Toxicology and Applied Pharmacology 339 (2018) 161–171

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Adrenergic and glucocorticoid receptor antagonists reduce ozone-induced lung injury and inflammation

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ARTICLE INFO

Keywords:
Stress response
Glucocorticoid receptor antagonist
Adrenergic receptor antagonist
Ozone
Lung injury
Lung inflammation

ABSTRACT

Recent studies showed that the circulating stress hormones, epinephrine and corticosterone/cortisol, are involved in mediating ozone-induced pulmonary effects through the activation of the sympathetic-adrenal-medullary (SAM) and hypothalamus-pituitary-adrenal (HPA) axes. Hence, we examined the role of adrenergic and glucocorticoid receptor inhibition in ozone-induced pulmonary injury and inflammation. Male 12-week old Wistar-Kyoto rats were pretreated daily for 7 days with propranolol (PROP; a non-selective β adrenergic receptor [AR] antagonist, 10 mg/kg, i.p.), mifepristone (MIFE; a glucocorticoid receptor [GR] antagonist, 30 mg/kg, s.c.), both drugs (PROP + MIFE), or respective vehicles, and then exposed to air or ozone (0.8 ppm), 4 h/d for 1 or 2 consecutive days while continuing drug treatment. Ozone exposure alone led to increased peak expiratory flow rates and enhanced pause (Penh); with greater increases by day 2. Receptors blockade minimally affected ventilation in either air- or ozone-exposed rats. Ozone exposure alone was also associated with marked increases in pulmonary vascular leakage, macrophage activation, neutrophilic inflammation and lymphopenia. Notably, PROP, MIFE and PROP + MIFE pretreatments significantly reduced ozone-induced pulmonary vascular leakage; whereas PROP or PROP + MIFE reduced neutrophilic inflammation. PROP also reduced ozone-induced increases in bronchoalveolar lavage fluid (BALF) IL-6 and TNF-α proteins and/or lung *Il6* and *Tnfα* mRNA. MIFE and PROP + MIFE pretreatments reduced ozone-induced increases in BALF *N*-acetyl glucosaminidase activity, and lymphopenia. We conclude that stress hormones released after ozone exposure modulate pulmonary injury and inflammatory effects through AR and GR in a receptor-specific manner. Individuals with pulmonary diseases receiving AR and GR-related therapy might experience changed sensitivity to air pollution.

1. Introduction

Ozone is a reactive secondary pollutant which oxidizes biomolecules in the respiratory tract upon inhalation (Bromberg, 2016). The accepted paradigm of ozone-induced lung injury and inflammation involves its direct interaction with lung lining components and generation of oxidized lipid and protein byproducts (Auerbach and Hernandez, 2012), which are responsible for activation of the inflammatory signaling cascade and mediating downstream effects such as lung function decrement, increased vascular permeability, and neutrophilic inflammation. These ozone-induced effects are reversible even if daily exposure continues over several days suggesting tolerance or adaptation (Miller et al., 2016a). The oxidatively-modified reactive byproducts generated locally within the lung are believed to promote local cellular effects, such as stimulating the release of cytokines and chemokines to promote recruitment of neutrophils via activation of NRF2 and NFκB pathways in the lung (Hollingsworth et al., 2007). These reactive byproducts are also thought to promote systemic effects through their release from the lung to circulation. The identity of circulating bioactive molecules and how they contribute to lung effects of air pollutants remains an area of interest.

Acute ozone exposure has also been shown to induce pulmonary sensory irritation and C-fiber activation. Upstream events that are involved in this response include neuron firing of the nucleus tractus solitarius (NTS) (Gackière et al., 2011), increases in circulating adrenocorticotropic hormone (ACTH) (Thomson et al., 2013), and cardiac

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https://doi.org/10.1016/j.taap.2017.12.006
Received 2 October 2017; Received in revised form 5 December 2017; Accepted 12 December 2017
Available online 13 December 2017
0041-008X/ Published by Elsevier Inc.

changes through autonomic reflex mechanisms (Arjomandi et al., 2015; Gordon et al., 2014). These events suggest that ozone inhalation is capable of triggering a centrally-mediated neuroendocrine stress response which results in increased release of stress hormones into the systemic circulation (Kodavanti, 2016; Snow et al., 2017). Indeed, we have recently shown that circulating stress hormones such as epinephrine and cortisol/corticosterone rise rapidly after acute ozone exposure in both rodents (Bass et al., 2013; Miller et al., 2015, 2016b) and humans (Miller et al., 2016c). Subsequent ozone-induced lung global gene expression changes mimic those induced by downstream events promoted by β_2 adrenergic and glucocorticoid receptor activation, further suggesting that circulating stress hormones might play a key role as mediators of pulmonary responses (Henriquez et al., 2017). The role of circulating adrenal gland-derived stress hormones in ozone-induced lung injury and inflammation in rats was further confirmed by the evidence that bilateral adrenalectomy diminished these ozone effects (Miller et al., 2016b) and associated global lung transcriptional changes (Henriquez et al., 2017).

Circulating adrenal-derived stress hormones, epinephrine and corticosterone mediate their tissue effects through adrenergic (AR; α and β) and glucocorticoid (GR) receptors. AR are widely distributed throughout the body and although epinephrine is a prototypical agonist for all types of AR, the selective activation of specific AR subtypes determines the physiological organ-specific response. β_1AR are mainly expressed in the cardiac tissue and are central in maintaining cardiac output and contractility of cardiac muscle (Kurz et al., 1991), hence βAR blockers are widely used to reduce blood pressure. On the other hand, β_2AR are primarily distributed in the smooth muscles of bronchi and blood vessels (Molinoff, 1984). Airway smooth muscle relaxation by β_2AR agonists is a common pharmacological intervention used for bronchodilation in asthma and chronic obstructive pulmonary disease (Cazzola et al., 2013). Propranolol (PROP) is a non-selective βAR antagonist capable of blocking both β_1AR and β_2AR and, unlike epinephrine, it readily crosses the blood brain barrier (Olesen et al., 1978).

Circulating cortisol/corticosterone binds to GR that are present in virtually all cells in the body. Nuclear translocation of these receptors up-regulates a variety of genes involved in homeostatic response(s) (Oakley and Cidlowski, 2013). Non-genomic actions of glucocorticoids on GR have also been identified (Duque and Munhoz, 2016). Although the anti-inflammatory and immunosuppressive actions of glucocorticoids are not completely understood, potent GR agonists are commonly used to treat inflammatory conditions (Petta et al., 2016). By contrast, other studies have shown pro-inflammatory actions of GR activation (Cruz-Topete and Cidlowski, 2015). Mifepristone (MIFE) is a GR antagonist used to examine cellular effects of GR (Kakade and Kulkarni, 2014).

Based on the previous observation that circulating stress hormones are likely involved in mediating pulmonary effects of ozone (Miller et al., 2016b), the goal of this study was to examine the role of stress hormone receptors, βAR and GR in ozone-induced local lung injury and inflammation using a pharmacological approach. PROP, a non-selective βAR antagonist, was used to antagonize the activity of epinephrine while MIFE, a GR antagonist, was used to antagonize the activity of corticosterone. We hypothesized that the blocking of βAR and/or GR would produce selective inhibition of lung injury and/or inflammation and associated signaling events caused by exposure to ozone in rats.

2. Materials and methods

2.1. Animals

Male Wistar Kyoto (WKY) rats (10 weeks of age), purchased from Charles River Laboratory (Raleigh, NC) were housed in pairs in polycarbonate cages containing beta chip bedding under controlled conditions (21 °C, 50–65% relative humidity and 12 h light/dark cycle). Rats were provided with standard Purina (5001) rat chow (Brentwood, MO) and water ad libitum, and housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved animal facility. Animal procedures were approved by the US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory Institutional Animal Care and Use Committee. Because we previously determined that 5–10% of male WKY rats develop spontaneous cardiac hypertrophy (Shannahan et al., 2010), all animals were evaluated for evidence of cardiac hypertrophy relative to body weights at necropsy. Those with 20% or greater increases in the heart to body weight ratio were removed from further data analysis.

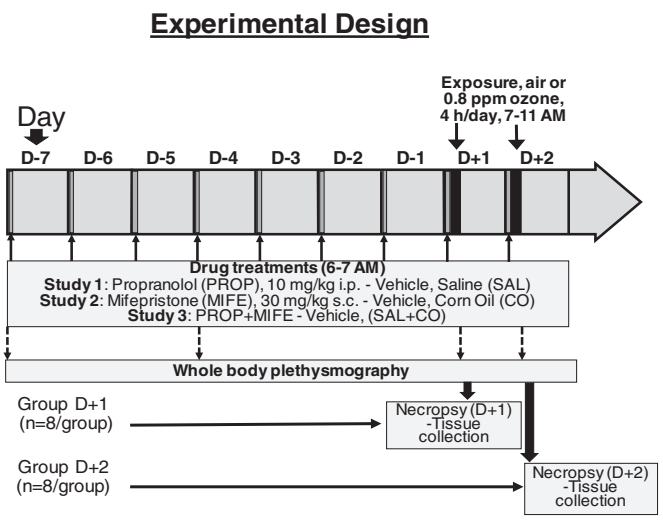

Fig. 1. Schema of the experimental design. For all three studies, the timing for drug pretreatments and the information on air or ozone exposure, plethysmography and necropsies are indicated by corresponding arrows. Animals assigned to 1 day (4 h) air or ozone exposure are referred as group D + 1 and those assigned to 2 consecutive days of exposure are referred as group D + 2. Animals assigned to group D + 2 were subjected to plethysmography prior to the start of drug pretreatment, after 3 days of drug pretreatment (D-4) and immediately after each day of air/ozone exposure. Necropsy and tissue collection were performed immediately after exposure (D + 1) or after exposure and plethysmography (D + 2) (within 1–2 h of exposure). Vehicles: SAL, saline; CO, corn oil; drugs: PROP, propranolol; MIFE, mifepristone.

2.2. Drug pretreatments and ozone exposures

Three studies were conducted for each protocol involving PROP, MIFE, and PROP followed by MIFE (PROP + MIFE) pretreatments (Fig. 1). For each study, rats were randomized by body weight into four groups (vehicle/air, drug/air, vehicle/ozone, drug/ozone) and time point (one day, D + 1; or two days, D + 2) (n = 8/group). In the first study, rats were injected i.p. with sterile saline (1 ml/kg) or propranolol hydrochloride (PROP, Sigma-Aldrich, St Louis, MO; 10 mg/kg in saline). In the second study, rats were injected s.c. with pharmaceutical grade corn oil (1 ml/kg) or mifepristone (MIFE, Cayman Chemical Co., Ann Arbor, MI; 30 mg/kg in corn oil). In the third study, control rats were injected with saline (1 ml/kg, i.p.) followed by corn oil (1 ml/kg, s.c.) while the drug-treated group was injected with PROP (10 mg/kg, i.p.) followed by MIFE (30 mg/kg, s.c.) (PROP + MIFE). For all three studies, the vehicle/drug pretreatments began seven days prior to the start of air or ozone exposure (from Day-7 to Day-1 in the morning) and continued each day of air or ozone exposure (D + 1 and D + 2) (Fig.1). The drug concentrations, the route of administration and the treatment durations were based on literature review of previously published papers examining the roles of AR and GR in mediation of cellular responses (Bible et al., 2015; Mohr et al., 2011; Sharrett-Field et al., 2013; Pitman et al., 2011). The dose levels we used for propranolol and mifepristone in rats were higher than what are used for humans. The maximum therapeutic dose for mifepristone in Cushing's syndrome patients is 20 mg/kg/day (https://reference.medscape.com/drug/