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Development and assessment of countermeasure formulations for treatment of lung injury induced by chlorine inhalation

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ABSTRACT

Chlorine is a commonly used, reactive compound to which humans can be exposed *via* accidental or intentional release resulting in acute lung injury. Formulations of rolipram (a phosphodiesterase inhibitor), triptolide (a natural plant product with anti-inflammatory properties), and budesonide (a corticosteroid), either neat or in conjunction with poly(lactic:glycolic acid) (PLGA), were developed for treatment of chlorine-induced acute lung injury by intramuscular injection. Formulations were produced by spray-drying, which generated generally spherical microparticles that were suitable for intramuscular injection. Multiple parameters were varied to produce formulations with a wide range of *in vitro* release kinetics. Testing of selected formulations in chlorine-exposed mice demonstrated efficacy against key aspects of acute lung injury. The results show the feasibility of developing microencapsulated formulations that could be used to treat chlorine-induced acute lung injury by intramuscular injection, which represents a preferred route of administration in a mass casualty situation.

1. Introduction

Chlorine is a gaseous chemical that is used in multiple types of industrial applications, including chemical syntheses, production of plastics, water treatment, and bleaching operations (Evans, 2005). Chlorine is one of the top ten chemicals produced by mass in the U.S., and much of it is transported from production sites to end use locations. Chlorine gas is highly reactive and produces respiratory toxicity when inhaled (White and Martin, 2010). Human exposure to chlorine can occur through industrial and household accidents. Large accidental releases of chlorine have resulted from train derailments, and these have led to human casualties (Joyner and Durel, 1962; Weill et al., 1969; Jones et al., 1986; Van Sickle et al., 2009). Chlorine is also considered a chemical threat agent, having been used as a chemical weapon in World War I and more recently in the Iraq War. Because chlorine is easily acquired and deployed, there are concerns that it could be used in a terrorist attack on the US populace. The U.S. Department of Homeland Security has estimated that a large-scale chlorine release in an urban area could produce as many as 100,000 hospitalizations for respiratory injuries (Homeland, H. S. C., 2004). Because casualties on this scale would likely overwhelm local health care capabilities, there is interest in developing medical countermeasures that could be used to treat acute lung injury induced by chlorine gas inhalation.

Chlorine damages cells lining the respiratory tract, and inhalation of chlorine at high concentrations can produce acute lung injury characterized by epithelial-endothelial barrier disruption, pulmonary edema, pneumonitis, and airway obstruction. Clinical symptoms include dyspnea, cough, hypoxemia, and bilateral infiltrates on chest X-ray (Van Sickle et al., 2009). Treatment for chlorine-induced lung injury involves primarily supportive care such as oxygen administration and mechanical ventilation. Although a variety of drugs, including β-adrenergic agonists, corticosteroids, and sodium bicarbonate, have been used off-label (Van Sickle et al., 2009), there are currently no FDA-approved medical countermeasures for chlorine-induced acute lung injury.

We are developing treatments that could be administered after chlorine exposure to ameliorate lung injury. We have shown previously that rolipram (a type 4 phosphodiesterase inhibitor), triptolide (a natural plant diterpenoid), and budesonide (a corticosteroid) inhibit various aspects of acute lung injury in mice exposed to chlorine gas. Rolipram inhibited pulmonary edema and airway hyperreactivity induced by chlorine inhalation (Chang et al., 2012). Triptolide inhibited chlorine-induced inflammation (Hoyle et al., 2010), and budesonide inhibited chlorine-induced inflammation and pulmonary edema (Chen et al., 2013). We previously administered rolipram, triptolide, and budesonide by different routes in initial experiments to establish efficacy of treatments. In the present study, we developed and tested formulations to optimize intramuscular delivery of the compounds of interest for treating chlorine-induced acute lung injury. The intramuscular route is attractive for

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administering countermeasures because it potentially allows for rapid treatment of large numbers of casualties by first responders. We developed a variety of formulations for each active compound and conducted *in vitro* and *in vivo* assessments in an effort to produce optimized countermeasures.

2. Materials and methods

2.1. Materials

Rolipram and triptolide were obtained from Tocris (Ellisville, MO), and budesonide was obtained from Medisca (Plattsburgh, NY). Poly(lactic:glycolic acid) (PLGA) was purchased from Lakeshore Biomaterials (Birmingham, AL), and polyethylene glycol 3350 was purchased from Spectrum Chemical (New Brunswick, NJ).

2.2. Production of spray-dried formulations

The active pharmaceutical ingredients, in addition to PLGA and polyethylene glycol (PEG) when present, were dissolved in methylene chloride or ethanol. These solutions were spray dried using a Pro-C-epT 4 M8 spray drier (Pro-C-epT, Zelzate, Belgium) with bifluid nozzle to produce microparticles. Payload analysis for the active compounds was performed by dissolution of formulations in DMSO and analysis by HPLC. Average deviation between actual and target payloads was 4.5%. The designations for the formulations refer to the target composition of the dried microparticles.

2.3. Scanning electron microscopy

Scanning electron microscopy was conducted using a model EVO 50 (Carl Zeiss, GmbH) environmental instrument operated at 20 keV. Both quadrant backscatter and secondary electron (Everhart-Thornley) detectors were used for this study. Samples were sputtered with AuPd prior to imaging in high-vacuum mode.

2.4. In vitro release assay

Release of active compounds from formulations into phosphate buffered saline at 37 °C over time was measured. Compounds were measured by HPLC using C18 columns with UV detection. For rolipram, the mobile phase was 40% acetonitrile in water with detection at 280 nm. For triptolide, the mobile phase was 40% acetonitrile in water with detection at 215 nm. For budesonide, the mobile phase was 70% methanol in water with detection at 240 nm.

2.5. Particle sizing

Particle sizing was determined using a Malvern Mastersizer with Hydro 2000S accessory. Approximately 200 mg of dry sample was suspended in 10 ml of a 0.1% (w/w) Tween 80 in water solution and mixed using a bench top vortex mixer. The suspension was added drop-wise to the sample chamber of the instrument until the proper obscuration was attained (6–20%). The sample was mixed to allow flow through the imaging cell with an impeller at 2800 rpm and sonication at 50% of maximum. A general purpose mathematical model that assumed spherical particles and a refractive index of 1.460 were used to calculate particle size distribution. An average of three sample measurements (5000 captures per measurement) was reported for each sample and plotted as a function of volume or number percent versus particle size.

2.6. Chlorine exposure and treatments

All animal experiments were approved by the University of Louisville Institutional Animal Care and Use Committee and were performed in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, 1996). Male FVB/NJ mice were purchased from The Jackson Laboratory at 8 weeks of age and housed for 1–2 weeks after receipt prior to chlorine exposure. Mice were housed under specific pathogen-free conditions with 3–5 mice per cage and were randomly assigned to treatment groups before chlorine exposure. Mice were exposed to a target dose of 240 ppm-h (240 ppm for 1 h) chlorine by whole body exposure as previously described (Chang et al., 2012). Actual doses averaged 237 ± 5 ppm-h (mean ± SD). Mice received 0.1 mg/kg buprenorphine subcutaneously for analgesia b.i.d. starting immediately after exposure until they were euthanized. Mice were treated with formulations containing rolipram, triptolide, or budesonide by intramuscular injection. Formulations were suspended in Dulbecco's phosphate buffered saline without calcium or magnesium containing 0.1% Tween 80, and 20 μl per mouse of each suspension was injected intramuscularly. As controls, placebo injections of the vehicle alone or PLGA microparticles without the active pharmaceutical component were performed. For analysis of pulmonary edema 6 h after exposure, mice received a single injection of rolipram 1 h after the end of the chlorine exposure. For analysis of airway hyperreactivity 1 day after exposure, mice received a total of three doses of rolipram: one dose 1 h after exposure, a second dose 10–12 h after exposure, and a third dose the following day 2 h before pulmonary function measurements were made. For analysis of neutrophils in lavage fluid 48 h after exposure or in tissue sections 6 h after exposure, mice were given a single treatment of triptolide formulations 1 h after chlorine exposure. Budesonide formulations were given b.i.d. for 2 days starting 1 h after chlorine exposure prior to analysis of lavage fluid neutrophils 48 h after exposure. In all cases, the labeling in the figures represents the amount of active compound administered in each dose.

2.7. Analysis of lung injury

Pulmonary edema was measured by analyzing extravascular lung water (Chang et al., 2012). Airway hyperreactivity was assessed by measuring respiratory system resistance at baseline and in response to increasing doses of methacholine as described (Chang et al., 2012). Lung lavage and analysis of the recovered cells was performed as described (Tian et al., 2008). Analysis of neutrophils in tissue sections from lungs collected 6 h after chlorine exposure was performed by immunostaining for the neutrophil marker Ly-6G as described (Hoyle et al., 2010).

2.8. Pharmacokinetic analysis

Male FVB/NJ mice (8 weeks old) were purchased from The Jackson Laboratory and used 1–2 weeks after receipt. Mice were injected intramuscularly with 360 μg of spray-dried neat rolipram formulation or 300 μg of spray-dried neat budesonide formulation. At various times after drug injection, blood was collected for preparation of plasma, following which the animals were euthanized for collection of lung tissue. Plasma and lung homogenates made in phosphate buffered saline were spiked with internal standard (dexamethasone) and extracted with methyl *tert*-butyl ether. Samples for LC-MS/MS analysis were prepared by drying the organic phase and dissolving the material in 50% methanol. HPLC separation was performed on a C8 column with gradient elution from 90% solution A (10 mM formic acid in water) 10% solution B (10 mM formic acid in methanol) to 10% solution A 90% solution B.

2.9. Data analysis

In vitro release data are shown as means of duplicate samples. Pharmacokinetic data are shown as means of 2–3 replicates for which rolipram or budesonide was detected. Other data are presented as group means ± standard error of the mean (SEM). Effects of treatment