European Journal of Pharmacology 744 (2014) 108–114

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Pulmonary, gastrointestinal and urogenital pharmacology

Role of mitogen-activated protein kinase phosphatase-1 in corticosteroid insensitivity of chronic oxidant lung injury

Mariona Pinart [a], Farhana Hussain [a], Sima Shirali [a], Feng Li [a], Jie Zhu [a], Andrew R. Clark [b], Alaina J. Ammit [c], Kian Fan Chung [a,*]

[a] Experimental Studies Unit, Airway Disease Section, National Heart and Lung Institute, Imperial College London, London, UK
[b] Kennedy Institute of Rheumatology Division, Imperial College London, London, UK
[c] Respiratory Research Group, Faculty of Pharmacy, University of Sydney, NSW, Australia

ARTICLE INFO

Article history:
Received 26 July 2014
Received in revised form
23 September 2014
Accepted 2 October 2014
Available online 12 October 2014

Key words:
Ozone exposure
Emphysema
Lung inflammation
Bronchial hyperresponsiveness
Mitogen-activated protein kinase phosphatase 1 (MKP-1)

ABSTRACT

Oxidative stress plays an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD) and in the induction of corticosteroid (CS) insensitivity. Chronic ozone exposure leads to a model of COPD with lung inflammation and emphysema. Mitogen-activated protein kinase phosphatase-1 (MKP-1) may underlie CS insensitivity in COPD. We determined the role played by MKP-1 by studying the effect of corticosteroids in wild-type C57/BL6J and MKP-$1^{-/-}$ mice after chronic ozone exposure. Mice were exposed to ozone (3 ppm, 3 h) 12 times over 6 weeks. Dexamethasone (0.1 or 2 mg/kg; intraperitoneally) was administered before each exposure. Mice were studied 24 h after final exposure. In ozone-exposed C57/BL6J mice, bronchial hyperresponsiveness (BHR) was not inhibited by both doses of dexamethasone, but in MKP-$1^{-/-}$ mice, there was a small inhibition by high dose dexamethasone (2 mg/kg). There was an increase in mean linear intercept after chronic ozone exposure in both strains which was CS-insensitive. There was lesser inflammation after low dose of dexamethasone in MKP-$1^{-/-}$ mice compared to C57/Bl6J mice. Epithelial and collagen areas were modulated in ozone-exposed MKP-$1^{-/-}$ mice treated with dexamethasone compared to C57/Bl6J mice. MKP-1 regulated the expression of MMP-12, IL-13 and KC induced by ozone but did not alter dexamethasone's effects. Bronchial hyperresponsiveness, lung inflammation and emphySEMa after chronic exposure are CS-insensitive, and the contribution of MKP-1 to CS sensitivity in this model was negligible.

1. Introduction

Oxidative stress is a feature of the airways and lungs of patients with chronic obstructive pulmonary disease (COPD), resulting from the release of reactive oxygen and nitrogen species from inflammatory and immune cells in the airways and from the direct exposure to oxidants present in cigarette smoke or environmental pollutants (Chung and Marwick, 2010). Oxidative stress plays an important role in the pathogenesis of COPD since cigarette smoke and particulate exposure are potent inducers of oxidative stress (Repine et al., 1997; Risom et al., 2005; Chung and Adcock, 2008). The mechanisms and pathways by which oxidative stress can lead to chronic inflammation and emphysema have been investigated in mouse models of cigarette exposure (Shapiro, 2007; Taraseviciene-Stewart and Voelkel, 2008). Thus, the importance of oxidative stress in inducing emphysema has been demonstrated in nuclear factor-E2-related factor-2 (Nrf2) knockout mice, which, through their diminished capacity to mount antioxidant defences, develop increased susceptibility to emphysema and lung inflammation following cigarette smoke exposure (Rangasamy et al., 2004). Furthermore, direct exposure of mice to an oxidant gas, ozone, results in emphysema and chronic lung inflammation reminiscent of COPD (Triantaphyllopoulos et al., 2011). Oxidant stress also causes bronchial hyperresponsiveness resulting from an increase in contractility of the airways (Li et al., 2011).

Corticosteroids (CSs) are widely used in the treatment of chronic airway inflammatory diseases. Although they are the most potent anti-inflammatory agent used in the treatment of asthma, they are not always effective as in patients with severe asthma and COPD. Several mechanisms may underlie CS insensitivity (Adcock et al., 2008), which includes the role of the mitogen-activated protein kinases (MAPK) (Chung, 2011). MAPK phosphatases terminate MAPK activation by dephosphorylating both threonine and tyrosine residues (Camps et al., 2000). MKP-1 or DUSP-1, the founding member of this group of at least 10 phosphatases, is an effective inhibitor of JNK and p38 MAPK. MKP-1 is up-regulated by oxidative stress and other stimuli such as ultra-violet light, TNFα,

* Corresponding author. Tel.: +44 207 3518174; fax: +44 207 3518126.
E-mail address: f.chung@imperial.ac.uk (K.F. Chung).

http://dx.doi.org/10.1016/j.ejphar.2014.10.003

IL-1 and several toll-receptor ligands (Keyse, 2000). MKP-1 may be involved in corticosteroid responses since corticosteroids inhibit p38 MAPK in macrophages from MKP-$1^{+/+}$ mice but not in those from MKP-$1^{-/-}$ littermates where there was an inability of corticosteroids to inhibit cytokine release from macrophages of these mice (Abraham et al., 2006). Thus, MKP-1 expression may underlie CS insensitivity found in various respiratory diseases such as severe asthma and COPD (Chung, 2011).

In order to investigate whether MKP-1 can modulate the effects of oxidative stress and their responses to CS, we used a model of chronic exposure to ozone that leads to alveolar space enlargement and destruction together with a chronic inflammatory process (6). Although CS have been shown to inhibit the effects of a single exposure to ozone (Salmon et al., 1998; Toward and Broadley, 2002), it is not known whether they inhibit the effect of multiple exposures to ozone. We therefore examined the effect of CS in a chronic ozone model, and determined the role played by MKP-1 by studying the MKP-$1^{-/-}$ mouse.

2. Materials and methods

2.1. Mice

Pathogen-free, 10–12 week old male C57/BL6J mice (Harlan, UK) and gender-matched MKP-$1^{-/-}$ mice (Kennedy Institute, Imperial College, UK) were housed within 'maximiser' filter-topped cages (Maximiser, Theseus caging system Inc., Hazelton, PA, USA). The original MKP-1 null strain provided by Bristol Myers Squibb (Dorfman et al., 1996) was on a mixed C57/BL6J-129Sv genetic background. This strain was back-crossed against C57/BL6J over nine generations, then intercrossed heterozygotes and MKP-$1^{+/+}$ and MKP-$1^{-/-}$ mice were identified by PCR-based screen of genomic DNA from tail snips. MKP-$1^{-/-}$ colonies are identical in genetic background (almost pure C57/BL6J), but differ only at the MKP-1 locus. We therefore used wild type C57/BL6J as controls. The protocols were approved by the Imperial College Biosciences group and performed under a license from the Home Office UK government.

2.2. Study design and methods

The experiments were performed within the legal framework of the United Kingdom under a Project License granted by the Home Office of Her Majesty's government. The researchers hold Personal Licenses to perform the experiments described here. MKP-$1^{-/-}$ and C57/BL6J were investigated and received ozone twice a week for a period of 6 weeks (a total of 12 exposures). Ozone was generated from an ozoniser (Model 500 Sander Ozoniser, Germany), mixed with air for 3 h at 2.5 parts per million (ppm) in a sealed Perspex container. Control animals received medical air only over the equivalent period. Ozone concentration was continuously monitored with an ozone probe (ATi Technologies, Ashton-U-Lyne, UK). Ozone exposure was carried out in 3 groups: (i) ozone and vehicle, (ii) ozone and 0.1 mg/kg dexamethasone and (iii) ozone and 2 mg/kg dexamethasone. During the final 4 weeks of ozone exposure, animals received either dexamethasone or vehicle 2 h prior to each exposure to ozone (i.e. 8 injections in all). After 2 weeks of ozone exposures, mice received intraperitoneal (i.p.) injections of either 0.1 mg/kg or 2 mg/kg dexamethasone (D4902-1G Sigma Aldrich, USA) dissolved in 0.1 ml Dulbecco phosphate buffered saline (PBS) (Sigma, Dorset, UK). One group exposed to ozone received the same volume (0.1 ml) of PBS as vehicle. Mice were studied 24 h after the last exposure to ozone.

2.3. Measurement of bronchial hyperresponsiveness

24 h following exposure, mice were anesthetized with an intraperitoneal injection of anesthetic solution containing midazolam (Roche Products Ltd., Welwyn Garden City, UK) and Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; Janssen Animal Health, Wantage, UK). Mice were tracheostomized and ventilated (Mini Vent type 845, Hugo Sach Electronic, Germany; rate: 250 breaths/min and tidal volume: 250 μl). Mice were monitored in a whole body plethysmograph with a pneumotachograph connected to a transducer (EMMS, Hants, UK). Transpulmonary pressure was assessed via an esophageal catheter. Instantaneous calculation of pulmonary resistance (R_L) was obtained. Increasing concentrations of acetylcholine chloride (ACh) (Sigma, Dorset, UK) (4–256 mg/ml) were administered with an Aeroneb® Lab Micropump Nebulizer (EMMS, Hants, UK), and R_L was recorded for a 3-min period following each concentration. R_L after each concentration was expressed as percentage change from baseline R_L measured following nebulized PBS (Sigma, Dorset, UK). The concentration of acetylcholine required to increase R_L by 100% from baseline was calculated (PC_{100}).

2.4. Measurement of inflammation and mean linear intercept, L_m

Following an overdose of pentobarbitone anesthetic, the lungs were dissected out and were inflated by injecting fresh 4% paraformaldehyde into the lungs to provide 25 cm of water pressure for at least 4 h. Lungs were processed using a histological automatic tissue processor and embedded in paraffin. Paraffin blocks were sectioned to expose the maximum surface area of lung tissue in the plane of the bronchial tree. 5 μm sections cut and stained with haematoxylin and eosin and Masson's trichrome stain were point-counted to assess morphological changes of airway epithelium, collagen deposition and airway smooth muscle (ASM) mass.

The mean linear intercept (L_m) is a measure of the surface area-to-volume ratio representing a stereological metric of alveolar size. Using a reticule with a Thurlbeck grid comprising 5 lines (each 550 μM long) and 10 fields per section was assessed at random. Fields with airways or vessels were avoided by moving one field in any one direction. The total score for each section was determined by counting the number of times the alveolar wall tissue intercepted each line. L_m was calculated by dividing the length of the line by the number of tissue intercepts counted.

The inflammatory response observed in the haematoxylin–eosin-stained lung sections was scored on a 0–3 scale as follows: 0=no inflammatory response, 1=mild inflammation with foci of inflammatory cells in bronchial or vascular wall and in alveolar septa; 2=moderate inflammation with patchy inflammation or localized inflammation in walls of bronchi or blood vessel and alveolar septa and less than 1/3 of lung cross-sectional area is involved; and 3=severe inflammation with diffuse inflammatory cells in walls of bronchi or blood vessels, and alveoli septa; between one third to two thirds of the lung area is involved.

All counts on histology sections were performed by one investigator who was unaware of the treatment protocol.

2.5. Reverse transcription, and real-time PCR of mouse lungs

RNA was extracted from frozen stored lung tissue using an RNeasy Mini kit (Qiagen). RNA yield was then amplified via PCR using an Omniscript Reverse Transcriptase kit (Qiagen) and stored at −80 °C until required. 0.5 μg per sample of RNA was used to synthesize single-stranded complementary DNA (cDNA) using random hexamers and an avian myeloblastosis virus reverse transcriptase (Promega). The cDNA generated was used as a template in subsequent real-time PCR analyses to determine transcript levels by using Rotor Gene (Rotor Gene 3000, Corbett Research) and QuantiTech SYBR