In Vitro Effects of Permethrin on Sinonasal Epithelia

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Abstract
Inhalant toxicants are postulated to contribute to the pathogenesis of chronic rhinosinusitis. Permethrin is a pesticide widely used in agricultural, industrial, and residential settings. The objective of this pilot study is to investigate the in vitro effects of permethrin on sinonasal epithelial cells (SNECs). Sinus mucosa was collected from 4 patients undergoing transsphenoidal pituitary surgery without a history of chronic rhinosinusitis. Cultured SNECs were exposed to varied concentrations of permethrin (0-156 μM) for 6 days. Cell viability and proliferation were determined via the MTT colorimetric assay and the Incucyte Live Cell Imaging System. Cellular reactive oxygen species (ROS) activity was measured by the DCFDA ROS detection assay. A statistically significant reduction in cell viability and proliferation was observed between the exposure and control groups at certain concentrations, and a dose-dependent increase in ROS activity was also observed. These findings indicate that permethrin may have deleterious effects on SNECs in a dose-dependent manner.

Keywords
chronic rhinosinusitis, epithelial cell, toxicant, pesticide, chemicals

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Inhalant toxicants are hypothesized to play a role in the pathogenesis of chronic rhinosinusitis (CRS).1 Combat zone and occupational chemical exposures are associated with an increased CRS prevalence.2-4 Farmers with pesticide exposures exhibit a higher incidence of sinusitis when compared with matched controls (n = 196).3 A dose-response relationship between pesticide exposure and sinusitis was demonstrated in a cross-sectional study of 261 healthy volunteers, independent of age, sex, and smoking status.4

Permethrin is a pyrethroid pesticide used in agriculture, urban areas, and the military. Aerosolized permethrin exposure has been associated with sore throat, rhinorrhea, cough, dyspnea, and respiratory failure.5 However, the impact of permethrin on the sinonasal cavity has not been previously explored. The purpose of this pilot study is to investigate the in vitro effects of permethrin on sinonasal epithelial cells (SNECs).

Methods
We established a cell culture system as previously described with modifications.6 Sphenoid sinus mucosal specimens were collected during transsphenoidal resection of pituitary lesions from 4 patients without a history of sinonasal disease, as approved by the Greater Los Angeles Department of Veterans Affairs Institutional Review Board. SNECs were expanded on tissue culture plates and transferred onto inserts, and confluent cells were cultured.6 The primary monolayer cell cultures of SNECs were used to maintain the homogeneity of the culture system. To determine the effect of permethrin on cell growth, SNECs were exposed for 6 days to various concentrations of permethrin (0-5 mM). For cell viability measurement, MTT colorimetric dye assay was performed. Optical density was measured at 570 nm. The Incucyte Live Cell Imaging System (Essen Bioscience) was used to assess cell proliferation.7 Live cells were monitored with real-time kinetic data. SNECs were exposed to different concentrations of permethrin, and wells were scanned every 2 hours from 0 hours to 6 days (144 hours) after exposure. Permethrin dosages were based on a prior in vitro study conducted on other human organs.8 The data are presented as fold changes in cell density from initiation (0 hours) to specific times points during the assay. Cell density was calculated with Incucyte software and phase

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Results

Permethrin Exposure Inhibits Cultured Cell Growth at 156 μM

When SNECS (1500 per well in quadruplicates) were treated with permethrin (Figure 1), a significant reduction in cell viability was observed with 156 μM (P = .004), although statistical significance was not observed at 39 or 78 μM in comparison with controls (t tests). There was no cell viability when permethrin was administered at higher concentrations (321 μM–5 mM).

Toxicant Treatment and Live Cell Analysis With the Incucyte Live Cell Imaging System

When cultured cells (1500 cells per well in quadruplicate; Figure 2) were treated with permethrin (39 μM–5 mM), cell viability was reduced in a dose-dependent manner from 78 μM to 5 mM. At lower concentrations, the toxicants do not appear to be killing the cells but may slow the cellular proliferation rate.

Generation of Oxidative Stress and ROS by Permethrin

SNECs were exposed to 0- to 5mM permethrin for 48 hours. Cellular ROS activity (1 × 10^5) was measured by the DCFDA ROS detection assay kit. Linear regression revealed that permethrin (Figure 3) caused a dose-dependent increase of ROS activity (B = 171.75, P < .001).

Discussion

The precise pathomechanism with which environmental toxicants contribute to CRS is not fully understood. Aerosolized permethrin is associated with upper and lower airway symptoms. Recent studies have demonstrated that permethrin activates adaptive immune responses of the peripheral and central nervous system, thereby contributing to inflammatory disorders. However, the effects of permethrin on SNECs has not been investigated.

In this pilot study, significant reduction in cell viability was observed at 156 μM but not at 39 or 78 μM, suggesting that lower doses may not cause cytotoxicity. Incucyte live cell imaging showed inhibition of SNEC proliferation from permethrin exposure in a dose-dependent manner from 78 μM to 5 mM. At lower concentrations, permethrin did not appear to be killing the cells but slowed the cellular proliferation rate. Permethrin was also shown to induce a dose-dependent increase of ROS activity of SNECs, similar to findings from previous studies on lymphocytes and cardiac tissues.
Commercially available pesticides contain 10% (100 mg/mL) permethrin to 40% (400 mg/mL). Neurotoxicity, including ataxia, convulsions, and respiratory arrest, has been reported in humans when 10% permethrin is ingested. However, toxicity thresholds from inhalant exposure in humans have not yet been determined. Since the dosages used in this in vitro study (0-5 mM, 1.956 mg/mL) are lower than the reported concentrations associated with human neurotoxicity, additional efforts are needed to determine the actual concentration of permethrin exposure in pesticides that can cause clinical morbidity.

Cytotoxicity contributes to the pathogenesis of lower airway inflammatory diseases. Toxicant exposures induce cell lysis, produce cellular debris, and generate ROS, causing proinflammatory cytokine release in lung tissue. Aldehydes and diesel exhaust increase secretion of IL-6, IL-8 and oxidative stress (hemeoxygenase 1) biomarkers in bronchial epithelial cells. Caspase 1–dependent IL-1β release is the primary pathomechanism for respiratory syncytial virus bronchiolitis as well as COVID-19–mediated lung injury and pneumonia. Our preliminary data demonstrate that permethrin exposure induces oxidative stress and cytotoxic effects in SNECs. However, additional research is necessary to explore whether cytotoxic-mediated inflammation contributes to CRS and serves as a potential pathomechanism with pesticide exposure.

Conclusion
This pilot study is the first to our knowledge to illustrate the deleterious effects of permethrin on sinonasal epithelia. In our sample, permethrin exposure significantly decreased SNEC proliferation and increased oxidative stress in a dose-dependent manner. Additional research is needed to confirm our findings and elucidate if such cytotoxicity incites chronic inflammation, as well as to determine the toxicity thresholds associated with sinonasal morbidity.

Author Contributions
Jivianne T. Lee, conception and design, data acquisition, analysis and interpretation, drafting and revising work critically for intellectual content, approval, agreement of accountability; Hong-Ho Yang, analysis and interpretation, drafting and revising work critically for intellectual content, approval, agreement of accountability; Daniel Sanghoon Shin, data acquisition, analysis and interpretation, approval, agreement of accountability; Eri Srivatsan, data acquisition, analysis and interpretation approval, agreement of accountability; Saroj Basak, conception and design, data acquisition, analysis and interpretation, drafting and revising work critically for intellectual content, approval, agreement of accountability.

Disclosures
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