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Comparison of *in vitro* toxicological effects of biomass smoke from different sources of animal dung

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ABSTRACT

Worldwide, over 4 million premature deaths each year are attributed to the burning of biomass fuels for cooking and heating. Epidemiological studies associate household air pollution with lung diseases, including chronic obstructive pulmonary disease, lung cancer, and respiratory infections. Animal dung, a biomass fuel used by economically vulnerable populations, generates more toxic compounds per mass burned than other biomass fuels. The type of animal dung used varies widely depending on local agro-geography. There are currently neither standardized experimental systems for dung biomass smoke research nor studies assessing the health impacts of different types of dung smoke. Here, we used a novel reproducible exposure system to assess outcomes related to inflammation and respiratory infections in human airway cells exposed to six different types of dung biomass smoke. We report that dung biomass smoke, regardless of species, is pro-inflammatory and activates the aryl hydrocarbon receptor and JNK transcription factors; however, dung smoke also suppresses interferon responses after a challenge with a viral mimetic. These effects are consistent with epidemiological data, and suggest a mechanism by which the combustion of animal dung can directly cause lung diseases, promote increased susceptibility to infection, and contribute to the global health problem of household air pollution.

1. Introduction

Biomass smoke, generated from the burning of solid fuels, such as animal dung, wood, and crop residues, for cooking and household heat, is the leading environmental risk factor for all-cause mortality (Martin et al., 2011). According to the Global Alliance for Cleaner Cookstoves, one person dies every 8 s due to biomass smoke inhalation (Yadama, 2013). Yet, 3 billion people worldwide, especially individuals with a low socioeconomic status, are exposed to biomass smoke (Martin et al., 2013). Women and children often breathe high levels of biomass smoke since they usually spend the most time near household fires based on cultural practices (Yadama, 2013; Gordon et al., 2014). The burning of biomass fuels is a global health disparity issue.

The respiratory tract, particularly the airway epithelium, is the primary target for inhaled biomass smoke. Epidemiological evidence associates biomass smoke exposure with lung diseases, including chronic obstructive pulmonary disease (COPD), lung cancer, and respiratory infections (Martin et al., 2011; Gordon et al., 2014; World Health Organization, 2014). Similarly, *in vitro* studies have shown that human lung cells exposed to certain types of biomass smoke have heightened inflammatory responses and impaired immune defenses (Mehra et al., 2012; Rylance et al., 2015; McCarthy et al., 2016). Biomass smoke exerts toxic effects on the lung, leading to human health problems.

Animal dung is a biomass fuel that is widely burned by people living in low-income countries, since it is cheap, easy to collect and prepare for burning, and available in areas with limited vegetation. However, the inhalation of dung biomass smoke is of particular concern to human

Abbreviations: 16-HBE, 16-human bronchial epithelial cells; AP-1, activator protein-1; ALI, air-liquid interface; AhR, aryl hydrocarbon receptor; CO, carbon monoxide; COPD, chronic obstructive pulmonary disease; Cox-2, cyclooxygenase-2; DSE, dung smoke extract; ELISAs, enzyme-linked immunosorbent assays; GM-CSF, granulocyte macrophage colony-stimulating factor; HEK, human embryonic kidney cells; IP-10, interferon-inducible gamma protein-10; ISRE, interferon-sensitive response element; IL-8, interleukin-8; JNK, c-jun N-terminal kinase; NFκB, nuclear factor kappa B; poly I:C, polyinosine-polycytidylic acid; SAECs, small airway epithelial cells; TPM, total particulate matter

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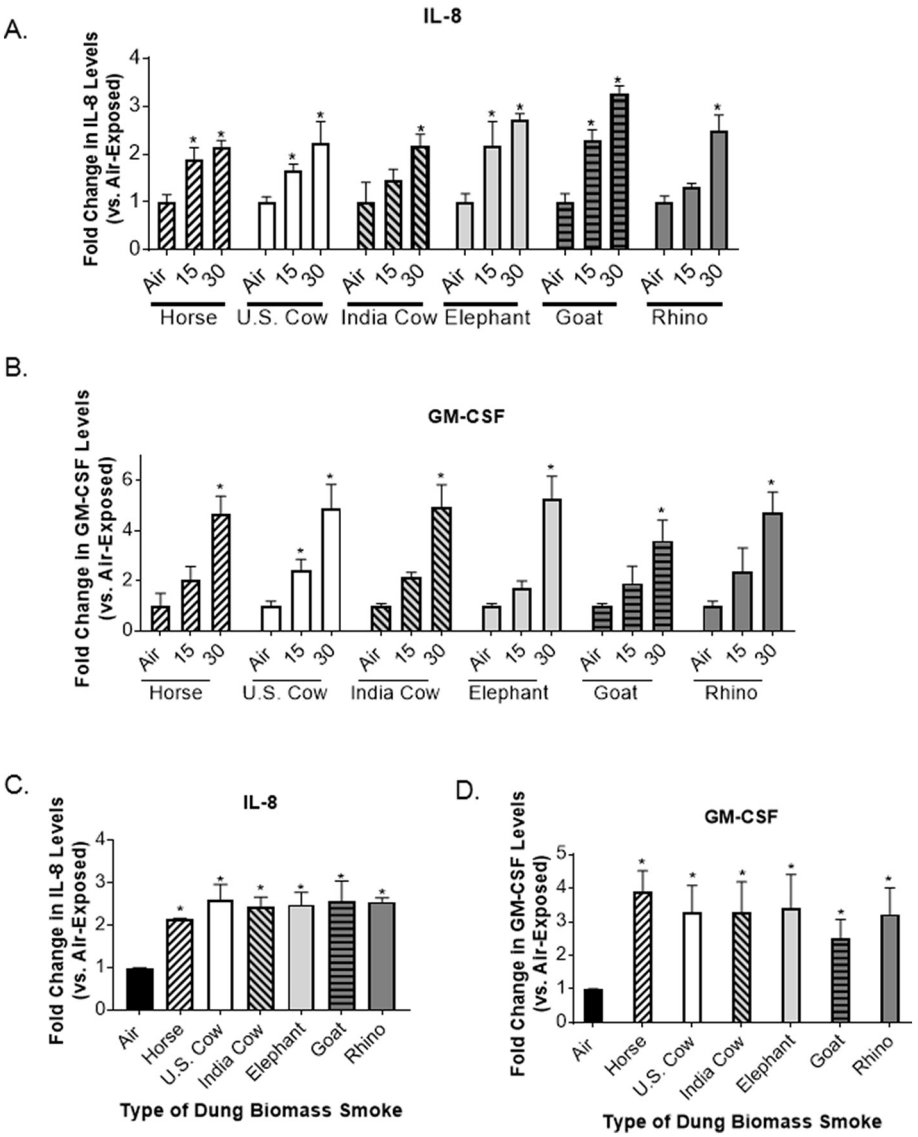

Fig. 1. Six different types of dung biomass smoke induce pro-inflammatory cytokine production in SAECs.
SAECs were exposed to air or dung smoke (horse, U.S. cow, India cow, elephant, goat, or rhinoceros (rhino)) for 15 or 30 min and cell supernatants were collected 24-h post-exposure. Dose-response effects of dung biomass smoke exposure on (A) IL-8 and (B) GM-CSF production in SAECs were determined by ELISA. Data represent mean ± SD (n = 3 – replicates per exposure group from an independent experiment), *p < 0.05 by two-way ANOVA (compared to air-exposed cells using Tukey's post-hoc analysis). (C) IL-8 and (D) GM-CSF levels were measured in SAECs exposed to 30 min of dung smoke in multiple experiments. Data represent mean ± SEM for n = 3 independent experiments with 3 replicate cultures per experiment. *p < 0.05 by one-way ANOVA (compared to air-exposed cells using a Dunnett's post-hoc analysis).

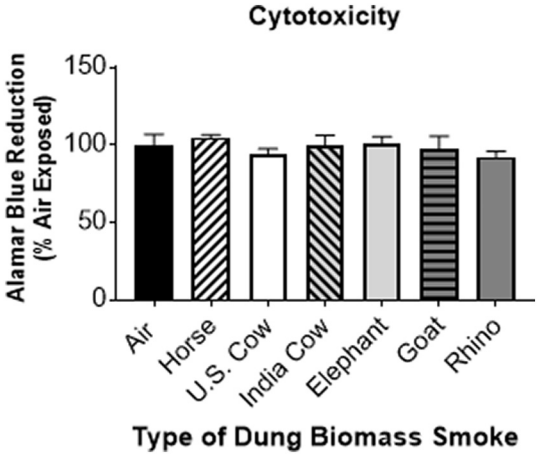

Fig. 2. Exposure to 30 min of six different types of dung biomass smoke does not cause cytotoxicity in SAECs.
SAECs were exposed to air or dung smoke (horse, U.S. cow, India cow, elephant, goat, or rhinoceros (rhino)) for 30 min. Alamar Blue reagent was added to the cells 24-h post-exposure. After a 4-hour incubation, cell supernatants containing Alamar Blue were collected. Reduction of Alamar Blue was determined by measuring the fluorescence of the samples (excitation = 560 nm and emission = 590 nm). Data represent mean (n = 3 replicates per exposure group from an independent experiment).

health. Cow dung biomass smoke was found to contain a greater oxidative capacity, more particulates per mass of fuel burned, and higher levels of microbial products compared to other combustion products, including wood smoke and diesel exhaust (Gordon et al., 2014; Mudway et al., 2005; Sussan et al., 2014).

We and others have reported the pro-inflammatory effects of dung biomass smoke *in vitro* and *in vivo*, which was generated by burning selected types of animal dung (Mehra et al., 2012; McCarthy et al., 2016; Mudway et al., 2005; Sussan et al., 2014; Air Pollution and Cancer, 2013). However, the type of animal dung burned in households varies depending on local geography, climate, and agricultural practices. While cow dung is the main biomass fuel source in some parts of India and East Asia, it is rarely used in other areas. People living in Africa, the Middle East, the Himalayas, and South America often burn dung from goats, horses, elephants, yaks, camels, llamas, and other local animals (Bruun, 2013; de Carle et al., 2015; Udzungwa Elephant Project, 2016.; Blakemore, 2015; Carroll, 2008; Xiao et al., 2015; J. Barnes, 2006). As a result, there is a critical knowledge gap concerning which type of animal dung should be used in the laboratory setting and whether different types of dung have different biological effects and health impacts. Currently, there are no studies examining the toxicological effects of different types of dung biomass smoke on lung cells. Here, we investigate the inflammatory effects and immune responses of