My Career in Toxicology: Research on Toxic Chemical Exposures and Medical Countermeasures

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Pharmacology
& Toxicology

My Journey....

Plant Biotechnology

Molecular Biology

Drug Discovery

Synthetic Biology

...but unique
Study of Toxic Chemical Exposures

Our lab focuses on understanding the mechanisms of toxicity and inflammation to effectively treat injuries or diseases from toxic chemical and environmental exposures.
Chemical Threat Agents

Environmental Agents: Adverse Health Effects

- **Air Pollution**- burning of fuels, fires and tobacco smoke (polycyclic aromatic hydrocarbons), gases (SO$_2$, CO)
- **Household products and chemicals**- formaldehyde, acids, ammonia
- **Pesticides/herbicides**- glyphosate, chlorpyrifos, chloropicrin
- **Water pollution**- fertilizers and pesticides; sewage; chemical waste
Chemical Threat Agents

Food/Drugs/Opioids and Industrial Agents: mass casualties by accidental, occupational or intentional exposure

- **Ammonia** - fertilizer, petroleum, mining
- **Chlorine** - disinfectant, pharmaceutical, bleaching agent
- **Hydrogen fluoride** - refrigerators, air conditioners
- **Methyl Isocyanate** - production of pesticides

**Bhopal Gas Tragedy 1984**

Union Carbide pesticide plant in Bhopal, India, 600,000 people and over 15,000 deaths

**Anhydrous Ammonia Tank Fire**

Food processing plant

https://www.epa.gov
Chemical Threat Agents

Warfare Agents: weapons of mass casualties by intentional exposure

- **Nerve Agents** - Acetylcholinesterase inhibitors disable enzymes responsible for transmitting nerve impulses [Tabun (GA), Sarin (GB), Soman (GD)]

- **Blood Agents** - Prevent exchange of oxygen between blood and body cells (cyanide)

- **Respiratory Agents** - Chlorine gas, Phosgene (CG), chloropicrin

- **Vesicating Agents** - Strong alkylating agents that effect eyes, skin, mucus membranes and internal organs [sulfur mustard, nitrogen mustard (HN-1,2 and 3), lewisite (L), phosgene oxime (CX)]
Chemical Warfare Agents in Various Attacks

5000 civilians killed in Halabja and 80,000 affected. The mustard and nerve agents used.

A subway passenger at Tokyo’s Akasaka Station, March 20, 1995

https://www.huffingtonpost.com/2015/03/20/tokyo-subway-sarin-attack_n_6896754.html

Syrian children after a poisonous gas attack in Eastern Ghouta, Damascus, April 7, 2018.

Vesicating Agents

- Priority chemical threats—blistering and strong alkylating agents
- Highly toxic effects to eyes, skin, mucus membranes and internal organs

<table>
<thead>
<tr>
<th>Chemical Agent</th>
<th>Pain</th>
<th>Tissue Damage</th>
<th>Blister</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mustard</td>
<td>Hours later</td>
<td>Onset of clinical effects is hours (24h)</td>
<td>Fluid filled</td>
</tr>
<tr>
<td>Lewisite</td>
<td>Immediate</td>
<td>Seconds to minutes</td>
<td>Fluid filled</td>
</tr>
<tr>
<td>Phosgene Oxime</td>
<td>Immediate</td>
<td>Seconds</td>
<td>Solid wheal</td>
</tr>
</tbody>
</table>

**MUSTARD AGENTS:** Sulfur mustard (HD, SM); Bis(2-chloroethyl) sulfide

Nitrogen mustard (bis(2-chloroethyl)methylamine

**ARSENICALS:** 2-chloroethenylidichloroarsine

**HALOGENATED OXIMES:** Dichloroformoxime
Dumping and Disposal

- In 1985 Congress directed the US Army to begin destroying the US stockpile of CA including SM
- SM at Army bases is being destroyed by burning or neutralization

Chemical dumping
(Photo courtesy of the U.S. Army) In 1964, mustard gas canisters are pushed into the Atlantic Ocean off New Jersey. Millions of pounds were dumped this way
Recent Use of SM in Chemical Warfare

**Terrorism and Warfare**

- **ISIS suspected of mustard attack against US and Iraqi troops** September 22, 2016
- **Top US general: ISIS shell that hit an Iraqi base contained a 'sulfur-mustard blister agent'.** Sep. 22, 2016
- **Tests show ISIS used mustard gas in Iraq, says diplomat at chemical watchdog.** February 2016
- **US official: 'IS making and using chemical weapons in Iraq and Syria'** September 2015

A projectile believed to have contained mustard gas, Aleppo, Syria

Phosgene Oxime (CX)

- Most dangerous among vesicating agents but least studied chemical warfare agent
- There were indications of Iraqi use of an agent whose effects resembled CX against Iran


March 20, 2019 (News9.com). FBI Investigation Finds Chemical Warfare Agent Inside Lawton Home. This was found to be CX

CP as a chemical threat agent in warfare

Employed in WWI: Russia in 1916, then British and German

Commander: Russia continues to use chemical weapons in Ukraine

Without giving evidence, Russia says it probes Ukraine use of chemical weapons

Ukraine's Armed Forces, in the statement, accused Russia of using "banned phosphorus and ammunition" and of using "disinformation as a weapon."
Available Treatments and Safety Gear and Clothing

- **PPE (protective clothing)**
- **Decontaminants**
  - Remove < 2 min
- **Barrier Cream (TSP)**
- **Medical Therapeutics**
  - Skin
  - Eye
  - Lung
  - Systemic

- **NBC Gloves**
- **Battle Dress Overgarment**
- **Protective Mask and Hood**
- **Vinyl Overboots**
• Pursuing the development of new and improved medical countermeasures
• Treat the acute and long-term conditions caused by potential and existing chemical threat agents
• Developing effective and targeted medical interventions is a critical component of the modern global strategy to overcome the challenges of chemical emergencies
Chemical Exposure Toxicity: Focus Study Tissues

**Skin**
Maximally exposed tissue; first line of defense

**Eye**
Severe injury; inflammation; blindness

**Lung**
Injury; long-term effects; respiratory failure and death

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Blood/Cardiovascular Toxicity  
Dermal Toxicity  
Epigenetic Alterations/Genetic Toxicity  
Eye Toxicity  
Respiratory Toxicity  
Reproductive Toxicity  
Neurotoxicity  
Nephrotoxicity  
Hepatotoxicity  
Immunotoxicity

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Javadi et. al., Ophthalmology, 2005  
Evison et al., British Journal of Medicine, 2002.  
Harrison’s Principles of Internal Medicine, 17th Edition.

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https://toxtutor.nlm.nih.gov/03-004.html
Chemical Exposure Toxicity: Study models

- Toxicology studies in the lab are conducted employing:
  - *in vivo* (mice, rats, rabbits, pigs)
  - *ex vivo* (rabbit and human tissues)
  - *in vitro* (cell culture) model systems
  - *in vitro* human tissue equivalents
Skin

- Part of the body’s enveloping membrane
  - Integument - Skin, hair, nails, glands
- Largest organ in the body by weight (ca. 10% B.W.)
- Surface area – 20 square feet (adult)
- Epidermis (derived from ectoderm)
  - avascular
- Dermis (derived from mesoderm)
  - Blood and lymph vessels, nerve fibers present
  - 90% thickness of skin
  - 15-20% T.B. weight; 18-40% T.B. water
- Hypodermis or subcutis
  - fat

https://upload.wikimedia.org/wikipedia/commons/2/27/Skin.png
Targeted Therapeutic Approaches to Counteract Toxicity from Vesicant Phosgene Oxime (urticant) Skin Exposure

Mast cells in sulfur mustard exposure: novel targets for modulation to develop therapies against the long-term health effects in Gulf War Veterans

Validate mast cells as key players and molecular targets in vesicant toxicity

Identifying a therapeutic strategy that can target mast cell activation and release of histamine to mitigate vesicant-induced skin injury
Why Mast Cells?

- Contribute in immune system-related inflammatory diseases
- Have effects on many physiological systems that are affected in GWI/inflammatory diseases
- Derived from distinct precursors in the bone marrow or other hematopoietic tissues which are reported to be targeted by vesicants

T.C. Theoharides et al. / Biochimica et Biophysica Acta 1822 (2012.)
CX exposure on mice skin tissue

MAST CELLS: Granulated leukocytes derived from the myeloid stem cell

Mice skin

Resting mast cell

Degranulating mast cell

Control

CX

Proteases

Chymase

Tryptase

Histamine

Proinflammatory enzyme in Azurophilic granules

Inflammatory Cytokines and Reactive Oxygen Species causing oxidative damage

Mast cells can regulate the activity of gelatinase enzyme matrix metalloproteinase 9 (MMP9)

SM or NM or CX exposure on mice skin tissue

Play a key role in the inflammatory response

Mast Cell KO mice

MPO, Myeloperoxidase
Mast Cells Promote Nitrogen Mustard-Mediated Toxicity in the Lung Associated With Proinflammatory Cytokine and Bioactive Lipid Mediator Production

Angela Cruz-Hernandez,† Ryan P. Mendoza,† Kathleen Nguyen,† Anna Harder,† Christopher M. Evans,† Alison K. Bauer,† Neera Tewari-Singh,§ and Jared M. Brown*†

Inhalation Toxicology
International Forum for Respiratory Research

A review of chemical warfare agents linked to respiratory and neurological effects experienced in Gulf War Illness

Angela Cruz-Hernandez, Andrew Roney, Dinesh G. Goswami, Neera Tewari-Singh & Jared M. Brown
Phosgene oxime: Injury and associated mechanisms compared to vesicating agents sulfur mustard and lewisite

Dinesh Giri Goswami, Rajesh Agarwal, Neera Tewari-Singh

Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

Cutaneous exposure to vesicant phosgene oxime: Acute effects on the skin and systemic toxicity

Neera Tewari-Singh, Dinesh G Goswami, Rama Kant, Claire R Crouth, Robert P Casillas, David J Orilsky, Rajesh Agarwal

Phosgene oxime: a highly toxic urticant and emerging chemical threat

Satyendra K. Singh, Joshua A. Klein, Holly N. Wright & Neera Tewari-Singh
Researcher at East Lansing’s MSU Working to Find Antidote to Deadly CX Chemical Agent

A researcher at Michigan State University in East Lansing was awarded a $1.4 million grant from the National Institutes of Health Countermeasures Against Chemical Threats to find an antidote to Phosgene oxime, or CX, one of the deadliest chemical agents ever manufactured.

By Grass Turner - December 11, 2019

A researcher at Michigan State University in East Lansing was awarded a $1.4 million grant from the National Institutes of Health Countermeasures Against Chemical Threats to find an antidote to Phosgene oxime, or CX, one of the deadliest chemical agents ever manufactured.

CX is categorized with blistering agents like mustard gas and causes serious allergic reactions that rapidly incapacitate and kill people. There’s no known antidote, according to Neera Tewari-Singh, assistant professor of pharmacology and toxicology in the College of Osteopathic Medicine at MSU.

COLDタイムスタンプ: Dec. 6, 2019

COULD COMMON ANTIHISTAMINES BECOME ANTIDOTE TO DEADLY CHEMICAL AGENT?

Contact(s): Kim Ward; Neera Tewari-Singh

In March 2019, the FBI entered an Oklahoma City apartment and found massive amounts of dangerous chemicals. Among the chemicals was Phosgene oxime, or CX, one of the deadliest chemical agents ever manufactured.

CX, categorized with blistering agents like mustard gas, is a nectar agent or irritant, a variety of chemical that causes serious allergic reactions that rapidly incapacitate and kill a person, but there’s no known antidote, according to Neera Tewari-Singh, assistant professor of pharmacology and toxicology in the College of Osteopathic Medicine at Michigan State University.

With a $1.4 million grant from the National Institutes of Health Countermeasures Against Chemical Threats, or the NIH-CounterACT program, Tewari-Singh is working on an antidote. CounterACT’s goal is to integrate research and technological advances in science and medicine to improve medical response to chemical emergencies, whether they are acts of terrorism or industrial disasters.

Created in 1929 by German chemists and stockpiled during World War II, CX was developed as a potential deadly chemical warfare agent, but its use in battlefield is not reported, Tewari-Singh said.

“Adding CX’s deadliness is that it’s easily manufactured and one of the least studied of all chemical threat agents,” she said. “Our study will provide the first major insight into its toxicity and effects on the body.”

Institute for Integrative Toxicology (https://msu.edu/~index.html)

Tewari-Singh Lab Investigating the Immune Mechanism of Mustard Gas Toxicity

November 23, 2020
Ocular Injury

The eye is one of the most sensitive organs to chemical exposure

Centers for Disease Control and Prevention (2013):
- Each day as many as 2,000 Americans suffer from ocular injury
- About 26% of these are due to chemical exposure

CORNEA
- Highly innervated
- Outermost part of the eye
- Tear film provides a reservoir
Treatment strategies for ocular toxicity from chloropicrin (CP)

- It is an aliphatic nitro compound also called trichloronitromethane
- Irritating and lacrimating properties and contaminates air, water, foo
- Study the mechanism of CP-induced corneal injury
**Chloropicrin**

- Incapacitating, chocking and lacrimating agent in World War 1
- In recent decades, is used as a pesticide and fumigating agent against strawberries, melons, tomatoes, almonds, raspberries, peppers, and melons
- Increased use of CP in recent years as an alternative to methyl bromide, which is being phased out under an international treaty

Chloropicrin in Agriculture

From 2002 to 2011, 787 people suffered

Symptoms: watery eyes, irritated lungs, coughing and headaches (Al Seib / Los Angeles Times)

Over 61,323 acres in California alone (2007)

A total of 1,015 cases from 1992-2007 reported to the California Pesticide Illness Surveillance Program

Water Chlorination
Chloropicrin

1. Pesonen et al, 2012. Toxicology letters
Chloropicrin Ocular injury models

- **In Vitro studies:**
  - HCE cells (Goswami DG, et al, 2020; Lehman JG et al, 2018; Ruff AL et al, 2022)

- **Ex Vivo**
  - Using Rabbits Cornea (Singh SK, et al, 2021)

- **In Vivo** – Rare (Causey et al, 2020)

- Develop a relevant In vivo Injury model (First objective)
Toxic consequences and oxidative protein carbonylation from chloropicrin exposure in human corneal epithelial cells

Dinesh G Goswami\textsuperscript{a,1}, Rama Kant\textsuperscript{a}, David A Ammar\textsuperscript{a,2}, Chapla Agarwal\textsuperscript{a}, Joe Gomez\textsuperscript{a}, Rajesh Agarwal\textsuperscript{a}, Laura M Saba\textsuperscript{a}, Kristofer S Fritz\textsuperscript{a}, Neera Tewari-Singh\textsuperscript{a,2,*}
Treatment strategies for ocular toxicity from chloropicrin (CP)

• Study the mechanism of CP-induced corneal injury

• Establish a useful mouse *in vivo* ocular injury model and ascertain if the nuclear erythroid 2-related factor 2 (Nrf2) signaling pathway is a key mediator in CP-induced corneal oxidative stress and inflammation using wild type and Nrf2 KO mice

• To evaluate the efficacy of Nrf2 activators and supersaturated oxygen emulsion alone, or in combination as an effective therapeutic strategy against CP-induced ocular injury
Ocular injury progression and cornea histopathology from chloropicrin vapor exposure: Relevant clinical biomarkers in mice


Summary and identified data gaps

Chloropicrin (CP)

Model
- In vitro (HCE cells)
- Ex vivo (rabbit cornea)

Relevant *in vivo* model that mimics the injury reported in human epidemiological literature

Mechanistic Studies to identify therapeutic target/s

Oxidative stress

Inflammation

? An effective treatment option that mitigates CP corneal toxicity by targeting both the oxidative stress and inflammatory pathways
Nuclear factor-erythroid factor 2-related factor 2 (Nrf2)

- It is a master defense mechanism protecting cells against oxidative and inflammatory stress; a key target of new approaches to treat ocular diseases
- Transactivates an battery of antioxidant enzymes (catalase and superoxide dismutase, glutathione and heme oxygenase-1)
- Important player in the maintenance of mitochondrial homeostasis and structural integrity
- Nrf2 protects mitochondria from oxidant injury likely through direct interaction with mitochondria

Test if the activation of the Nrf2 pathway can inhibit both oxidative stress and inflammation from chemical exposures
Preliminary studies show that CP causes oxidative stress and inflammation.

Mechanistically understanding the link between Nrf2 pathway activity and CP-induced corneal injury.
Assessing the severity of corneal injury in Nrf2 KO mice compared to WT mice: clinical markers

6-8 weeks old Balb/C Male mice
WT from Jackson labs and inbred Nrf2 Knockout (KO)
n = 10/group
P<0.05
~0.7652ppb CP vapor for 1min
Nrf2 KO results in a more severe and early appearance of CP-induced corneal ulceration compared to WT mice.

*WT Vs Control, #KO Vs control, +WT Vs KO.

P<0.05.
T-test.
n= 10
H, Hours
D, Days
Nrf2 KO results in a more severe CP-induced cornea neovascularization compared to WT mice

*WT Vs Control,
#KO Vs control,
+WT Vs KO.

P<0.05.
T-test
n= 10
D, Days
To develop an in vivo CP-induced ocular injury model in mice.

• Here, we plan to establish and ocular CP vapor exposure model.
• Scoring for clinical observations and assessments.
• Clinical, biological, molecular biomarkers.

RNA Sequencing

To determine if the Nrf2-ARE pathway is a key mediator in CP-induced ocular injury using wild type and Nrf2 KO mice

To evaluate the efficacy of Nrf2 activators and supersaturated oxygen emulsion alone, or in combination using mice.

Synthetic oleanane triterpenoids as Nrf2 activators

• Triterpenoids can activate the Nrf2 pathway by disrupting cysteine residues between Keap1 and Nrf2

2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO)

CDDO

CDDO-Me

CDDO-TFEA
Supersaturated Oxygen Emulsion for Topical Treatment of Ocular Trauma

**Goal:** To utilize supersaturated oxygen emulsion treatment in conjunction with an eye cup/wound chamber to preserve tissue and improve wound healing outcomes following ocular trauma.
Effect of supersaturated oxygen emulsion treatment on chloropicrin-induced chemical injury in ex vivo rabbit cornea

Satyendra K. Singh, Dinesh G. Goswami, Holly N. Wright, Rama Kant, Izza A. Ali, Leah N. Braucher, Joshua A. Klein, Madeline G. Godziela, David A. Ammar, Kathryn M. Pate, Neera Tewari-Singh.

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A Supersaturated Oxygen Emulsion for the Topical Treatment of Ocular Trauma

Kathryn M Pate, PhD†; Dinesh G Goswami, PhD†; Mark Lake, PhD†; Sharon Lake, BS‡; Rama Kant, PhD†; David Ammar, PhD†; Neera Tewari-Singh, PhD§

ABSTRACT Introduction: Roughly 13% of all battlefield injuries include some form of ocular trauma. Ocular tissue...
A novel therapeutic approach that can more effectively mitigate acute and chronic ocular injuries from mustard vesicating agents which can impact the care of battlefield ocular trauma.
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