Pharmacological Reports 67 (2015) 1061–1067

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

Protective effect of gallic acid against bleomycin-induced pulmonary fibrosis in rats

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ARTICLE INFO

Article history:
Received 29 December 2014
Received in revised form 22 March 2015
Accepted 25 March 2015
Available online 11 April 2015

Keywords:
Pulmonary fibrosis
Bleomycin
Gallic acid
Oxidative stress
Rats

ABSTRACT

Background: Bleomycin (BLM), a chemotherapeutic agent is indicated in the management of some types of cancers. This drug produces a dose-dependent pulmonary fibrosis (PF) in most patients as well as experimental animals through oxidative injury. This study aimed to investigate the effect of gallic acid (GA), a polyphenolic compound, against PF-induce by BLM in rats.

Materials and methods: The rats were given GA orally at doses (50, 100, and 200 mg/kg/day) for 7 consecutive days before the administration of single intratracheal (*it*) instillation of BLM at 7.5 IU/kg. GA doses were continued for 21 days after BLM exposure. The regulatory effects of GA on BLM-induced pulmonary toxicity were determined by assaying oxidative stress biomarkers, lung and serum cytokine levels, and by histopathological examination of lung tissue.

Results: The results showed that intratracheal BLM administration significantly increased the inflammatory or fibrotic changes, collagen content, levels of malondialdehyde (MDA), and pro-inflammatory cytokines such as TNF-α and IL1β in lung. Also, it significantly decreased non-enzymatic (total thiol) and enzymatic (glutathione peroxidase (GPx)) antioxidant contents in the rats' lung tissue. However, oral administration of GA reversed all of these biochemical indices as well as histopathological alterations induced by BLM.

Conclusion: Results of the present study demonstrate that GA, by its antioxidant properties, attenuates oxidative damage and fibrosis induced by BLM. Thus, an effective supplement with GA as an adjuvant therapy may be a very promising compound in reducing the side effects of BLM.

Introduction

Idiopathic pulmonary fibrosis (IPF), featured by chronic exacerbating dyspnea and respiratory failure [1,2], which caused by environmental toxins, radiation, or chemotherapy of cancers or many chronic inflammatory diseases [3,4]. Pulmonary fibrosis leads to reduced lung function and has a high mortality rate [5]. The processes that drive fibrosis in lung tissue are complex which involved the leukocytes infiltration, proliferation of fibroblast cells, damage of the alveolar structures, and the deposition of extracellular matrix proteins. Moreover, reactive oxygen species (ROS) such as hydrogen peroxide, peroxynitrite, superoxide, and hydroxyl radical are also play a pivotal role in lung inflammatory processes that can induce fibrosis [6]. Many xenobiotics that stimulate the overproduction of ROS such as paraquat [7], butylated hydroxytoluene [8] and bleomycin [9] are able to produce lung fibrosis. There is currently no Food and Drug Administration-approved drug for the treatment of pulmonary fibrosis or other fibrosing disorders [5].

Bleomycin is an antitumor drug that used in the management of some human cancers, including lymphomas, squamous cell carcinomas and testicular tumors. Its cytotoxicity occurs by induction of free radicals that cause DNA breaks leading to cell death. This drug can be given by several routes: *iv*, *im*, or *sc*; in case of malignant effusion, it can be administered intrapleurally or intraperitoneally. Moreover, some studies suggest that the route

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http://dx.doi.org/10.1016/j.pharep.2015.03.012
1734-1140/

by which bleomycin is administrated may affect bleomycin-induced toxicity. Bleomycin continuously infused may induce less toxicity than bolus injection. However, other studies failed to show a relation between route of administration and toxicity [10].

The application of bleomycin is featured by the occurrence of pulmonary fibrosis that limits its clinical use. Moreover, bleomycin has been extensively used to prepare animal model of lung fibrosis [11]. So, a growing body of evidence has been reported that there is a linear positive relation between intratracheal dose and severity of pulmonary fibrosis induced by bleomycin in animals. Also, several studies suggested a similar relation in humans as well [10,12]. The lung is selectively influenced because this tissue lacks an enzyme that hydrolyzes the bleomycin, which prevents its metabolite from binding to metal ions and DNA [13]. The complex metabolites can generate ROS such as superoxide and hydroxyl radicals [11]. In mice, bleomycin produces DNA strand breakages that are lung selective and can be stimulated with oxygen exposure [14]. The breakage of DNA strand by bleomycin can be inhibited *in vitro* by the supplementation with variety of antioxidants, such as superoxide dismutase [15], glutathione [16], and also some herbal constituents such as cucumin [17], epigallocatechin-3-gallate [18], l-carnitine, *Ginkgo biloba* [19], and resveratrol [20].

Gallic acid (GA; 3,4,5-trihydroxybenzoic acid) and its derivatives are considered the main polyphenolic compounds in grapes, mango, areca nut, walnut, different berries, green tea and other fruits as well as in wine [21]. Preclinical studies have shown that GA possesses different pharmacological effects including antioxidant, anticancer, antimicrobial, anti-inflammatory [22] and neuroprotective activities [21,23–27]. In animal studies, GA reduces oxidative stress damages and enhances the levels of glutathione (GSH), GSH peroxidase, GSH reductase, and GSH S-transferase in hepatic and neural tissues, as well as catalase in serum [21,23,24,28]. It can also inhibit the polyunsaturated fatty acid saturation [29] and has anti-angiogenesis activity [30]. Exposure of human stomach cancer KATO-III cells and human colon adenocarcinoma COLO-205 cells to GA led to both growth inhibition and induction of apoptosis [31]. Moreover, Hsu et al. also reported that GA induces apoptosis in preadipocyte cells [28] and also in A549, a human lung adenocarcinoma cell line [32]. Since GA has the ability to induce apoptosis in tumor and preadipocyte cells, we attempted to investigate the inhibition of lung fibrosis by this compound in an experimental model of pulmonary fibrosis using intratracheal administration of bleomycin in rats.

Materials and methods

Chemicals

DTNB (2,2′-dinitro-5,5′-dithiobis-2-nitrobenzoic acid), TBA (2-thiobarbituric acid), n-butanol, Tris base, ethylendiaminetetraacetic acid disodium, glacial acetic acid, phosphoric acid, potassium chloride, 1,1′-3,3′-tetramethoxypropane (purity 99%) were obtained from Merck Company (Darmstadt, Germany). GA and bleomycin hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Nippon Kayaku Co. (Tokyo, Japan), respectively. Glutathione peroxidase (GPx) kit was obtained from Randox (Randox Labs, Crumlin, UK). The kits of TNF-α and IL-1β were purchased from eBiosource International, Inc. (Camarillo, CA, USA). All other chemicals were of analytical grade and prepared from Merck Company (Darmstadt, Germany). All drugs were dissolved in normal saline (0.9% NaCl). Drug concentrations were freshly prepared in such a way that the necessary dose could be injected in a volume of 5 ml/kg by oral route. Doses and drug administration schedules were selected based on previous report [21,33] and on pilot experiments in our laboratory.

Animals

Adult male Wistar rats weighing 220–250 g were used throughout the study. All of them were kept in the same room under a constant temperature (22 ± 2 °C), humidity (55–60%) and illuminated 7:00 a.m. to 7:00 p.m. with free access to food pellets and water. The rats were acclimatized to the laboratory conditions one day before the experimental session. All animal experiments were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals. The Institutional Animal Ethical Committee of Jundishapur University, formed under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Reg. No. APRC-9312) approved the pharmacologic protocols.

Induction of bleomycin-induced pulmonary fibrosis

An animal model of bleomycin induced pulmonary fibrosis was used as previously described [19,34,35]. Briefly, rats were intratracheally injected with bleomycin hydrochloride (7.5 IU/kg body weight in 0.25 mL normal saline) under ether anesthesia. The rats were killed 21 days after bleomycin injection. Control group was intratracheally given the same volume of saline instead of bleomycin. Lung fibrosis was assessed by lung hydroxyproline level as well as lung histopathological examination.

Experimental groups

Animals were randomly divided into the following five groups (eight each). Group 1 was the sham group in which normal saline (5 ml/kg) was given by oral gavage; group 2 was fibrosis group in which bleomycin was administered intratracheally and received normal saline as the same as group 1 (BLM-treated). In groups 3–5, GA (50, 100 and 200 mg/kg, *po*) was administered for 28 consecutive days started 7 day before bleomycin administration.

Lung sample collection and biochemical assays

At the end of experiments, the animals were sacrificed by decapitation. The lung tissues were removed quickly, rinsed with saline, and then kept in a freezer (−80 °C) until used. The weight of the lungs was recorded for each animal. The lung tissues were used to measure the levels of collagen, pro-inflammatory cytokines and oxidative stress parameters. A small portion of both lungs was cut and used for histopathological examination. The tissues were homogenized in a cold KCl solution (1.15%) to give a 10% homogenate suspension used for assessment of thiobarbituric acid reactive substances (TBARS) value, expressed as malondialdehyde equivalents (MDA), total thiol contents and GPx activity. Also, serum was withdrawn and kept at −80 °C to measure the pro-inflammatory cytokines levels.

Assessment of hydroxyproline content in the lung tissue

To evaluate the oxidant-induced tissue fibrosis, we measured the total tissue collagen contents using a colorimetric assay as lung hydroxyproline level as previously described [36]. Briefly, 100 mg of left lung samples was homogenized and then hydrolyzed in 100 ml of 6 N HCl for 18 h at 120 °C. The hydrolysate was then neutralized with 2.5 N NaOH. Aliquots (2 ml) were analyzed for hydroxyproline content after the addition of 1 ml chloramine-T, 1 ml perchloric acid, and 1 ml para-dimethylaminobenzaldehyde to produce colored complex. The absorbance of each sample at 550 nm was measured using spectrophotometer (Shimadzu UV-1650CT). Results are expressed as μg of hydroxyproline per gram lung tissue.