 and MDA-MB-231 cells) as well as in *in vivo* model of DMBA-induced mammary tumor animals.

Introduction

Breast cancer is a complex, multi-stage disease involving the deregulation of a number of different signaling cascades. It is a global health problem affecting one of every eight women in the United States (Wolf et al., 2006). Among the presently available cytotoxic drugs, anthracyclines play an undisputed role in the treatment of various malignancies (Minotti et al., 2004). Doxorubicin (DXR) obtained from soil actinomycetes *Streptococcus peucetius* is used for the treatment of solid tumors such as those arising in the breast, bile ducts, endometrial tissue, esophagus and liver, osteosarcomas, soft-tissue sarcomas and non-Hodgkin's lymphoma (Kalyanaraman et al., 2002). Despite its therapeutic benefits, its clinical use is limited due to dose-related cardiotoxicity. DXR-induced cardiotoxicity occurs due to the formation of free reactive oxygen radicals, direct DNA damage and/or interference with DNA repair and induction of immune reactions involving antigen-presenting cells in the heart (Chlebowski, 1979; Di Cosimo and Baselga, 2008). The enzymatic pathway of DXR-induced free radical generation involves the mitochondria also and is an important mechanism of DXR cardiotoxicity. DXR has high affinity for cardiolipin, a phospholipid that is enriched in the inner mitochondrial membrane. Due to this, DXR concentrates inside the myocytes (Goormaghtigh et al., 1990). Further, DXR-induced mitochondrial damage might lead to respiratory chain defects, resulting in continuous production of free radicals. In addition, mitochondrial damage may result in the release of cytochrome *c*, leading to the induction of apoptosis. DXR is also believed to reduce the activity of respiratory complexes, adenine nucleotide translocator or the voltage-dependent anion channel, which are important in the generation and transport of ATP from the mitochondria to the cytosol. Increased oxidative stress is a common factor implicated across the numerous hypotheses of the cardiotoxicity of DXR (Heide and L'Ecuyer, 2007).

Activation of the nuclear enzyme poly (ADP-ribose) polymerase (PARP) by oxidant-mediated DNA damage is an important pathway of cell dysfunction and tissue injury in conditions associated with oxidative stress (Catalgol et al., 2010). PARP overactivation has been implicated in DXR-induced cardiotoxicity and subsequent chronic cardiomyopathy (Ying et al., 2001; Pacher et al., 2002). PARP inhibitors are effective in a number of diseases such as diabetes, cancer, neuropathy, nephropathy, cerebral ischemia and cardiac myopathy (Tikoo et al., 2007b). Another way to inhibit PARP is to

Abbreviations: DMBA, Dimethylbenz [a]anthracene; DXR, Doxorubicin; LDH, Lactate Dehydrogenase; MTT, 3-(4,5-Dimethylthiazal-2-yl)-2,5-diphenyltetrazolium bromide; PARP, Poly(ADP-ribose) polymerase; NADH, Nicotinamide Adenine Dinucleotide; TBARS, Thiobarbituric Acid Reactive Substances.

* Corresponding author. Laboratory of Chromatin Biology, Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar, Mohali-160 062, Punjab, India. Fax: +91 172 2214692.
E-mail address: tikoo.k@gmail.com (K. Tikoo).

0041-008X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2010.12.012

inhibit poly (ADP)-ribose glycohydrolase (PARG) and thus, prevent the cleavage of poly (ADP-ribose) synthetase (PARS), which subsequently leads to PARP inhibition (Tikoo et al., 2007b). It has been reported that co-administration of PARP-1 inhibitor with cytotoxic drugs potentiates the activity of these agents and causes persistent DNA single strand breaks ultimately leading to cell death (Tentori and Graziani, 2005). Tannic acid is a PARG inhibitor and prevents PARP-1 mediated cell death by slowing the turnover of PAR chain and thus, limiting NAD^+ depletion. It is a mixture of polygalloyl esters of glucose and is known to exert various biological effects *viz.* anti-inflammatory, anti-cancer and anti-viral effects (Zhao et al., 2005). Formentini et al. (2008) had reported that mono-galloyl glucose moiety form the pharmacophore of tannins which is responsible for the PARG inhibitory activity. Interaction of PARG with PAR occurs through multiple binding sites which can be differently occupied by chemical inhibitors (Koh et al., 2003). Tannins might interact with the extended PAR-binding domain of PARG in a complex manner. Tannic acid also acts as an anti-oxidant and scavenges the free radicals and superoxide radicals. The ability to chelate iron and copper ions has also been attributed to its anti-oxidant effect (Andrade et al., 2005).

Based upon the above facts, we hypothesize that treatment of tannic acid along with DXR may reduce its cardiotoxicity and synergize or potentiate its anti-cancer activity both *in vitro* as well as *in vivo*. Furthermore, we investigated the mechanism underlying the effect of tannic acid on DXR-induced cardiotoxicity and its chemotherapeutic efficacy.

Materials and methods

Chemicals. DXR, dimethylbenz [a]anthracene (DMBA) was purchased from Sigma (St. Louis, MO, USA) and tannic acid was purchased from Merck Ltd. (Mumbai, India). H9c2 embryonic rat heart myoblast cell line was procured from the National Centre for Cell Science (NCCS, Pune, India). Dulbecco's Modified Eagles Medium (DMEM), antibiotic solution, fetal bovine serum and trypsin-EDTA were purchased from GIBCO (USA). MTT was purchased from Himedia Laboratories (Mumbai, India). All the other chemicals were purchased from Sigma (St. Louis, MO, USA), unless otherwise specified.

Cell culture. H9c2 rat heart myoblasts and MDA-MB-231 breast cancer cells were cultured in DMEM supplemented with 10% fetal bovine serum and antibiotics (penicillin G 100 IU/ml and streptomycin 100 mg/ml) in 5% CO_2 at 37 °C. At 80–90% confluence, H9c2 and MDA-MB-231 cells were treated with DXR (1 μM and 5 μM) and tannic acid (25 μM) for the indicated time interval. Cell supernatant was taken for LDH measurement and further processing was done for western blotting.

Animals. All the experiments were approved by the Institutional Animal Ethics Committee (IAEC) and complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) on handling of experimental animals. Female SD rats (8 weeks old; 160–180 g) were procured from the Institute's central animal facility (CAF) and kept under controlled environmental conditions of temperature (22 ± 2 °C), relative humidity (50 ± 5%) and a 12 h light/dark cycle. Standard animal feed (Pranaw Agro Industries, New Delhi) and water were provided to the animals *ad libitum*. The chemically induced mammary tumor animals were daily examined for signs of distress or pain. Special attention was given at 12 weeks, when the tumors were developed. The overall clinical condition, including appearance, posture, body temperature, presence of persistent anorexia and/or labored respiration, behavioral and physiological responses of every tumor bearing animal were routinely monitored. The food and water intake, body weight and tumor volume were assessed frequently. The

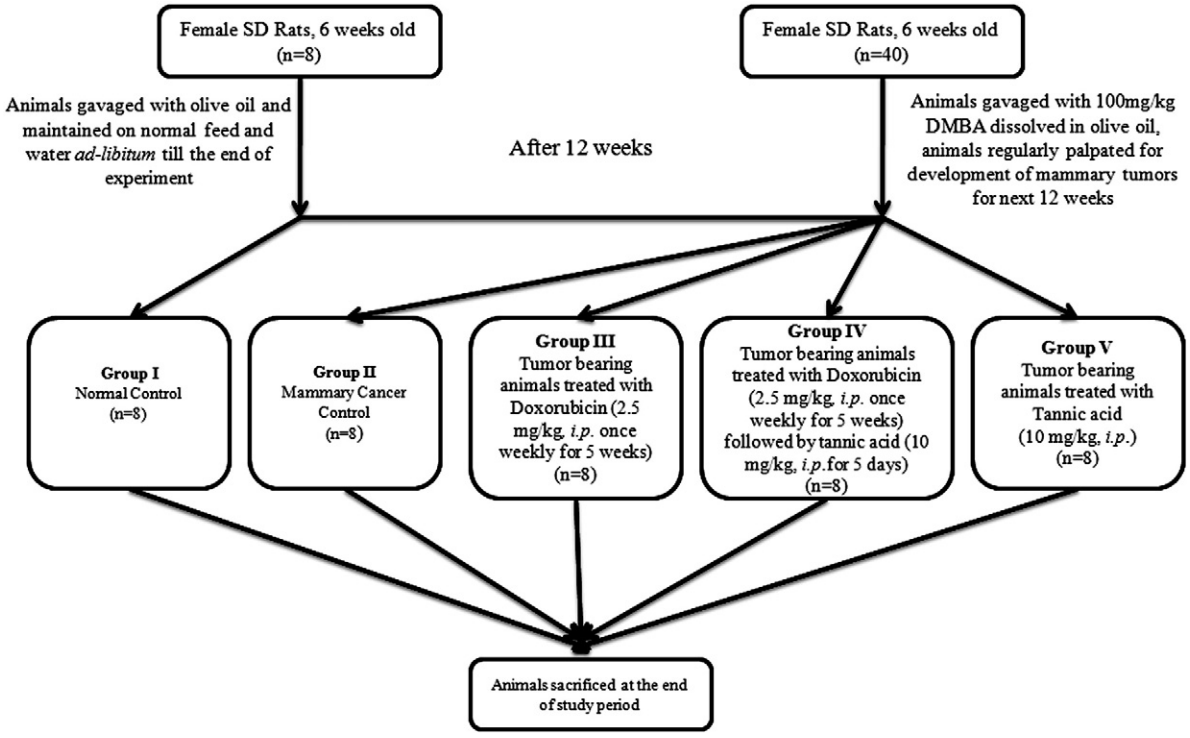

Fig. 1. Schematic representation illustrating the detailed experimental design. Female SD rats were divided initially into two groups namely, normal control (Group I) which received olive oil and DMBA treated group receiving DMBA (100 mg/kg, orally), dissolved in olive oil. After 12 weeks, DMBA treated rats were then divided into four different groups on the basis of their tumor volume. Group II (n = 8) received normal saline and served as mammary cancer control rats. Group III (n = 8) received DXR (2.5 mg/kg, once weekly for 5 weeks, i.p.) dissolved in normal saline. Group IV (n = 8) received DXR (2.5 mg/kg, once weekly for 5 weeks, i.p.), followed by post-treatment with tannic acid (10 mg/kg for five days, i.p.). Group V (n = 8) received tannic acid (10 mg/kg, i.p.) dissolved in phosphate buffered saline (PBS) and served as tannic acid control group. Animals were sacrificed at the end of the study period.