Toxicology Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Toxicology Letters

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

Transient receptor potential (TRP) channels as a therapeutic target for intervention of respiratory effects and lethality from phosgene

Devon Andres[a,*], Brian Keyser[a], Betty Benton[a], Ashley Melber[a], Dorian Olivera[b], Wesley Holmes[b], Danielle Paradiso[b], Dana Anderson[b], Radharaman Ray[a,*]

[a] Research Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5400, USA
[b] Analytical Toxicology Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5400, USA

HIGHLIGHTS

- Phosgene (CG) increases intracellular Ca^{2+} ($[Ca^{2+}]_i$) in cultured BSMC and HPMEC.
- TRP channel inhibitors (general TRP, TRPV) prevent CG-induced $[Ca^{2+}]_i$ increase.
- TRP channel inhibitors protect mice against a 24-h lethal CG inhalation dose.
- TRP channels appear to play a role in CG toxicity both *in vitro* and *in vivo*.
- TRP channels are possible targets for intervention against CG toxicity.

ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form 3 November 2015
Accepted 5 November 2015
Available online xxx

Keywords:
Phosgene
TRP channels
Inhalation
Calcium signaling
Respiratory toxicology
SKF
Therapeutic

ABSTRACT

Phosgene (CG), a toxic inhalation and industrial hazard, causes bronchoconstriction, vasoconstriction and associated pathological effects that could be life threatening. Ion channels of the transient receptor potential (TRP) family have been identified to act as specific chemosensory molecules in the respiratory tract in the detection, control of adaptive responses and initiation of detrimental signaling cascades upon exposure to various toxic inhalation hazards (TIH); their activation due to TIH exposure may result in broncho- and vasoconstriction. We studied changes in the regulation of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in cultures of human bronchial smooth muscle cells (BSMC) and human pulmonary microvascular endothelial cells (HPMEC) exposed to CG (16 ppm, 8 min), using an air/liquid interface exposure system. CG increased $[Ca^{2+}]_i$ ($p < 0.05$) in both cell types, The CG-induced $[Ca^{2+}]_i$ was blocked ($p < 0.05$) by two types of TRP channel blockers, SKF-96365, a general TRP channel blocker, and RR, a general TRPV (vanilloid type) blocker, in both BSMC and HPMEC. These effects correlate with the *in vivo* efficacies of these compounds to protect against lung injury and 24 h lethality from whole body CG inhalation exposure in mice (8–10 ppm × 20 min). Thus the TRP channel mechanism appears to be a potential target for intervention in CG toxicity.

Published by Elsevier Ireland Ltd.

1. Introduction

1.1. Phosgene as a toxic inhalation hazard

Phosgene (CG, $COCl_2$) is an industrial chemical used in the manufacturing of multiple compounds *e.g.*, pharmaceuticals, dyes, and polyfoam rubber products. However, CG is highly toxic and its inhalation exposure can compromise the entire respiratory tract (Miller and Chang, 2003; Tuorinsky and Sciuto, 2008). As such CG was used as a chemical weapon during World War I. Moreover, since CG is very easy to make, it also is a concern as a potential terrorist threat. Following a latent period of about 6–8 h), CG

* Corresponding author at: Research Division, U.S. Army Medical Research Institute of Chemical Defense, 2900 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, USA.
E-mail addresses: devon.k.andres.ctr@mail.mil (D. Andres), radharaman.ray.civ@mail.mil (R. Ray).

http://dx.doi.org/10.1016/j.toxlet.2015.11.004
0378-4274/Published by Elsevier Ireland Ltd.

initiates detrimental physiological effects such as bronchoconstriction, bronchitis, vasoconstriction, pulmonary edema, *etc*., followed by late effects such as inflammation, pulmonary and airway remodeling and asphyxia. All these eventually may develop into chronic obstructive pulmonary disease (COPD) leading to death by suffocation (Borak and Diller, 2001; Pauluhn et al., 2007). However, current therapeutic options are limited to symptomatic treatment for pulmonary edema and pulmonary dysfunction, *e.g*., administration of leukotriene inhibitor, ibuprofen, aminophylline, or isoproterenol *N*-acetyl cysteine, but mainly by mechanical ventilation (Sciuto and Hurt, 2004; Pauluhn et al., 2007).

1.2. TRP inhibitors as targeted therapy against CG inhalation toxicity

The mechanism of CG-induced lung injury has eluded investigators for decades. TRP channels consisting of a group of non-selective cation channels with some subtypes preferentially conducting calcium ions are expressed throughout the airways from nasal mucosa to the alveolar-capillary system (Owsianik et al., 2006; Grace et al., 2014). These TRP channels are involved in many physiological and pathological systems in the airways and also throughout the body (Nilius et al., 2007; Billeter et al. 2014; Grace et al., 2014). Certain TRP channels are known to be involved in both chemosensory functions and detrimental signaling cascades following exposures to toxic inhalation hazards (*e.g*., Cl_2, CG, sulfur mustard, methyl isocyanate, SO_2,*etc*.) (Bessac and Jordt, 2010; Li et al., 2011; Buch et al., 2013). This study looked at subtypes of TRP channels, TRPC (canonical type), TRPV1 and TRPV4 (vanilloid type), and TRPA1 (ankyrin type) that have been reported to play a role in physiological and pathological mechanisms in the airway (see Table 1). These are cation conducting channels with some preference for Ca^{2+}. TRPV5 and TRPV6 channels have relatively higher specificity for Ca^{2+} ions; however, they were not studied here. Intracellular Ca^{2+} homeostasis is important in the regulation of respiratory function. Alterations in intracellular Ca^{2+} homeostasis following inhalation exposure to respiratory toxicants or other stimuli cause chemosensory responses (*i.e*., cough, sneezing, pain) and may contribute to bronchoconstriction, vasoconstriction and altered alveolar and vascular membrane barrier permeability; this may cause enhanced bronchopressure and pulmonary hypertension (Nilius et al., 2007; Bessac and Jordt, 2010; Li et al., 2011; Buch et al., 2013; Billeter et al., 2014; Grace et al., 2014).

TRP channels integrate multiple stimuli and activate downstream cellular signal pathways *via* Ca^{2+} entry and/or membrane depolarization and play an essential role in contraction and relaxation of the vascular system (Firth et al., 2007; Guibert et al., 2007, 2011; Yue et al., 2015). CG has been reported to cause a substantial increase in intracellular free calcium (Werrlein et al., 1999). A direct link between TRP channel mediated increase in intracellular Ca^{2+} and vasoconstriction and/or bronchoconstriction has not been established. We propose here a hypothetical mechanism suggesting a possible regulatory role of TRP channels in the overall mechanism of intracellular Ca^{2+} increase *via* different types of Ca^{2+} channels to include the voltage-gated Ca^{2+} channels as follows. The possible mechanism for the change in intracellular calcium may involve a combination of influx of extracellular Ca^{2+} ions and triggered release of intracellular Ca^{2+} ions stores. CG interaction with TRPA1 and/or T-type calcium channels is thought to cause the influx of Ca^{2+} ions through the plasma membrane. Consequently an increased intracellular Ca^{2+} concentration may somehow activate Ca^{2+} release from intracellular stores. The depletion of intracellular stores of Ca^{2+} ions may then signal extracellular Ca^{2+} entry through TRPV/C channels and/or L-type calcium channels in order to replenish the depleted Ca^{2+} stores. There has been evidence that TRPV and TRPC channels contribute to vasoconstriction *via* both store operated calcium channels and receptor mediated channels (Firth et al., 2007; Guibert et al., 2007, 2011; Yue et al., 2015). Moreover, it has been proposed that a substantial increase in intracellular free Ca^{2+} activates signaling pathways involved in cellular function that could be harmful, including depolarization of muscle cells causing vaso- and broncho-constriction (Firth et al., 2007; Guibert et al., 2007; Guibert and Ducret, 2011; Yue et al., 2015).

Inhibitors of TRPA1, TRPV1 and TRPV4 have been developed to prevent activation of chemosensory TRP channels that are believed to be involved in pulmonary edema (Nilius et al., 2007; Billeter et al., 2014; Grace et al., 2014; Jurek et al., 2014) and abnormal blood pressure as well as abnormal fluid and electrolyte balance resulting from COPD (Li et al., 2011), heart failure (Thorneloe et al., 2012), chronic asthma (Lee and Gu, 2009), exposure to isocyanates and tear gases (Bessac et al., 2009), and exposure to chlorine gas (Balakrishna et al., 2014). Typically acute reactions to CG from its exposure at levels higher than the odor threshold and standard permissible levels include irritation, bronchoconstriction, and vasoconstriction, disruption of the alveolar membrane barrier function, pulmonary edema, asthma-like symptoms and respiratory distress. TRP channels have been proposed to represent specific targets of TIHs *e.g*., gases such as Cl_2, CG, sulfur mustard, methyl isocyanate, SO_2, chlorine, *etc*. (Bessac and Jordt, 2010; Li et al., 2011; Buch et al., 2013; Balakrishna et al., 2014). Involvement of different subtypes of TRP channels in different respiratory and cardiac disorders have been proposed as shown in Table 1.

We investigated the possible role of TRP channels in the mechanism of CG toxicity first using *in vitro* cell culture models and selected TRP inhibitors. These studies were designed for a proof of concept that TRP channels might be involved in the toxicity of CG and that the TRP channels could be possible therapeutic targets for intervention against CG-induced pulmonary injury and lethality *in vivo* in mice. The particular TRP inhibitors studied were SKF-96365

Table 1
Specific TRP channels related to respiratory diseases.

Channel	Possible disease connection	Reference
TRP C1/C6	Asthma, bronchial hyper responsiveness, COPD, idiopathic pulmonary arterial hypertension, heart hypertrophy, mucus hypersecretion	(Nilius et al., 2007; Sel et al., 2008; Banner et al., 2011; Grace et al., 2014; Kaneko and Szallasi, 2014)
TRP V1	Asthma, chronic pain, chronic cough, enhanced airway inflammation and bronchial hyper-reactivity, important sensor of noxious stimuli and tissue damage, bronchoconstriction	(Caterina et al., 2000; Helyes et al., 2007; Nilius et al., 2007; Banner et al., 2011; Billeter et al., 2014; Grace et al., 2014; Kaneko and Szallasi, 2014)
TRP V4	Chronic pain, asthma, bronchial hyperresponsiveness, hypertension, lung edema by control of epithelial and endothelial barrier function, acute respiratory distress syndrome, COPD, inflammatory airway disease, ventilator induced airway injury	(Michael et al., 2010; Freichel et al., 2001; Liedtke and Friedman 2003; Suzuki et al., 2003; Nilius et al., 2007; Banner et al. 2011; Grace et al., 2014; Jurek et al., 2014; Kaneko and Szallasi, 2014)
TRP A1	Chronic pain, asthma, COPD, important sensor of noxious stimuli and tissue damage, allergic and non-allergic airway hyper-responsiveness, increased bronchoconstriction	(Bautista et al., 2006; Nilius et al., 2007; Caceres et al., 2009; Banner et al., 2011; Balakrishna et al., 2014; Billeter et al., 2014; Grace et al., 2014; Kaneko and Szallasi, 2014)

Please cite this article in press as: D. Andres, et al., Transient receptor potential (TRP) channels as a therapeutic target for intervention of respiratory effects and lethality from phosgene, Toxicol. Lett. (2015), http://dx.doi.org/10.1016/j.toxlet.2015.11.004