Toxicology Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Toxicology Letters

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

Conceptual approaches for treatment of phosgene inhalation-induced lung injury

Wesley W. Holmes[a,*], Brian M. Keyser[b], Danielle C. Paradiso[a], Radharaman Ray[b], Devon K. Andres[b], Betty J. Benton[b], Cristin C. Rothwell[b], Heidi M. Hoard-Fruchey[b], James F. Dillman[b], Alfred M. Sciuto[a], Dana R. Anderson[a,*]

[a] *Analytical Toxicology Division, US Army Medical Research Institute of Chemical Defense, 2900 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, United States*
[b] *Research Division, US Army Medical Research Institute of Chemical Defense, 2900 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, United States*

HIGHLIGHTS

- Describes proposed mechanisms of phosgene induced-lung injury.
- Provides rational treatment strategies for phosgene inhalation injury.
- Several therapeutics significantly increased 24 h survival.

ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form 1 October 2015
Accepted 12 October 2015
Available online xxx

Keywords:
Phosgene
Inhalation
Whole-body
Mechanism
Survival
Treatment
Toxicity
Mice
Exposure

ABSTRACT

Toxic industrial chemicals are used throughout the world to produce everyday products such as household and commercial cleaners, disinfectants, pesticides, pharmaceuticals, plastics, paper, and fertilizers. These chemicals are produced, stored, and transported in large quantities, which poses a threat to the local civilian population in cases of accidental or intentional release. Several of these chemicals have no known medical countermeasures for their toxic effects. Phosgene is a highly toxic industrial chemical which was used as a chemical warfare agent in WWI. Exposure to phosgene causes latent, non-cardiogenic pulmonary edema which can result in respiratory failure and death. The mechanisms of phosgene-induced pulmonary injury are not fully identified, and currently there is no efficacious countermeasure. Here, we provide a proposed mechanism of phosgene-induced lung injury based on the literature and from studies conducted in our lab, as well as provide results from studies designed to evaluate survival efficacy of potential therapies following whole-body phosgene exposure in mice. Several therapies were able to significantly increase 24 h survival following an LCt_{50-70} exposure to phosgene; however, no treatment was able to fully protect against phosgene-induced mortality. These studies provide evidence that mortality following phosgene toxicity can be mitigated by neuro- and calcium-regulators, antioxidants, phosphodiesterase and endothelin receptor antagonists, angiotensin converting enzymes, and transient receptor potential cation channel inhibitors. However, because the mechanism of phosgene toxicity is multifaceted, we conclude that a single therapeutic is unlikely to be sufficient to ameliorate the multitude of direct and secondary toxic effects caused by phosgene inhalation.

Published by Elsevier Ireland Ltd.

Abbreviations: FTIR, Fourier transform infrared spectrometer; PE, post-exposure; ip, intraperitoneal; im, intramuscular; tBHQ, tertbutylhydoquinone; VPA, valproic acid; SS-31, Szeto-Schiller-31; PC, phosphotidylcholine; GSH, glutathione; ROS, reactive oxygen species; TRP(A1), transient receptor potential cation channel; NMDA, *N*-methyl-D-aspartic acid or *N*-methyl-D-aspartate; VSMC, vascular smooth muscle cells; ET-1, endothelin 1; ET_A, endothelin receptor A; ET_B, endothelin receptor B; IP3, inositol trisphosphate; AA, arachidonic acid; cAMP, cyclic adenosine monophosphate; RBC, erythrocytes; P_{mv}, pulmonary microvascular pressure; SOCE, store-operated calcium entry; GABA, gamma-aminobutyric acid; ACE, angiotensin converting enzyme; cGMP, cyclic guanosine monophosphate; PDE, phosphodiesterase; AMPK, AMP-activated protein kinase; NAC, *n*-actylcysteine; CAPE, caffeic acid phenethyl ester.

* Corresponding authors. Fax: +1 410 436 1960.
E-mail addresses: wesley.w.holmes.civ@mail.mil (W.W. Holmes), dana.r.anderson.civ@mail.mil (D.R. Anderson).

http://dx.doi.org/10.1016/j.toxlet.2015.10.010
0378-4274/Published by Elsevier Ireland Ltd.

1. Introduction

Toxic chemicals are a potential threat to unprotected populations, particularly in urban environments where residential, commercial, and industrial areas are intermingled. The possibility exists for portions of the civilian population to be exposed to chemical agents through industrial accidents, natural disasters, and terrorist attacks. The potential of mass casualties by an industrial accident was brought to the forefront in the 1984 Bhopal, India, disaster when approximately 3600 people died as a result of an accidental release of 40 ton of methyl isocyanate, and possibly phosgene and hydrogen cyanide (Dhara, 1992). For many of these toxic chemicals, inhalation is the primary route of exposure and typically, there are no identified medical countermeasures. Supportive care usually is the only option for treatment.

Phosgene ($COCl_2$) is a highly reactive and extensively produced toxic industrial gas that poses a substantial risk to public health. In the US, phosgene is used primarily as a chemical intermediate in the production of dyes, pesticides, plastics, polyurethanes, isocyanates, and pharmaceuticals. In 2002, the estimated annual usage in the US was over 1 million metric tons with over 10,000 workers at risk (Council, 2002). The manufacture of phosgene is mostly captive, where phosgene is produced and consumed on site; however, some phosgene is still transported by rail. For industrial applications, phosgene is produced by reacting carbon monoxide with chlorine gas in the presence of activated charcoal. In addition, phosgene can be formed by the thermal decomposition of chlorinated hydrocarbons during fires (Brown and Birky, 1980; Gerritsen and Buschmann, 1960). As a result of this extensive usage, thousands of chemical and industrial workers, welders, and firemen are potentially at risk of exposure. Phosgene was used as a chemical warfare agent during WWI and proved to be extremely effective and lethal. Phosgene exposure has been implicated as the cause of roughly 80% of all chemical-related deaths in WWI (Winternitz and Yale, 1920). Several characteristics of phosgene make it viable as a potential chemical weapon for terror groups: phosgene is cheap and easily produced, it can elude detection by human olfactory senses, and it has been demonstrated to be an effective weapon of mass destruction.

At low doses, odor and/or irritation by phosgene is of little use for exposure avoidance. The smell of phosgene is described as that of musty, moldy or new mown hay, and/or green corn. The odor recognition threshold ranges from 0.4 ppm to 1.5 ppm (A.I.H. Association, 1989). Although phosgene has an odor, it is not effective as a deterrent to exposure because olfactory fatigue occurs rapidly, and the odor may be masked by other smells (Borak and Diller, 2001). While phosgene exposure can cause irritation of mucosal membranes, physiological effects can manifest from concentrations well below the threshold concentration (>3 ppm) for phosgene-induced mucosal irritation. Thus, mild or delayed irritation can result in a lack of avoidance and subsequent prolonged exposure. At higher concentrations (>150 ppm min) phosgene exposure can cause life-threatening and latent non-cardiogenic pulmonary edema that is seen 6–24 h post-exposure (Diller, 1978). According to the Medical Management Guidelines for Phosgene issued by the Agency for Toxic Substances Disease Registry, there is currently no effective medical countermeasure for phosgene exposure, and emergency medical treatment consists of support of cardiopulmonary functions via supplemental oxygen and possibly bronchodilators and corticosteroids (Agency for Toxic Substances and Disease Registry, 2015). Corticosteroids are known to depress several biological processes that are involved in phosgene-induced lung injury such as cytokine production/release, inflammation, and vascular leakage; there is little supportive evidence that corticosteroid treatment following phosgene exposure has any positive effect on the outcome of the injury (Smith et al., 2009; Liu et al., 2014; Mautone et al., 1985).

Inhalation of phosgene results in distal lung damage and life-threatening pulmonary edema. Chemically, the slow rate of hydrolysis and low solubility of phosgene favor penetration into the deep lung regions, where pathophysiological changes occur. The diffusion length of phosgene in an aqueous solution has been measured to be about 8.8 μm, which is 4–8 times the thickness of the air/blood barrier (Nash and Pattle, 1971). The ability of phosgene to enter the capillary circulation following inhalation has been shown (Sciuto et al., 1996a), thus providing evidence that phosgene can exert toxic effects on tissues, blood, and cellular components throughout the lung. Biologically, acylation and free radical-mediated reactions are the most relevant reactions that occur between phosgene and important cellular constituents. Acylation reactions involving phosgene occur with biological molecules containing sulfhydryl, amino, and hydroxyl moieties (Babad and Zeiler, 1973). In addition, phosgene can undergo heterolytic and homolytic dissociation to form a highly reactive carbamoyl monochloride radical (Arroyo et al., 1993). Taken together, these reactions are responsible for the alteration and dysfunction of proteins and phospholipids and the generation of pernicious reactive oxygen and nitrogen species (ROS). In laboratory animals, exposure to phosgene causes edema, affects type I pneumocytes (Diller et al., 1985; Frosolono and Pawlowski, 1977), and alters energy metabolism (Frosolono and Pawlowski, 1977; Currie et al., 1985), gene transcription, and expression of proteins involved the glutathione (GSH) redox cycle (Sciuto et al., 1995; Sciuto et al., 2003; Sciuto et al., 2005), enhances leukotriene production (Sciuto et al., 1996c; Guo et al., 1990; Kennedy et al., 1989a), stimulates ET-1 release (Zhang et al., 2008), increases lipid peroxidation (Sciuto, 1998), and decreases 3′–5′-cyclic adenosine monophosphate (cAMP) levels (Kennedy et al., 1989a). Based on these observations we have developed a proposed mechanism for phosgene-induced lung injury and have used this model to select and evaluate potential therapeutics.

2. Methods and materials

2.1. Animals

Male CD-1 mice weighing 25–30 g were purchased from Charles River Laboratory (Wilmington, MA).

2.2. Experimental design

Groups of 404 CD-1 male mice were placed in a Plexiglas cylinder (25 cm in height, 28 cm in diameter), 15.4 L in volume and exposed whole-body to a concentration time amount of 32–40.5 mg/m^3 (8–10 ppm) × 20 min (640–810 mg × min/m^3) phosgene and filtered clean house air at a rate of 20 L/min followed by a 5-min room air washout. Phosgene was metered through a Brooks mass flow controller (Brooks Instruments, Fremont, CA) at a rate dependent upon the desired concentration, mixed with air, and then passed through an infrared spectrometer (Miran 1A, Foxboro Co, Sharon, MA) equipped with a real-time analog output prior to entering the exposure chamber. Out-flowing gas from the chamber was passed through a Gasmet Fourier transform infrared spectrometer (FTIR) to determine the concentration of phosgene exiting the chamber. Exhaust from the FTIR is passed through an M18 charcoal canister before being passed through a standard chemical agent fume hood. Under these conditions, the coefficient of variation of exposure to phosgene has been calculated to be 4 ± 0.5% (n 25–30). Exposures were run utilizing a randomized block design where each treatment and treatment dose was represented. For each exposure, mice received

Please cite this article in press as: W.W. Holmes, et al., Conceptual approaches for treatment of phosgene inhalation-induced lung injury, Toxicol. Lett. (2015), http://dx.doi.org/10.1016/j.toxlet.2015.10.010