Respiratory Response to Toxic Injury: Upper Respiratory Tract (Nasal Toxicology)

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Nose and Society

• 40 million cases of chronic sinusitis
• Cases of allergic rhinitis also increasing
• Increase in the identification of nasal toxicants
• New understanding of the molecular makeup of olfaction: 2004 Nobel Prize (Drs. Axel and Buck)

Outline

• Nasal Airway Anatomy and Histology
• Nasal Toxicity of Inhaled Ozone
• Nasal Toxicity of Inhaled Nanoparticles
• Olfactory Toxicity of Inhaled Satratoxin-G (Mycotoxin in Stachybotris chartarum)
• Future Studies, Summary and Conclusions
Comparative Anatomy of the Respiratory Tract

Rat
- Nasal Airway
- Nasopharynx
- Larynx
- Trachea
- Bronchi
- Terminal Bronchiole
- Respiratory Bronchiole

Human
- Pulmonary Artery
- Pulmonary Vein
- Alveolar Capillary Bed
- Alveolus

Comparative Nasal Airway Structure and Function

<table>
<thead>
<tr>
<th></th>
<th>HUMAN</th>
<th>MONKEY</th>
<th>RAT</th>
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<tbody>
<tr>
<td>Volume (cm³)</td>
<td>16-19</td>
<td>8</td>
<td>0.4</td>
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<tr>
<td>Turbinate Anatomy</td>
<td>Simple</td>
<td>Simple</td>
<td>Complex</td>
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<tr>
<td>Olfactory Epithelial Surface Area</td>
<td>Small &lt;10%</td>
<td>Moderate 20-30%</td>
<td>Large 50%</td>
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<tr>
<td>Breathing</td>
<td>Oronasal</td>
<td>Oronasal</td>
<td>Nasal</td>
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Computer Assisted Reconstruction of Rat Nasal MRI

Proximal T2 Nasal Section

Distal T4 Nasal Section
Comparative Anatomy of Nasal Airways


Nasal Airway of the Monkey Nose


Nasal Mucosa of the Monkey Nose

Proximal Nasal Airway of the Mouse


Distal Nasal Airways of the Mouse

Olfactory System

1) Main Olfactory Organ (odors in general)
2) Vomeronasal Organ (chemicals in social and sexual activities)
3) Septal Olfactory Organ (same odors as main olfactory system and an alerting role)

Olfactory Mucosa

- Olfactory Epithelium
  - Olfactory sensory neurons (OSN)
  - Axons are unmyelinated (0.1-0.7 mm in diameter)
  - Sustentacular cells
  - Basal cells
  - Microvillous cells
- Lamina Propria
  - Olfactory nerve fascicles (bundles of OSN axons)
  - Schwann cells surrounding bundles of OSN axons
  - Bowman’s Glands
  - Blood vessels
  - Connective tissue


Unique Characteristics of the Olfactory Chemosensory Neurons

- Dendrites exposed to the external environment
- Axons project directly to the forebrain without synapsing in the thalamus (conduit to the CNS)
- Capacity for continued postnatal neurogenesis
Olfactory Marker Protein

Olfactory Sensory Neuron (Receptor Cells)
Dendrite: Knob and Cilia

Odorant Receptors and Organization of the Olfactory System

2004 Nobel Award
Drs. Axel and Buck
Olfactory Genes & Receptors

- 3% of human genome devoted to identifying odors (1000 odorant receptor genes ORG)
- Humans and mice have 5 million olfactory sensory neurons (OSN)
- Bloodhound has 220 million OSN
- 1 ORG per OSN
- 1,800 olfactory axon convergence centers in the Olfactory Bulb = Glomeruli
- Each ORG corresponds to 2 glomeruli

Ozone (O₃)

- One of the most reactive chemicals
- Oxidant gas in photochemical smog
- 131 million people (50% of the U.S. population) live in communities where average ambient concentrations exceed the NAAQS
- Respiratory toxicant causing airway inflammation and remodeling
- Long-term exposure causes an increase in mortality

Comparative Nasal Toxicity of Ozone


Ozone-Induced Remodeling of Maxilloturbinate in Rat

Kinetics of Nasal Tissue Responses to Inhaled Ozone

Kinetics of Ozone-Induced Mucous Cell Metaplasia in Rat Transitional Epithelium

Cho HY et al., Toxicol Sci 51:135-145, 1999
Nasal Morphometry of Ozone-Induced Nasal epithelial Injury in Infant rhesus Monkeys


Effect of 1- and 11-cycle O3 exposure on the intracellular concentrations of GSH (A), GSSG (B), ascorbate (AH2; C), and uric acid (UA; D) in the nasal mucosa from the anterior MT, posterior MT, and anterior ET of infant monkeys.


Correlations Among Sites of Ozone-Induced Nasal Airway Injury, Predicted Ozone Flux, and Epithelial Type

Fractional Deposition of Inhaled Particles in the Human Respiratory Tract (ICRP Model, 1994; Nose-breathing)

Is the nose a potential target for toxic injury caused by inhaled nanoparticles?

Carbon Black Nanoparticles

- Combustion derived nanoparticles
- Industrially produced for colouring enamels, acrylcs, and plastics, as well as inks and paints
- Low toxicity, low solubility without organics or metals
- Pulmonary carcinogen in rats with long-term exposures and under overload conditions

Donaldson et al. Particle and Fibre Toxicology 1:1-14, 2005
Effects of Inhaled Carbon Black in Three Rodent Species: Study Design and Methods

• Female F344 rats, B6C3F1 mice, and Syrian Golden Hamsters
• Rodents exposed by inhalation to 0, 1, 7, or 50 mg/m³ of high surface area carbon black (HSCB; 17nm primary particle diameter) for 6 h/day, 5 days/wk for 13 wk
• Some rats exposed to 50 mg/m³ of low surface area CB (LSCB; 70nm primary diameter) for 6 h/day, 5 days/wk for 13 wk
• Rodents were sacrificed 1 day, 13 wk, or 11 mo post-exposure
• Nasal and pulmonary airways were processed for microscopic and morphometric examination

Elder et al., Toxicol Sci. 2005; 88(2), 614-629

Pulmonary Effects of Inhaled Carbon Black in Laboratory Rats

• Initial lung burdens were similar in high-dose HSCB and LSCB
• Surface area burdens were equivalent for mid-dose HSCB and high-dose LSCB
• LSCB cleared faster from the lung (less retention) than HSCB
• More pulmonary pathology in HSCB than in LSCB
• Particle surface area is an important factor in target tissue dose and, therefore, toxic effects
• Toxicity in Rats>Mice>Hamsters

Elder et al., Toxicol Sci. 2005; 88(2), 614-629

Nasal Histopathology in Rodents Exposed to Inhaled Carbon Black

• Particles in nasal mucosa
• Epithelial hyperplasia
• Mucous cell metaplasia
• Inflammation (rhinitis)
• Atrophy of turbinate bone
• Hyalinosis (chitinase)
• Dose-dependent severity
• No LSCB related lesions
HSCB-Induced Histopathology in Maxilloturbinates of Rats

H&E

AB/PAS

HSCB in Nasal Mucosa

In Nasal Transitional and Respiratory Mucosa

In Nasal Mucosal Macrophages

HSCB in Olfactory Epithelium, Nerves, and Bulb

Olfactory Nerves

Dorsal Meatus
11 Months After HSCB Exposure: Maxilloturbinate

HSCB-Induced Mucous Cell Metaplasia in Transitional Epithelium Lining the Rat Maxilloturbinate

Mucous Cell Metaplasia in Rats and Mice, but not Hamsters, Exposed to HSCB
Interspecies Differences in the Severity of HSCB-Induced Mucous Cell Metaplasia at One Day after Exposure

<table>
<thead>
<tr>
<th>Species</th>
<th>T1</th>
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<td>Hamster</td>
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HSCB-Induced Rhinitis in Rats, but not in Mice or Hamsters

- Neutrophils in Nasal Mucosa Lining the Metaplasia (T2) of Rats Exposed to Carbon Black
- Neutrophils in Nasal Mucosa Lining the Metaplasia (T2) of Rats Exposed to Carbon Black Particles

Summary of Results

- Rats exposed to 7 or 50 mg/m³ HSCb had chronic rhinitis, epithelial hyperplasia, and mucous cell metaplasia at one day post-exposure
- Magnitude of HSCb-induced nasal lesions was site- and dose-dependent, and attenuated with time post exposure
- Aggregates of HSCB found in nasal mucosa, olfactory nerves and olfactory bulb
- Rats exposed to 1 mg/m³ HSCb had no nasal lesions
- Rats exposed to 50 mg/m³ LSCb had minimal nasal lesions
- HSCB-induced nasal toxicity was species dependent: Rat > Mice > Hamsters
Conclusions

- The nose is a potential target tissue for nanoparticle-induced toxicity and a possible route of exposure to the brain
- As in the lung, particle surface area is an important determinant in the nasal toxicity caused by inhaled carbon black
- Future studies are needed to determine the underlying mechanisms responsible for nasal toxicity caused by nanoparticles
- The effects of nanoparticles on the human nasal airways is yet unknown.

Black Mold, Trichotheccenes and Satratoxins

- 'Black Mold' Stachybotrys chataeum – saprophytic fungus that grows on cellulosic materials
- Trichotheccenes – low molecular weight (200-5000D) mycotoxins produced by Stachybotrys, Fusarium, and other molds
- Bind to ribosomes, inhibit protein synthesis, and induce ribotoxic stress
- Satratoxins – macrocyclic trichotheccenes produced by Stachybotrys

Experimental Design and Methods

- 7-8 wk old, C57Bl/6 Mice
- Single intranasal instillation of Satratoxin G (5G; 500 µg/kg BW) in saline or saline alone (controls)
- Necropsy at 6h, 1 day, 3 days, 7 days or 28 days after instillation
- Nasal and olfactory bulb tissues were processed for microscopic, morphometric, and molecular analysis

Summary of SG-Induced Histopathology in Nose and Brain


Kinetics of SG-Induced Atrophy of OE


SG-Induced Apoptosis of OE at 24h Post Instillation

**SG-induced Loss of OMP-Positive Olfactory Sensory Neurons**


- Single Instillation
- Multiple Instillations
- Days Post Instillation

**SG-Induced Atrophy of Olfactory Marker Protein (OMP)-Positive Olfactory Sensory Neurons**


A) Normal OMP expression in olfactory nerve and glomerular layers of olfactory bulb (OB)
B) Expression of OMP in OB 7 days after SG instillation
C) Site of SG-induced atrophy of OB (*)

**Effects of Trichothecenes on OE**

A) Saline, ISO-3ATRAKIN, DEUXHYDROX, SATRATON G
B) VERRUCARIN A, T-2 TOXIN
STG-Induced Inflammatory Responses in Nose and Brain

Islam Z, Harkeama JM, and Pestka JJ. Environ Health Perspect. 2006; 114: 1099-1107

Satratoxin-G from the Black Mould Stachybotrys chartarum Induces Rhinitis and Apoptosis of Olfactory Sensory Neurons in the Nasal Airways of Rhesus Monkeys


Table 1. - Expression of Satratoxin-G from the nose to the brain:

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<tr>
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<th>Nose</th>
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Carey SA et al. Toxicol Pathol 2012 online, in press

Satratoxin G-Induced Olfactory Injury in Rhesus Monkeys

Carey SA et al. Toxicol Pathol. 2012; online, in press
Satratoxin G-Induced Loss of OSN in Rhesus Monkeys

Satratoxin G-Induced Apoptosis of OSN in Rhesus Monkeys

Satratoxin G-Induced Rhinitis in Rhesus Monkeys
Conclusions

• Nasal airways and brain are potential target sites for toxicity caused by inhalation exposure to macrocyclic trichothecene mycotoxins that may be found at high concentrations in the indoor air of mold-contaminated, water-damaged buildings.

• Quantitative assessments of human exposures and epidemiological studies are needed to determine the potential risks to human health.

Future Studies:
Multi-Scale Modeling for Airway Dosimetry
Rat vs. Human Nasal Breathing

Slide courtesy of Dr. Rick Corley

Summary and Conclusions

✓ Nasal Airway Anatomy and Histology
✓ Nasal Toxicity of Inhaled Ozone
✓ Nasal Toxicity of Inhaled Nanoparticles
✓ Olfactory Toxicity of Inhaled Satratoxin-G (Mycotoxin in Stachybotris chartarum)

• The nose is a target organ for many inhaled toxicants.
• Nasal responses to toxicants are dependant on many factors (e.g., physicochemical characteristics, dosimetry, tissue sensitivity, host, species).
• Results from future laboratory animal studies will be able to better predict human risk to toxic injury.