Biomedicine & Pharmacotherapy 106 (2018) 443–453

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Morin attenuates doxorubicin-induced heart and brain damage by reducing oxidative stress, inflammation and apoptosis

Muslum Kuzu[a,*], Fatih Mehmet Kandemir[b], Serkan Yildirim[c], Sefa Kucukler[b], Cuneyt Caglayan[d], Erdinc Turk[e]

[a] Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, İbrahim Çeçen University of Ağrı, Ağrı, Turkey
[b] Department of Biochemistry, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey
[c] Department of Pathology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey
[d] Department of Biochemistry, Faculty of Veterinary Medicine, Bingol University, Bingöl, Turkey
[e] Department of Pharmacy Professional Sciences, Faculty of Pharmacy, İbrahim Çeçen University of Ağrı, Ağrı, Turkey

ARTICLE INFO

Keywords:
Apoptosis
Doxorubicin
Inflammation
Morin
Oxidative stress

ABSTRACT

Doxorubicin (DOX) is an effective antineoplastic agent of the anthracycline group. However, as with most anticancer drugs, they cause some toxic effects, including major cardiotoxicity and cognitive impairment. In this study, protective effects of morin against DOX-induced cardiotoxicity and neurotoxicity in rats were investigated. Morin was orally administered to rats at a dose of 50 and 100 mg/kg body weight for 10 days. DOX was administered 40 mg/kg body weight by single dose intraperitoneal injection on the 8th day of the study. Both the levels of glutathione (GSH) and malondialdehyde (MDA) were assessed and enzyme activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were assessed to determine the protective effect of morin against oxidative stress. To determine the anti-inflammatory effect, the levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), nuclear factor kappa B (NF-κB) were assessed in the heart and brain tissues. Lactate dehydrogenase (LDH) and creatine kinase isoenzyme-MB (CK—MB) activities, which are cardiac function markers, and cardiac troponin-I (cTn-I) levels were also determined. Anti-apoptotic effect was determined by anti-apoptotic protein B-cell lymphoma-2 (Bcl-2) and pro-apoptotic protein cysteine aspartate specific protease-3 (caspase-3) changes. The regulatory role of morin in signal transduction in the brain tissue was assigned with the determination of amount of acetylcholinesterase (AChE), and its healing effect on the central nervous system was determined with imuinohistochemical detection of glial fibrillar acidic protein (GFAP) level. Histopathological evaluation of heart and brain tissues was performed in all groups.

1. Introduction

According to a report on cancer, which is the leading source of morbidity and mortality, it is estimated that there were 8.2 million cancer-related deaths and 14 million new cases worldwide in 2012, and new cases will be increased by 70% in the next 20 years [1]. Chemotherapy drugs, which are used primarily in the fight against cancer in the clinical setting, are used to kill cancer cells. However, chemotherapy drugs are cytotoxic agents that cause side effects such as loss of appetite, baldness, shortness of breath, cardiotoxicity by not only killing cancer cells but also harming healthy cells [2]. These side effects can harm cancer patients and reduce their quality of life or even cause their deaths [3].

Doxorubicin (DOX) is an anthracycline, a typical anticancer, and was first isolated from *Streptomyces peucetius* in the 1960s [2]. DOX is one of the most effective cytotoxic drugs against solid tumors such as ovarian, breast, gastrointestinal, Wilms tumor and hematologic malignant tumors such as Hodgkin's and non-Hodgkin's lymphoma and pediatric leukemia. DOX is also a common agent for adjuvant and neoadjuvant chemotherapy in breast cancer treatment [4,5]. Two mechanisms suggested for the anticancer effect of DOX have been confirmed [6]. One of these interact with the DNA and as a result, it causes degradation of DNA repair mediated with topoisomerase II [7]. The second mechanism involves oxidative stress for the cell membranes and DNA, and for proteins including NADH-dehydrogenase, NO synthase, xanthine oxidase, glutathione peroxidase, catalase and superoxide dismutase [8,9]. However, the clinical practice of DOX is limited to cumulative and dose-related cardiotoxicity and it causes congestive heart failure. Myelosuppression, acute nausea and vomiting, alopecia, stomatitis and extravasation reactions have been reported as other DOX

* Corresponding author at: İbrahim Çeçen University of Ağrı, Faculty of Pharmacy, 04100, Ağrı, Turkey.
E-mail address: mkuzu@agri.edu.tr (M. Kuzu).

https://doi.org/10.1016/j.biopha.2018.06.161
Received 14 April 2018; Received in revised form 26 June 2018; Accepted 27 June 2018

toxicities reported for DOX. The emergence of multidrug resistance and the low specificity of cancer cells are other problems [10]. In addition to all of these, cognitive impairments were reported to have adverse effects on patients' daily activities [11]. DOX is converted to the semiquinone radical by NADPH-cytochrome P-450, which initiates production of superoxide anion and hydroxyl radicals resulting in lipid peroxidation [12]. DOX also increases inflammatory effects in the myocardium and the vasculature by increasing the expression of nuclear factor kappa-B (NF-κB), a key regulator of genes that is involved in the inflammatory responses and immune responses [13].

Flavonoids are ubiquitous compounds and are a family of phenolic compounds found in many fruits, vegetables, fruit juices and herbal dietary supplements [14], and a number of which have revealed free radical extinguishing and antioxidant properties. Some epidemiological studies support the hypothesis that antioxidant behavior of flavanoids may reduce the risk of developing cardiovascular diseases [15]. Morin (3,5,7,2′,4′-pentahydroxyflavone), which is known to exhibit antioxidant, anti-inflammatory, antidiabetic, anti-carcinogenic, neuro-protection, and anti-proliferative effects in vivo and in vitro, is a natural bioflavonoid. It is first isolated from the *Moraceae* family and is found in most of the plants, fruits and wines [15,16]. In one study, morin was reported to have a neuroprotective effect in the ischemic brain injury model, and this effect was due to antioxidant and anti-apoptotic properties [17]. In addition, morin is considered to be an important bioactive compound that can be used to prevent hepatotoxicity [18]. However, the curative effects of morin against DOX-induced heart and brain damage have not been investigated yet. In this study, the healing effect of morin on heart and brain damage created on DOX-exposed rats was examined in the light of some biochemical, immunohistochemical and histopathological data.

2. Materials and methods

2.1. Drugs and chemicals

DOX was obtained as Adrimisin® (50 mg/25 mL injectable solution) from Saba Pharmaceuticals (İstanbul, Turkey). Morin hydrate and other chemicals were obtained from Sigma–Aldrich (St Louis, MO) and were of high analytical grade. The DOX dose was determined according to the previous study [12].

2.2. Animals

Male Wistar albino rats weighing 220–250 g for 10 weeks were used in the study. The rats were obtained from Ataturk University Experimental Research and Application Center. Animals were housed in standard cages under well-regulated conditions (relative humidity range: 45 ± 5%, temperature: 24 ± 1 °C and a 12-h light/12-h dark cycle). During the experiment, rats were fed with standard rat diet and water ad libitum. The experiments were designed and conducted according to ethical norms approved by the Local Animal Care Committee of Ataturk University, Erzurum, Turkey (Protocol No: 2017–1/9).

2.3. Experimental design

The study consisted of 5 different groups with 7 male rats of the genus Wistar Albino in each group. The selection of the rats was random. The groups were designed as follows.

Group I (Control); oral saline was administered for 10 days.

Group II (Morin); 100 mg/kg morin hydrate was orally administered to rats for 10 days.

Group III (DOX); On day 8 intraperitoneal 40 mg/kg single dose DOX was administrated.

Group IV (DOX + Morin 50 mg/kg); 50 mg/kg morin hydrate was orally administered to rats for 10 days, and intraperitoneal 40 mg/kg DOX was administrated on the 8th day.

Group V (DOX + Morin 100 mg/kg); 100 mg/kg morin hydrate was orally administered to rats for 10 days, and intraperitoneal 40 mg/kg DOX was given on the 8th day.

At the end of study period (10th day), the rats were sacrificed under mild sevoflurane anesthesia (Sevorane liquid 100%, Abbott Laboratory, İstanbul, Turkey). The heart and brain tissues from rats were evaluated for biochemical, immunohistochemical and histopathological analysis.

2.4. Examination of lipid peroxidation, GSH and antioxidant enzyme activities

MDA level was measured according to the method of Placer et al. [19]. Reduced glutathione (GSH) level was measured according to the method of Sedlak and Lindsay [20]. The MDA and GSH levels were expressed as nmol/g tissue. Superoxide dismutase (SOD) activity was measured by the method of Sun et al. [21], catalase (CAT) activity was determined according to the method of Aebi [22], glutathione peroxidase (GPx) activity was determined according to Lawrence and Burk's method [23]. The obtained results were presented as U/g protein for SOD and GPx and catal/g protein for CAT. Protein assays were performed according to the method of Lowry et al. [24].

2.5. Analysis of inflammation markers

Inflammation markers in the heart and brain tissues were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits. Heart and brain NF-κB, TNF-α and IL-1β levels were determined by using rat ELISA kit of Sunred Biological Technology. Analysis was performed with Elisa Plate Reader (Bio-Tek, Winooski, VT) according to the manufacturer's instruction.

2.6. Analysis of cardiac function markers

The cardiac lactate dehydrogenase (LDH) and creatine kinase isoenzyme-MB (CK-MB) activities and cardiac troponin-I (cTn-I) level were determined by using rat ELISA kit from Sunred Biological Technology (Shanghai, China). Analysis was performed with Elisa Plate Reader (Bio-Tek, Winooski, VT) according to the manufacturer's instruction.

2.7. Determination of Bcl-2 level

B-cell lymphoma-2 (Bcl-2) level was determined by using commercial rat ELISA kits obtained from Sunred Biological Technology Company. Analysis was performed with Elisa Plate Reader (Bio-Tek, Winooski, VT) according to the manufacturer's instruction.

2.8. Determination of AChE enzyme level

Acetylcholine Esterase (AChE) enzyme activity was determined by using commercial rat ELISA kits obtained from Sunred Biological Technology Company. Analysis was performed with Elisa Plate Reader (Bio-Tek, Winooski, VT) according to the manufacturer's instruction.

2.9. Histopathological analysis

As a result of the conducted necropsy, heart and brain tissue samples taken for histopathological evaluation were determined in 10% formalin solution for 48 h. They were buried in paraffin blocks as a result of routine tissue monitoring. Cross sections were taken from each block with a thickness of 4 μm. Preparations for histopathological examination were stained with hematoxylin-eosin (HE) and were examined by light microscopy (Leica DM 1000, Germany). The sections were evaluated as no (−), mild (+), moderate (++) and severe (+++) according to their immunopositivity