Effects of Methamphetamine on Lung Inflammation

Sarah Hamblock[1], Ray Hamilton, Mary Buford, Andrij Holian
STEER[1], Center for Environmental Health Sciences, Department of Biomedical & Pharmaceutical Health Sciences
University of Montana-Missoula[1]

Abstract

Methamphetamine (MA) is an increasingly popular illegal stimulant in the United States. Smoking MA has increased in popularity since it causes a rapid and sustained high. When MA is smoked, a pyrolysis product, *trans*-phenylpropene (TPP), is produced. The mechanisms responsible for the physiological effects of MA on the lung (increased airway resistance (asthma like) and reduced clearance of infectious agents) and the relative contributions of MA and TPP to the lung effects are unknown. Therefore in this study, the effects of MA were tested in two different models, in vivo and in vitro.

Resistin and atrial natriuretic peptide (ANP) have been associated with asthma and in a previous study mRNA levels were found to be upregulated in lungs exposed to MA. Therefore, embedded lung samples from Balb/c mice were sectioned and stained using immunohistochemistry with antibodies specific for both proteins. However, neither protein presented a significant difference in expression between the control and treated lung samples.

The NLRP3 inflammasome with IL-1β release is central to the macrophage innate immune response to infectious agents and other danger signals such as crystalline silica. To model the effect of MA on this pathway, in vitro studies used human THP-1 cells incubated for 24 hours with increasing doses (0.05, 0.1, 0.5, and 1.0 mM) of MA and TPP. Some samples were also treated with crystalline silica particles, a positive control. A MTS assay was used to determine the toxicity of the chemicals on the cells, and IL-1β levels were measured using an ELISA kit. MA and TPP were not found to be significantly toxic to cells. However, both MA and TPP were found to decrease IL-1β production, consistent with the reported effect of MA on the immune system.

Introduction

Methamphetamine (MA) is the most widely used illegal stimulant in the United States according to a 2006 National Drug Intelligence Center report. The number of users has been steadily increasing over the last 14 years nationwide. The National Drug Threat Assessment Report stated that smoking is the leading mode of administration, as it produces an almost instantaneous and long-lasting high. When MA is heated in the process of smoking, some of the solid is chemically broken down, forming a pyrolysis product, *trans*-phenylpropene (TPP). Once inhaled, TPP is metabolically activated into an epoxide that causes cell degradation and death (Sanga et al, 2005).

The effects of MA on the central nervous system have been and continue to be investigated; however, the effects on the respiratory system have not been thoroughly studied.

It has been noted that people who have been indirectly exposed to MA present asthma-like symptoms (Thrasher et al, 2009). In a recent study, it was found that acute exposure to vaporized MA caused lung injury in mice (Holian et al, 2008). Unpublished microarray analysis from the same study showed that in lung tissues from mice exposed to MA, the mRNA of two proteins, atrial natriuretic peptide (ANP) and resistin were upregulated. Studies have shown that both proteins have elevated levels in people with moderate to severe asthma (ANP-Mohapatra et al, 2004)(Resistin-LaRochelle et al, 2007). If these proteins are found in MA/TPP treated lung epithelial cells, then the relationship between asthma and MA exposure can further be supported.

Studies have shown that MA use causes reduced function of the immune system (Sang-Whan In et al, 2005). One mechanism involved in the innate immune response of inflammation is the dual pathway-activation of the NLRP3 inflammasome, a multi-protein complex found in macrophages. Uptake of particles, such as silica, leads to inflammasome activation when coupled with NF-κB activation by LPS. When both pathways are activated pro-IL-1β is cleaved and the cytokine IL-1β is secreted. IL-1β has been shown to be the most potent cytokine causing fever in humans (Dinarello 2010) and indicates inflammasome activation. If MA or TPP affects the inflammasome, a mechanistic explanation for reduced innate immune system may evolve.

Based on the limited MA/respiratory system studies and importance of the inflammasome in the innate immune system, this study is testing the hypothesis that there is an association of environmental exposure to MA and its pyrolysis product, TPP, with reduced immune responses.

In Vivo Model

Airway epithelium acts as a physical barrier against inhaled particles, as well as producing pro-inflammatory cytokines as part of an immune response. One of the symptoms of asthma is bronchial constriction, which can be caused by smooth muscle constriction or airway inflammation.

Resistin
- Protein found in adipose tissue and human lung tissue
- Associated with the inflammatory response
- In plasma, increasing levels correspond with increasing asthma severity

ANP
- Peptide found in cardiovascular, bronchial and lung systems
- Can induce anti-inflammatory response in epithelial cells
- Increases in concentration with increasing asthma severity

Ovalbumin (OVA) was the positive control in the in-vivo study. DO11.10 mice are transgenic, enhancing their T-cell specificity for OVA.

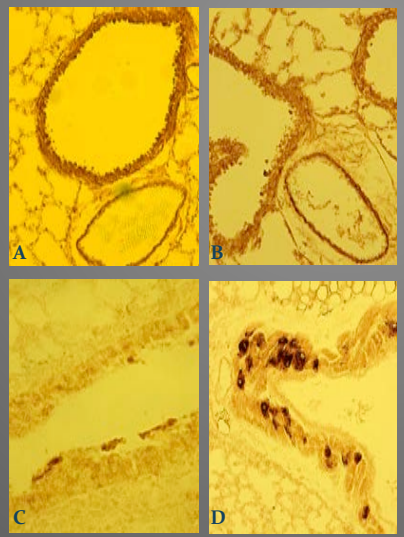
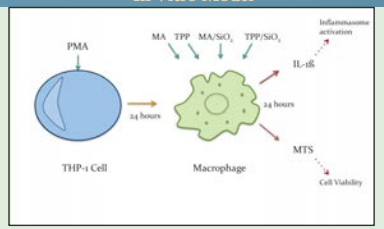


Figure 1. Control (A) and OVA treated (B) lungs stained for resistin show dark staining in the epithelial cells, indicating the presence of the protein. Control (C) and OVA treated (D) lungs stained for ANP have limited but distinct staining of airway epithelial cells.

In Vitro Model



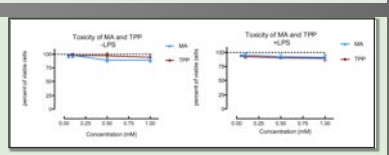


Figure 2. These data show minimal toxicity of MA and TPP, both with and without endotoxin, after a 24 hour incubation with the treatment.

Crystalline silica particles (SiO_2) were a positive control in the in vitro study of inflammation. SiO_2 is known to cause inflammasome activation when coupled with LPS.

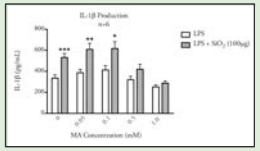


Figure 3. This data represents IL-1β production after a 24 hour incubation with the treatment, showing a decrease in cytokine production with an increase in MA concentration.

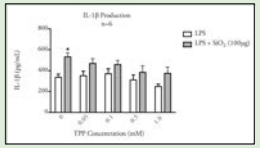


Figure 4. This data represents IL-1β production after a 24 hour incubation with the treatment, showing a slight decrease in cytokine production with an increase in TPP concentration.

Methods

Human Cell Line
The cells used in this experiment were human THP-1 monocyte cells. The cells were cultured in 1640 RPMI with 10% fetal bovine serum. Cells were differentiated into macrophages 24 hours before use by adding phorbol ester (PMA).

Cytokine Measurements
Human IL-1β levels were measured using an ELISA kit from R&D Systems, following the manufacturers protocol.

Cell Viability
Cell viability was measured using a MTS colorimetric assay.

Histology and Immunohistochemistry
Paraffin embedded lungs of DO11.10 mice with a Balb/c background were sectioned on a micrometer into 7 μm sections. Lung sections were stained using an ABC Vectastain Kit and an ImmPACT DAB Peroxidase Substrate, both from Vector Laboratories and used according to manufacturers protocol.

Results

In Vivo
- Staining of both resistin and ANP was predominantly on large airway epithelial cells. There is no significant difference in expression of either resistin or ANP between control lungs and lungs treated with OVA. Furthermore, there was no difference in staining following MA exposure.

In Vitro
- Cell death does not significantly contribute to injury of the lungs after MA exposure.
- MA and TPP affect the immune system by decreasing inflammasome activation.

Future Directions

- Determine the specific component of the inflammasome that is inhibited by MA.

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