Aerosolized Vitamin D Attenuates Ozone-Induced Inflammation and Immune Dysfunction

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Ground-Level Ozone

Ozone (O₃) is an oxidant gas formed through photochemical reactions with primary pollutants

Although ozone levels have been decreasing in the US, worldwide ozone levels continue to increase

Zhang et al. 2019
Health Effects of Ozone

Molecular Mechanisms
- Oxidative stress
- Inflammation
- Epithelial barrier disruption

Health Effects
- Increased risk of infection
- Pathogenesis/exacerbation of lung disease
- Cardiovascular and systemic health effects

Can we attenuate these cellular responses?

Ozone

Nuvolone et al. 2018
Circulating VitD and Ozone Exposure

Vitamin D is **positively** correlated with:
- Lung Function (FEV1, FVC)
- Uteroglobin
- %Macrophages, %Lymphocytes

Vitamin D is **negatively** correlated with:
- IFN\(\gamma\), IL-10, IL-12p70, IL-13, IL-1\(\beta\), IL-2, IL-8, TNF\(\alpha\), GM-CSF, IL-12p40, IL-16, IL-17, IL-1\(\alpha\)
- %Total cell count, %Neutrophils

Baseline
\(\uparrow\) Plasma VitD
\(\uparrow\) Lung Function

Post-Ozone Exposure
\(\downarrow\) Inflammation

Perryman et al. 2023
Why Inhaled/Aerosolized Vitamin D?

- Vitamin deficiency has been linked to chronic lung diseases and microbial infections
- Oral supplementation studies with Vitamin D to treat lung disease have produced mixed results

Inhaled Vitamin D may allow for direct delivery to the airway epithelium
Objectives

1. Does Vitamin D provide protection against ozone?

2. What is the optimal dose timing, form, and route?

3. Does aerosolized Vitamin D boost antimicrobial defenses?
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Experimental Outline

VitD Treatment → Ozone Exposure → End of exposure → Sample Collection

30 minutes → 4 hours → 4 hours

Pre-Treatment
- Apical/Aerosol
- Basolateral
  - Vitamin D vs. 1,25-dihydroxyvitamin D

Exposure
- Air Control Ozone Exposure

Assays
- RNA-seq
- IL-8 ELISA
Vitamin D Reduces Ozone-Induced Transcriptional Changes

Air vs. Ozone

- TNFA SIGNALING VIA NFkB
- MTORC1 SIGNALING
- HYPOXIA
- INFLAMMATORY RESPONSE
- APOPTOSIS
- REACTIVE OXYGEN SPECIES PATHWAY
- IL2 STAT5 SIGNALING
- COMPLEMENT

Ozone upregulates inflammation, oxidative stress, innate immunity, and cell death pathways

Ozone vs. Ozone + VitD

- TNFA SIGNALING VIA NFkB
- MTORC1 SIGNALING
- HYPOXIA
- IL2 STAT5 SIGNALING
- INFLAMMATORY RESPONSE
- REACTIVE OXYGEN SPECIES PATHWAY
- APOPTOSIS
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Vitamin D pre-treatment downregulates pathways
Vitamin D Attenuates Ozone-Induced Inflammation

30 Minute Pre-treatment

Basolateral VitD reduced IL-8 secretion
Objectives

1. Does Vitamin D provide protection against ozone?

Vitamin D reverses ozone-induced transcriptional changes and IL-8 release.
Objectives

1. Does Vitamin D provide protection against ozone?

2. What is the optimal dose timing, form, and route?

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Vitamin D Aerosols Attenuate IL-8 Release

24 Hour Pre-treatment

Apical VitD reduced IL-8 secretion
Potential Mechanisms

Decoy-mediated

Ozone → Vitamin D

Receptor-mediated

1,25-VitD → VDR, RXR

↑ Antioxidant genes/pathways

Girgis et al. 2012
2. What is the optimal dose timing, form, and route?

With 30 minute pre-treatment, basolateral vitamin D reduces inflammation

With 24 hour pre-treatment, aerosolized vitamin D reduces inflammation
Objectives

1. Does Vitamin D provide protection against ozone?

2. What is the optimal dose timing, form, and route?

3. Does aerosolized Vitamin D boost antimicrobial defenses?
Vitamin D has been shown to increase expression of cathelicidin, an antimicrobial peptide.

Oral supplementation of vitamin D in humans is unable to increase levels of cathelicidin in the lung.

Inhaled supplementation may be able to directly increase lung levels of cathelicidin.
Vitamin D Upregulates Cathelicidin Expression

24 Hour Pre-treatment

VitD aerosols increased CAMP expression
3. Does Vitamin D boost antimicrobial defenses?

Yes, aerosolized vitamin D increased the expression of cathelicidin, an antimicrobial peptide.
Conclusions

Inhaled VitD may have utility as a prophylactic for acute ozone exposure
Can a VitD aerosol be taken to minimize the impact of pollutant exposure?
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Questions?