Biochemical Pharmacology 150 (2018) 24–34

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

PARP-1 inhibition ameliorates elastase induced lung inflammation and emphysema in mice

Vivek Dharwal, Amarjit S. Naura*

Department of Biochemistry, Panjab University, Chandigarh, India

ARTICLE INFO

Keywords:
COPD
Lung inflammation
Emphysema
NF-κB
Olaparib
PARP-1

ABSTRACT

COPD is associated with high morbidity and mortality and no effective treatment is available till date. We have previously reported that PARP-1 plays an important role in the establishment of airway inflammation associated with asthma and ALI. In the present work, we have evaluated the beneficial effects of PARP-1 inhibition on COPD pathogenesis utilizing elastase induced mouse model of the disease. Our data show that PARP-1 inhibition by olaparib significantly reduced the elastase-induced recruitment of inflammatory cells particularly neutrophils in the lungs of mice when administered at a dose of 5 mg/kg b.wt (*i.p.*). Reduction in the lung inflammation was associated with suppressed myeloperoxidase activity. Further, the drug restored the redox status in the lung tissues towards normal as reflected by the levels of ROS, GSH and MDA. Olaparib administration prior to elastase instillation blunted the phosphorylation of P65-NF-κB at Ser 536 without altering phosphorylation of its inhibitor IκBα in the lungs. Furthermore, olaparib down regulated the elastase-induced expression of NF-κB dependent pro-inflammatory cytokines (TNF-A, IL-6), chemokine (MIP-2) and growth factor (GCSF) severely both at the mRNA and protein levels. Additionally, PARP-1 heterozygosity suppressed the recruitment of inflammatory cells and production of TNF-A, IL-6, MIP-2 and GCSF in the BALF to the similar extent as exhibited by olaparib administration. Finally, PARP-1 inhibition by olaparib or gene deletion protected against elastase-induced emphysema markedly. Overall, our data strongly suggest that PARP-1 plays a critical role in elastase induced lung inflammation and emphysema, and thus may be a new drug target candidate in COPD.

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is one of the leading causes responsible for worldwide mortality and morbidity. Owing to its rising prevalence, the management of the disease has become a formidable challenge for the present healthcare systems. Currently, COPD has around 11.7% global prevalence and is the fourth leading cause of worldwide deaths [1–3]. Further, with the rise in ratio of smokers, aging population in developed nations, prevalence of obesity, and outdoors pollution, projections are that the disease will be the third leading cause of worldwide deaths by 2020 [3–7]. Surprisingly, the available therapies against the disease have limited efficacy [8–11]. Furthermore, lesser effectiveness of steroids in managing COPD associated inflammation makes it imperative to better understand the disease pathogenesis and find new therapeutic targets [12–14].

PARPs comprise 18-membered family of proteins that carry out post-translational modifications of the target proteins by transfer of mono/poly ADP-ribose moieties, using NAD^+ as a substrate [15]. Among the different members of family, PARP-1 is the most important and extensively studied member. It is responsible for the majority (85–90%) of poly(ADP-ribosyl)ation in cell [15,16]. Originally, it was assumed that the protein is primarily involved in dealing with cellular stresses and plays an active role in DNA repair but in recent past pro-inflammatory role of this protein has emerged. It is now known that the over activation of PARP-1 (under severe/persistent DNA damage conditions) leads to depletion of its substrate .i.e. NAD^+ and thus causes necrosis [17]. Additionally, there are growing numbers of reports showing that PARP-1 regulates the expression of several NF-κB dependent cytokines, chemokines, adhesion molecules, inducible nitric-oxide synthase (iNOS), which play a critical role in manifestation of inflammatory cycle [18–22]. Since cigarette smoke, chronic inflammation, oxidative stress, nitrosative stress are associated with COPD pathogenesis, the reactive oxygen species (ROS) induced-DNA damage is also increased in patients with COPD [23–25]. Thus, persistent activation of PARP-1 during the disease progression has been reported [26,27]. Interestingly, several studies have reported key role of PARP-1 in asthma and acute lung injury pathogenesis [28–32]. In addition, pro-inflammatory role of the protein has been reported in

* Corresponding author at: Department of Biochemistry, Panjab University, Chandigarh 160014, India.
E-mail address: anaura@pu.ac.in (A.S. Naura).

https://doi.org/10.1016/j.bcp.2018.01.027
Received 14 November 2017; Accepted 12 January 2018

bleomycin-induced lung fibrosis [33]. However, only few studies have been conducted evaluating the roles of PARP-1 in COPD and research has been limited primarily to patients' blood samples [26,27,34]. The present study was designed with an aim to decipher role of PARP-1 in COPD pathogenesis using elastase-induced mouse model of the disease. Olaparib (Lynparza/AZD2281), a new generation competitive PARP inhibitor approved by US food and drug administration (FDA) and the European medicines agency (EMA) in ovarian cancer patients, was used in this study, and effects of PARP-1 inhibition on elastase-induced inflammation and emphysema were analyzed.

2. Materials and methods

2.1. Animals

Male BALB/c mice weighing 25–30 g (4–6 week old) were obtained from central animal house, Panjab University. The animals were housed in polypropylene cages, and were allowed full access to standard chow and water. All the experimental protocols involving animal manipulations were approved by the Panjab University institutional animal ethics committee (PU/IAEC/S/14/53). Additionally, C57BL/6 wild type, PARP-$1^{+/-}$ and PARP-$1^{-/-}$ (generated by backcrossing with C57BL/6 wild type) mice were also used in this study. Animals were housed and bred in a pathogen-free animal care facility at LSUHSC (New Orleans, LA), and were allowed full access to standard mouse chow and water. All experimental protocols were approved by the LSUHSC animal care and use committee (AICUC#2986).

2.2. Chemicals

All the chemicals used in the study were of analytical grade. Porcine pancreatic elastase (PPE) was purchased from Sigma-Aldrich, St. Louis, MO, USA. Olaparib was purchased from Selleck chemicals, Houston, TX, USA.

2.3. Experimental design

Animals were randomly divided into four groups with each group having 5–6 animals. Mice were anesthetized using intra-peritoneal (*i.p*) injection of ketamine (Thermis Medicare Limited, Haridwar, India) (90 mg/kg) and xylazine (Indian Immunological Limited, Hyderabad, India) (20 mg/kg), and were subjected to the following treatments:

2.3.1. Control group

Mice were administered 50 µl of saline intratracheally (*i.t*).

2.3.2. Olaparib group

Mice were administered 50 µl of saline (*i.t*) and were given olaparib (5 mg/kg b.wt) intraperitoneally (*i.p.*) 60 min prior to saline administration.

2.3.3. Elastase group

Mice were administered elastase (1U/mouse) (*i.t*).

2.3.4. Elastase + Olaparib group

Mice were administered elastase (1U/mouse) (*i.t*) and were given olaparib (either 1, 2.5, 5, or 10 mg/kg b.wt) (*i.p*) 60 min prior to elastase administration.

Furthermore, a group of mice were treated with vehicle alone (mixture of DMSO and saline at ratio of 1.5: 200). Olaparib stock was prepared by dissolving 8.6 mg of the drug in 100ul of DMSO (Sigma-Aldrich, St. Louis, MO, USA). For 5 mg/kg b.wt dosing 1.5 µl of the stock solution was dissolved in 200 µl saline and was administered *i.p*. Animals were sacrificed at different time points i.e. 24 h, 72 h, or 21 days after elastase administration. Additionally, some of the animals were given dexamethasone (Sigma-Aldrich, St. Louis, MO, USA) at different doses to evaluate effectiveness of steroids in our model.

2.4. Broncho-alveolar lavage fluid (BALF) analysis

2.4.1. Estimation of inflammatory cells in BALF

Animals were sacrificed 24 or 72 h after elastase administration by cervical dislocation. The lungs were subjected to broncho-alveolar lavage and microscopic slides were prepared using cytospin centrifuge as previously described [31]. Total and differential numbers of inflammatory cells were counted. Lung tissues of sacrificed animals were preserved at −80 °C and further analyses were carried out as explained later.

2.4.2. Cytokines estimation

Cytokines assessment was done in BALF supernatant samples for mouse TNF-A, IL-6, IL-1β, KC, GCSF, GMCSF, IL-12 (P40), IL-2, IL-4, IL-5, and MCP-1 using Bio-Plex assay kit and Bio-Plex® Multiplex Immunoassay System, and following manual instructions (Bio-Rad, Hercules, CA, USA).

2.5. Extraction of RNA and conventional PCR analysis

RNA extraction was performed in samples stored in RNA *later,* using phenol-chloroform isolation method [35]. cDNA was prepared using iScript cDNA synthesis kit, Bio-rad, Hercules, CA, USA. *Expression* of pro-inflammatory genes *TNF-A, IL-6, MIP-2, KC,* and *GCSF* was analyzed. Additionally, expression of cell adhesion molecules *ICAM-1* and *VCAM-1* was analyzed. *β-ACTIN* gene expression was used as internal control. The following primer sequences were used: ***TNF-A*****:** Forward-5′-TAT GGC TCA GGG TCC AAC TC-3′, Reverse-5′-CTC CCT TTG CAG AAC TCA GG-3′; ***IL-6*****:** Forward- 5′-CCG GAG AGG AGA CTT CAC AG-3′, Reverse- 5′-TCC ACG ATT TCC CAG AGA AC-3′; ***MIP-2*****:** Forward- 5′-AAG TTT GCC TTG ACC CTG AA-3′, Reverse- 5′-AGG CAC ATC AGG TAC GAT CC-3′; ***KC*****:** Forward- 5′-GCT GGG ATT CAC CTC AAG AA-3′, Reverse- 5′-TCT CCG TTA CTT GGG GAC AC-3′; ***GCSF*****:** Forward- 5′-CCT TCA CTT CTG CCT TCC AG-3′,Reverse- 5′-GCT CAG GTC TAG GCC AAG TG-3; ***ICAM-1*****:** Forward- 5′-AGC ACC TCC CCA CCT ACT TT-3′, Reverse- 5′-AGC TTG CAC GAC CCT TCT AA-3′; ***VCAM-1***: Forward- 5′-ACA GAC AGT CCC CTC AAT GG-3′, Reverse- 5′-ACC TCC ACC TGG GTT CTC TT-3′; ***β-ACTIN***: Forward- 5′-TAC AGC TTC ACC ACC ACA GC-3′, Reverse- 5′-TCT CCA GGG AGG AAG AGG AT-3′.

The resulting PCR products were subjected to electrophoresis in a 2% agarose gel and were stained with ethidium bromide. Bands were visualized and images were captured using Bio-Rad ChemiDoc, Hercules, CA, USA.

2.6. Western blot analysis

Proteins were extracted from the stored lung tissue samples using radio-immuno-precipitation assay (RIPA) buffer. Protein concentration was determined using the Lowry assay [36]. Equal amount of denatured proteins (40 µg) from different samples were loaded to SDS-PAGE gel and proteins were separated. Separated proteins were transferred to membrane [37]. Levels of poly(ADP-ribosyl)ation were measured using poly(ADP-ribose) monoclonal antibody (Enzo Life Sciences, Farmingdale, NY, USA) antibody. Assessment of NF-κB activation was done using monoclonal antibodies against pIKBα (Ser32/36) mouse (Cell Signaling Technology, Danvers, MA, USA) and Ph-p65NF-κB Rabbit (Cell Signaling Technology, Danvers, MA, USA). Expression of ICAM-1 was evaluated using ICAM-1 specific antibody (Santa Cruz Biotechnology, USA). Horseradish peroxidase-conjugated secondary antibodies (Bio-Rad, Hercules, CA, USA), specific to each primary antibody, were used and protein bands were visualized with enhanced chemiluminescence labeling (ECL) solution (Bio-Rad, Hercules, CA, USA) using FluorChemM (ProteinSimple, San Jose, USA). For each protein of interest, western blot was done twice.