Biomedicine & Pharmacotherapy 101 (2018) 123–128

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Grape seed proanthocyanidin reverses pulmonary vascular remodeling in monocrotaline-induced pulmonary arterial hypertension by down-regulating HSP70

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ARTICLE INFO

Keywords:
Grape seed proanthocyanidin
Monocrotaline
Pulmonary arterial hypertension
Pulmonary arterial smooth muscle cell
HSP70
NF-κb

ABSTRACT

Heat shock protein 70 (HSP70) is a molecular chaperone which has a low content in cytoplasm under normal physiological conditions. A higher intracytoplasmic HSP70 level can be observed in pulmonary arterial smooth muscle cell (PASMC) in pulmonary arterial hypertension (PAH), and this up-regulation can promote pho-IκBα expression, which is an NF-κB signaling pathway inhibitor. NF-κB signaling pathway up-regulation can promote PASMC proliferation and pulmonary vascular remodeling in PAH, resulting in elevation of pulmonary pressure and the subsequent right heart failure caused by right ventricular hypertrophy. Grape seed proanthocyanidin (GSP) is effective in vascular protection and several tumor treatments, and its effect on PAH treatment remains to be elucidated. In this study, we made observations and contrasts in monocrotaline(MCT) -induced PAH rats, and found decrease in mPAP, PVR and RVHI after GSP administration. Our study also proved GSP's effect on down-regulating the intracytoplasmic HSP70 content both in cellular and animal levels. The results indicate a possible mechanism of GSP reversing pulmonary vascular remodeling by down-regulating HSP70, and this change may influence pho-IκBα expression. Therefore, inhibition of NF-κB signaling pathway caused by GSP can lead to inhibition of PASMC proliferation in PAH.

1. Introduction

Pulmonary arterial hypertension (PAH) is a pulmonary vascular disease characterized by elevation in pulmonary arterial pressure [1]. It contains various types of pathological features, but has similar histological changes in lung tissue such as intimal fibrosis, tunica media thickening, pulmonary arteriole obstruction and plexiform lesions predominate [2]. The current therapeutic and strategic targets of PAH symptomatically remain to be pulmonary vascular structure, whose mechanisms include anti-inflammatory action, vascular remodeling reversing, vasodilatation, antiplatelet aggregative activity and anti vascular obstruction [1]. PAH plays a role of a common cause of a series of cardiopulmonary diseases such as chronic pulmonary heart disease, chronic obstructive pulmonary disease (COPD) and pulmonary embolism etc., which appears a high mortality rate and brings severe adverse effects on patients [3]. The existing drugs for the treatment of PAH are of high cost and often with toxic side effects [4]. Therefore, drugs with both effectiveness and safety for PAH treatment share broad prospects.

Grape seed proanthocyanidin (GSP) is a pure natural substance extracted from grape seeds, which has functions of anti-inflammatory action [5], antopxidation, cardiovascular endothelium protection [6] and repair promotion after myocardial ischemia [7]. Remarkably, it appears no evident toxic effect in long term drug dosage on animals [8]. GSP is found to have anti-cancer effects on non-small cell lung cancer [9], colorectal cancer [10], breast cancer [11], skin squamous cell carcinoma [12], pancreatic cancer [13], and a capacity of inducing leukocyte apoptosis [14]. GSP also has therapeutic functions in the respiratory system, as its antopxidation effect has been proved to promote repair of pulmonary vascular injury caused by diabetes in rats [15] and can inhibit viral replication in respiratory epithelium, respiratory mucin formation and inflammation of pulmonary epithelial cells [16]. Obviously, GSP has a protective effect on pulmonary

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https://doi.org/10.1016/j.biopha.2018.02.037
Received 29 September 2017; Received in revised form 8 February 2018; Accepted 9 February 2018

vascular endothelium. Results carried out by GSP anticancer studies suggested that GSP has a function of inducing apoptosis in abnormal proliferation cells.

The use of GSP in PAH remains to be elucidated. Recently, new theories came out that PAH could be considered as a cancer-like disease, mainly for the abnormal proliferation of pulmonary arterial smooth muscle cell (PASMC), and this feature contributes to the pulmonary vascular remodeling [17,18]. In the micro-environment of PAH, pulmonary vascular endothelial cells get injured, increasing local inflammatory mediators, and PASMC in tunicae media abnormally reproduce [19]. The protective effect of GSP on pulmonary vascular endothelium is significant, while inhibition of PASMC proliferation is also worth investigating and exploring. Heat shock protein 70 (HSP70), as a member of heat-shock protein family, is involved in the regulation of apoptosis [20]. The increase of HSP70 is often associated with the promotion of cell proliferation and protection against apoptosis [21]. In this study, we investigated the inhibition effect of GSP on PASMC in monocrotaline(MCT)-induced PAH rat and hypothesized the possible mechanism related to HSP70.

2. Materials and methods

2.1. Drugs and reagents

GSP was purchased from Shanghai Yuanye Company. Monocrotaline(MCT) was purchased from Shanghai Tongtian Biotechnology and the specification was 150 mg/bottle. CCK-8 was purchased from Japan Dojindo Company. Anti-HSP70 polyclonal antibody and rabbit anti-β-actin monoclonal antibody were purchased from the United States Thermo company.pho-IκBα was purchased from Cell Signaling Technology Company. HSP70 was purchased from Beijing Boao Sen Company.

2.2. Animals and ethics statement

27 healthy adult male SD rats (obtained from Laboratory animal center of Wenzhou Medical University, Wenzhou, Zhejiang, China) weighing 180–220 g were used throughout the study. Animals were kept under appropriate environment with room temperature (22 ± 2 °C) and suitable humidity (55 ± 5%). Standard feed and water were provided for 1 week as adaptive culture. Animal experiments and administration strictly complied with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All experimental operations were committed to ensure that animal suffering is minimized.

2.3. Groups and dosage

27 SD rats were divided into 3 groups with a random number table (Con group, MCT group, GSP + MCT group): ① Con group (n = 9): rats received an subcutaneous injection of physiological saline, and the administration was continued for 3 weeks as a variable control parallel to the experimental group. ② MCT group(n = 9): rats received a single subcutaneous injection of MCT (60 mg/kg). An equal volume of physiological saline was given afterwards. The administration was continued for 3 weeks. ③ GSP + MCT group: rats received a single subcutaneous injection of MCT (60 mg/kg), and considering GSP has a low absorption rate [22], we applied intraperitoneal injection of GSP (10 ml/kg) afterwards. These operations were continued for 3 weeks.

2.4. Pulmonary artery hemodynamics measure

Rats were anesthetized by an intraperitoneal injectionof 20% ethylurethanm(dosage 1.2 ± 0.6 g/kg). An heparin-filled poly-ethylene (PE) catheter with its end burned hooked was straightened by a silver needle. It was applied to external jugular vein catheterization with its direction of the hook adjusted towards the pulmonary artery. The terminal of the PE catheter was connected with PowerLab biological signal acquisition and analysis system through the pressure transducer, which showed the pulmonary artery pressure. Data were used to calculate mean pulmonary arterial pressure(mPAP). To calculate pulmonary vascular resistance(PVR), the cardiac output (CO) was measured by thermodilution with an CO detector(ml313C, AD Instruments) and PowerLab/4SP biological signal recording and analysis system. PVR was calculated by formula: PVR = mPAP/CO.

2.5. Right ventricular hypertrophy index measure

After rats were executed and dissected, hearts were separated with the atrial appendage and the connective tissue cut off. The right ventricle (RV) was cut along the edge of the ventricle and the interventricular septum. After drained by filter paper, the weight of RV, left ventricle plus interventricular septum (LV + S) were respectively weighed. And right ventricle hypertrophy index(RVHI) was calculated by formula: RV/(LV + S) × 100%.

2.6. Morphological observation of pulmonary artery

Paraffin sections were made and stained with HE for observation of the pathological changes after lavage of lung tissues. To calculate the percentage of medial wall area (WA%) and the percentage of medial wall thickness (WT%), an Image-Pro analysis system was applied to measure the external diameter (ED), internal diameter (ID) and medial wall thickness (MWT). Pulmonary arteriole media thickness (PAMT) was calculated by the formula: PAMT = 2 × MWT/ED, and the value of wall area (WA), total area (TA), lumen area (LA) were calculated as well. WT% was calculated by the formula: WT% = 2 × PAMT/ED × 100%, and WA% was calculated by the formula: WA% = (TA-LA)/TA × 100%. Both indexes were considered for morphological observation of pulmonary artery.

2.7. CCK-8 assay

Primary cultured PASMCs were divided into 3 groups: Con group, MCT group, GSP + MCT group. The cell suspension was prepared by a concentration of 2×10^4 /ml, and was seeded in a 96-well plate by 100 μl/well with 3 groups of parallel control. MCT group and GSP + MCT group received an administration of MCT (0.75 g/L). GSP + MCT group received an extra administration of GSP (4 g/L), and MCT group received the same amount of physiological saline. Con group received the same amount of physiological saline as a controlled variable. Cell in each group received 10 μl CCK-8 solution before they were incubated. After 4 h, cell proliferation in each group were measured by optical density (OD) value at 450 nm.

2.8. Western blotting

The total proteins in cell and tissues in each group were extracted and prepared before they were quantitatively determined. Proteins were separated by 10% SDS-PAGE and transferred to an NC membrane. ECL imaging system was applied to collect the images after gel image analysis. The expressions of HSP70 and pho-IκBα were detected by corresponding antibodies, and expression levels were presented by the stripe gray scales.

2.9. Data were analyzed by SigmaPlot

Data were analyzed by SigmaPlot 12.0 and GraphPad Prism 6.01. The experimental procedures were repeated 3 times. The data were presented as mean ± standard deviation ($\bar{x}$ ± D). $P < 0.05$ is regarded to be significantly different.