Available online at www.sciencedirect.com
ScienceDirect
Pulmonary Pharmacology & Therapeutics 20 (2007) 642–649
PULMONARY PHARMACOLOGY & THERAPEUTICS
www.elsevier.com/locate/ypupt

Resveratrol alleviates bleomycin-induced lung injury in rats

Göksel Şener[a,*], Nurhayat Topaloğlu[b], A. Özer Şehirli[a], Feriha Ercan[c], Nursal Gedik[d]

[a]Marmara University, School of Pharmacy, Department of Pharmacology, Istanbul, Turkey
[b]Marmara University, School of Medicine, Department of Internal Medicine, Istanbul, Turkey
[c]Marmara University, School of Medicine, Department of Histology-Embryology, Istanbul, Turkey
[d]Kasımpasa Military Hospital, Division of Biochemistry, Istanbul, Turkey

Received 9 May 2006; received in revised form 17 July 2006; accepted 31 July 2006

Abstract

Antioxidant therapy may be useful in diseases with impaired oxidant–antioxidant balance such as pulmonary fibrosis. This study was designed to examine the effects of resveratrol, an antioxidant agents, against bleomycin-induced pulmonary fibrosis and oxidative damage. Wistar albino rats were administered a single dose of bleomycin (5 mg/kg; via the tracheal cannula) followed by either saline or resveratrol (10 mg/kg; orally) for 14 days. The effect of resveratrol on pulmonary oxidative damage was studied by cell count and analysis of cytokine levels (TGF-β, TNF-α, IL-1β and IL-6) in the bronchoalveolar lavage fluid (BALF) and biochemical measurements of malondialdehyde (MDA), an end product of lipid peroxidation; glutathione (GSH), a key antioxidant; and myeloperoxidase (MPO) activity, an index of neutrophil infiltration, in the lung tissue. Bleomycin-induced lung fibrosis was determined by lung collagen contents and also microscopically.

Bleomycin caused a significant decrease in lung GSH, which was accompanied with significant increases in MDA level, MPO activity, and collagen contents of the lung tissue concomitant with increased levels of the pro-inflammatory mediators and cell count in BALF. On the other hand, resveratrol treatment reversed all these biochemical indices as well as histopathological alterations induced by bleomycin. The results demonstrate the role of oxidative mechanisms in bleomycin-induced pulmonary fibrosis, and resveratrol, by its antioxidant properties, ameliorates oxidative injury and fibrosis due to bleomycin. Thus, an effective supplement with resveratrol as an adjuvant therapy may be a very promising agent in alleviating the side effects of bleomycin, an effective chemotherapeutic agent.

Keywords: Bleomycin; Resveratrol; Fibrosis; Cytokine; Lipid peroxidation; Glutathione

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic inflammatory interstitial lung disease with a high mortality rate and poor response to available medical therapy [1,2]. It is characterized by an accumulation of alveolar macrophages and neutrophils in the lower respiratory tract, paranchymal cell injury and fibrosis of the alveolar walls [3].

Fibrosis is a feature of tissue remodeling that arises as a result of homeostatic repair mechanism but it may impact adversely the physiological function of the lungs [4]. It is well known that accumulated macrophages and neutrophils, releasing of toxic oxidants, are capable of inducing oxidant-mediated lung paranchymal cell toxicity [2,5]. Activated neutrophils can also release myeloperoxidase (MPO), an enzyme that interacts with H_2O_2, to form the highly toxic hydroxyl radicals [6]. In this regard, it is reasonable to hypothesize that the alveolar epithelial cell injury that characterizes IPF may result, at least in part, from an enhanced oxidant burden that may exist in the lower respiratory tract of patients. Thus, supplementing patients with agents that have antioxidant and anti-inflammatory properties as an adjuvant therapy may have beneficial effects in the treatment of IPF.

Resveratrol (3,5,4′-*trans*-trihydroxystilbene), a natural phytoalexin present in grapes, peanuts, mulberries and red

*Corresponding author. Tel.: +90 216 414 29 62; fax: +90 216 345 29 52.
E-mail addresses: gsener@marmara.edu.tr, gokselsener@hotmail.com (G. Şener).

1094-5539/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pupt.2006.07.003

wine, has various pharmacological effects including anti-inflammatory properties, modulation of lipid metabolism and prevention of cancer [7,8]. Its anti-inflammatory effect is related to inhibiting oxidation, leukocyte priming and expression of inflammatory mediators. Recently, it has been found to prevent and cure cardiovascular diseases [9] and improve microcirculatory disorders by protecting the vascular endothelium, modulation of lipid metabolism, increasing cellular nitric oxide levels, as well as inhibiting platelet aggregation [10]. Most of the studies have focused on the beneficial effects of resveratrol in the prevention of coronary hearth diseases and, cancer; however there are a limited number of studies considering its possible use as a therapeutic agent against drug induced oxidative organ damage. On the other hand, the use of alternative therapies, herbs, and supplements occurs at a very high rate among patients attending a variety of care settings [11].

Since bleomycin, one of the clinically important causative agents in pulmonary fibrosis, is widely used in experimental models of human disease resembling pulmonary fibrosis [12], in the present study we aimed to investigate the possible protective effect of resveratrol against bleomycin-induced oxidative lung injury in rats.

2. Materials and methods

2.1. Animals

Male Wistar albino rats (200–250 g) were housed in an air-conditioned room with 12-h light and dark cycles, where the temperature ($22 \pm 2\,^{\circ}C$) and relative humidity (65–70%) were kept constant. All experimental protocols were approved by the Marmara University School of Medicine Animal Care and Use Committee.

2.2. Experimental model for pulmonary fibrosis and treatment protocols

Following an overnight fasting, the rats were anesthetized (0.5 mg/kg ketamine hydrochloride and 1 mg/kg xylazine) and a midline incision was made in the neck and the trachea was exposed by blunt dissection. A tracheal cannula (i.d. = 2 mm, length = 2.5 cm) was inserted into the trachea under direct visualization. To produce pulmonary fibrosis, animals ($n = 16$) received a single dose of 5 mg/kg bleomycin (dissolved in 0.25 ml saline (0.9% NaCl)) via the tracheal cannula while control rats ($n = 16$) were administered 0.25 ml of saline intratracheally. Immediately after bleomycin administration, tracheal cannula was removed and rats were treated orally with either saline (1 ml/kg) or resveratrol (10 mg/kg/day; Mikrogen Pharmaceutical, Istanbul, Turkey) for 14 days. Fourteen days after bleomycin administration, BALF was obtained and then animals were decapitated to obtain lung tissues.

2.3. Bronchoalveolar lavage fluid

BALF was obtained by washing the airways four times with 5 ml of saline through a tracheal cannula. BALF was pooled and centrifuged at 1200*g* for 10 min. The supernatant was harvested for cytokine analysis and the pellet was smeared onto slides for cell classification and counting in BALF. A total cell count was performed with a haemocytometer and differential cell counts were performed on smeared preparations stained with Wright–Giemsa. Two hundred cells were counted for determination of the differential cell count.

BALF levels of transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), IL-1β and IL-6 were quantified using enzyme-linked immunosorbent assay (ELISA) kits specific for the previously mentioned rat cytokines according to the manufacturer's instructions and guidelines (Biosource International, Nivelles, Belgium). These particular assay kits were selected because of their high degree of sensitivity, specificity, inter- and intra-assay precision and small amount of plasma sample required to conduct the assay.

2.4. Biochemical analysis of lung tissue

After decapitation, lung tissues of rats were obtained, washed in 0.9% NaCl and stored at –70 °C. Afterwards, MDA, (an end product of lipid peroxidation) and glutathione (GSH, a key antioxidant) levels were measured in these samples. Tissue-associated MPO activity, as an indirect evidence of neutrophil infiltration, was measured in the tissue samples, while oxidant-induced tissue fibrosis was determined by tissue collagen contents. For histological analysis, samples of the tissues were fixed in 10% buffered *p*-formaldehyde and prepared for routine paraffin embedding.

Tissue samples were homogenized with ice-cold 150 mM KCl for the determination of MDA and GSH levels. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously [13]. Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of 1.56×10^5/M/cm and results were expressed as nmol MDA/g tissue. Glutathione measurements were performed using a modification of the Ellman procedure [14]. Briefly, after centrifugation at 2000*g* for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/l $Na_2HPO_4 \cdot 2H_2O$ solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of 1.36×10^5/M/cm. Results were expressed in μmol GSH/g tissue.

MPO is an enzyme that is found predominantly in the azurophilic granules of polymorphonuclear leukocytes (PMN). Tissue MPO activity correlates significantly with the number of PMN determined histochemically in