REPURPOSING TO SPEED CHEMICAL AND BIOLOGICAL MEDICAL COUNTERMEASURE DISCOVERY AND DEVELOPMENT

Biomarkers, Drug Targets, And Repurposed Medical Countermeasures Against Phosgene Inhalation Injuries

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Phosgene gas (CG, military designation) has been used as a terrorist weapon, in warfare, and has injured many in transportation or industrial accidents. CG targets the lungs, causing severe edema and lung injury after inhalation, with high lethality in exposed victims. Despite these devastating effects, no mechanism-based treatments of phosgene injury or biomarkers have been developed. Additionally, reproducible animal models that recapitulate human acute respiratory distress syndrome (ARDS) after CG exposure are scarce. Here, we developed a highly reproducible mouse model of CG inhalation injuries, identified biomarkers and potential drug targets, and screened potential medical countermeasures.

Briefly, 8-9 week old male BALB/c mice were exposed to 20 ppm CG in a nose-only exposure manifold for 15 minutes. Potential medical countermeasures (transient receptor potential ion channel vanilloid 4 (TRPV4) inhibitor, Soluble epoxide hydrolase inhibitors (sEHIs), or angiotensin-converting enzyme (ACE) inhibitors were administered post-CG exposure. In cohorts of mice, we evaluated therapeutic efficacy either until 8 or 24 hours post-CG exposure. At the end of the study time point, we collected bronchoalveolar lavage fluid (BALF) for total and differential leukocyte counts, vascular injury markers, and pro-inflammatory cytokines; conducted methacholine airway challenge pulmonary functional tests; or collected lung tissues for qPCR analysis of pro-inflammatory cytokine transcripts and histopathology. The protein levels of highly expressed pro-inflammatory cytokine transcripts identified in the qPCR analysis were determined using a custom-designed multiplexed solid-phase electrochemiluminescence assay panel.

Exposure to CG resulted in a median survival rate of 18.6 hours in 24-hour observation studies. CG exposure caused a significant increase in BALF protein and albumin, suggesting vascular protein leakage, as well as BALF leucocytes. Protein levels of pro-inflammatory cytokines such as IL-1β, IL-6, KC, MIP-2, MMP9, and TNFα were significantly increased in CG-exposed mice. Exposure to CG resulted in the elevation of vascular injury markers such as serum amyloid protein, adiponectin, soluble vascular cell adhesion molecule-1, and fibrinogen, and coagulation disorder markers such as thrombin-antithrombin complex in BALF. CG exposure resulted in airway hyperresponsiveness with increasing methacholine concentrations. Lungs of CG-exposed mice revealed histopathological characteristics consistent with key human ARDS features, such as increased numbers of alveolar macrophages, thickening of alveolar septal walls, and intra-alveolar accumulation of neutrophils and proteinaceous debris. Taqman array analysis of newly identified alveolar endothelial types (alveolar-capillary and general capillary cells) in cDNA transcripts in CG-exposed lungs revealed possible damage to key alveolar endothelial cell types. Treatment with TRPV4 inhibitor, sEHIs, or ACE inhibitors ameliorated CG-induced pulmonary injury and improved Kaplan-Meier survival curves.