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MORPHOLOGICAL FEATURES OF THE EFFECT OF PESTICIDES ON TRACHEOBRONCHIAL LYMPH NODES

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Abstract. This study investigates the profound morphological and systemic changes induced in laboratory rabbits by chronic aerosol exposure to the organophosphate pesticide Chlorpyrifos (CPF). The objective was to characterize the regional immunotoxicity by analyzing the tracheobronchial lymph nodes (TBLNs) and lung tissue. The intoxication, administered as an aerosol (1:50 dilution, twice every three days for four months), resulted in severe negative systemic effects, including substantial body weight loss (up to 20% decline) and marked clinical signs of poisoning. Pathohistological analysis revealed significant pulmonary pathology (edema, hypertrophy of Club/Clara cells, peribronchial inflammation) and distinct structural shifts in the TBLNs. Key changes in TBLNs included cortical widening, lymphoid follicular dystrophy, a narrowed medulla, and severe accumulation of free macrophages in the medullary sinusoids. These findings establish that chronic CPF aerosol exposure exerts a high degree of toxicity on both the respiratory and immune systems, critically impairing regional immune competence.

1. Introduction

1.1. Pesticides, Exposure Routes, and Public Health

Pesticides, particularly organophosphates (OPs) such as Chlorpyrifos (CPF) and pyrethroids like Cypermethrin (CYP), are globally utilized agricultural chemicals. While effective in pest control, their widespread use leads to substantial environmental contamination, posing chronic health hazards, especially in agricultural regions. Inhalation of aerosolized residues represents a critical, often unavoidable, exposure route, directly affecting the respiratory system. The toxicity of CPF is primarily associated with the inhibition of acetylcholinesterase, leading to cholinergic crisis; however, its non-cholinergic immunotoxic and structural effects are increasingly recognized as significant contributors to systemic disease.

1.2. The Role of Tracheobronchial Lymph Nodes in Respiratory Immunity

The **tracheobronchial lymph nodes (TBLNs)** are strategically positioned along the main airways, serving as the primary regional site for processing inhaled particulate matter and antigens. TBLNs are crucial for initiating and regulating respiratory immune responses, integrating innate and adaptive immunity. Therefore, morphological alterations in TBLNs provide a sensitive and direct indicator of the immunotoxic impact of inhaled environmental pollutants. Any disruption to the architecture or cellular dynamics of the TBLNs can compromise pulmonary defense mechanisms, increasing susceptibility to respiratory infections and chronic inflammatory conditions.

1.3. Study Objectives

The main objective of this study is to investigate the morphological changes occurring in the tracheobronchial lymph nodes under the influence of various pesticides (Chlorpyrifos and Cypermethrin), to better understand the mechanism of regional immunotoxicity.

Research Tasks:

1. To characterize the specific morphological shifts in TBLNs using general histological (H&E) and histochemical methods (Van Gieson, PAS reaction) and immunohistochemical markers (Ki-67, CD-68, CD-20), and conduct a comparative analysis with the control group.
2. To investigate the morphometric indicators of histological shifts in TBLNs induced by pesticides and compare them with the morphological parameters of the control group.

2. Materials and Methods

2.1. Object, Subject, and Ethical Compliance

The study was conducted at the Fergana Medical Institute of Public Health, adhering strictly to the European Union and European Parliament Directive 2010/63/EC. Non-pedigreed rabbits (Total $n = 47$; $n = 28$ for final samples) weighing $2.4 \text{–} 2.6 \text{ kg}$ were housed under standard vivarium conditions. The object of study was the TBLN, and the subject was the tissue isolated after acute aerosol intoxication.

2.2. Intoxication and Monitoring Protocol

Experimental animals were exposed to aerosolized Chlorpyrifos solution (1:50 dilution in boiled water) applied twice every three days for four months. Animals were monitored daily for changes in general condition and body weight.

2.3. Histological Sample Preparation and Analysis

Animals were euthanized using chloroform. TBLN fragments (1 cm^3) were fixed in 10% neutral formalin, dehydrated using ascending ethyl alcohol series, and embedded in paraffin. Sections were cut to $6 \text{–} 7 \text{ } \mu m$ thickness. Staining included:

- **General Morphology:** Hematoxylin-Eosin (H&E).
- **Histochemical Analysis:** Van Gieson's stain and Periodic acid–Schiff (PAS) reaction.
- **Immunohistochemistry (IHC):** Markers for proliferation (Ki-67), macrophages (CD-68), and B-lymphocytes (CD-20) were planned for expression assessment.

Microscopic and morphometric evaluations were performed using an MT 5300L light microscope at $\times 40$, $\times 100$, and $\times 400$ magnifications.

3. Results

3.1. Systemic and Clinical Observations

The Chlorpyrifos exposure induced significant chemical stress and moderate to severe clinical signs of poisoning:

- **Clinical Toxicity:** Observed signs included excessive nasal mucous discharge, changes in the nasal mucosa, labored breathing, lacrimation, and marked ptyalism.
- **General Condition:** Experimental animals displayed progressive coat dullness, sparsity, and fragility after the first week.
- **Body Weight Dynamics:** Control animals showed a healthy weight increase ($1.0 \text{–} 1.5 \text{ kg}$). In stark contrast, the experimental group showed a dramatic body weight **decrease of up to 20%** within the first ten days, with continued, gradual decline over the four-month period.

3.2. Macroscopic and Pulmonary Pathological Findings

A. Macroscopic Findings:

- **TBLNs:** Experimental TBLNs were macroscopically **hypertrophied** (larger than the control's 0.5 cm size), exhibiting a medium-dense, **firm consistency**, and presented a **darker, light-brownish hue**, contrasting with the light bluish-grey of controls.
- **Lungs:** The lung tissue showed extensive **edema** and **plethora (hyperemia)**, feeling dense, hardened, and tense upon palpation. Microcirculatory congestion and localized hemorrhages were evident.

B. Pulmonary Pathohistology:

Microscopic examination confirmed severe toxic damage:

- **Cellular Injury: Hypertrophy and numerical decrease of Club (Clara) cells** in the respiratory bronchioles, and focal exfoliation of the respiratory epithelium.
- **Inflammation:** Extensive **peribronchial inflammatory infiltrates** and thickening of the inter-alveolar septa.
- **Vascular Changes:** Severe microcirculatory plethora, particularly in the capillary network, accompanied by an increased number of **macrophages and neutrophils** within the connective tissue of the alveolar septa.

3.3. Tracheobronchial Lymph Node Histopathology

Pathohistological analysis of the hypertrophied TBLNs revealed key immunopathological shifts:

- **Structural Rearrangement:** The demarcation line between the cortex and medulla was shortened due to the expansion and **widening of the paracortical zone**.
- **Follicular Atrophy:** A pronounced **reduction in the number and presence of dystrophy** in the lymphoid follicles. This was linked to a **decrease and slowdown of proliferative processes** within the germinal centers.
- **Medullary Changes:** The medullary substance was **narrowed**, while the medullary sinusoids were **widened** and displayed thinning of their walls (lymphoid, endothelial, reticular cells).
- **Cellular Accumulation:** The most striking feature was the significant accumulation of **lymphoid cells and free macrophages** within the wide lumen of the sinusoids.
- **Edema:** Small **vesicles (vacuolation)** were observed throughout the cortical and medullary substances, indicative of interstitial edema.

4. Discussion

4.1. Mechanism of Systemic Toxicity and Stress Response

The severe systemic deterioration—marked by clinical signs of cholinergic crisis and persistent, dramatic weight loss—confirms that the chronic, low-dose CPF aerosol exposure functioned as both a direct toxicant and a major chemical stressor. The sustained catabolic state indicated by weight loss is often linked to chronic inflammation and a dysfunctional neuroendocrine response triggered by persistent chemical exposure, extending beyond the acute cholinergic phase.

4.2. Linking Pulmonary and Lymph Node Pathology

The pathology observed in the lungs (interstitial pneumonitis, Clara cell injury, and inflammatory infiltrates) confirms that the respiratory tissue absorbed significant toxic insult. This massive influx of inflammatory mediators and CPF residues into the lymph system directly drives the morphological changes in the TBLNs.

The TBLN response shows a clear picture of **immune dysregulation**:

- **Impaired Adaptive Immunity:** The **follicular dystrophy** and decreased proliferation in germinal centers (suggesting reduced B-cell activity and decreased Ki-67 expression) point to a compromised ability to mount a robust, long-term adaptive immune response. This lymphoid depletion is a classic sign of immunotoxicity.
- **Compensatory Innate Immunity:** The **macrophage accumulation** in the sinusoids (implying heightened CD-68 activity) demonstrates a frantic, yet possibly ineffective, effort by the innate system to clear the toxic load and cellular debris. The widening of the paracortical zone suggests a sustained effort by T-cells to manage the continuous antigen presence.

4.3. Conclusion and Public Health Implications

The findings establish a direct and critical link between chronic aerosol exposure to Chlorpyrifos and the compromise of the regional immune and structural integrity of the respiratory defense system. The observed pathology provides a clear framework for understanding the potential long-term consequences, including increased susceptibility to respiratory infections and chronic obstructive diseases.

The research unequivocally demonstrates the urgent need for:

- **Revising safety protocols** and strengthening regulatory control over pesticide use in agriculture.
- **Developing strategies** to minimize environmental and occupational aerosol exposure to protect public respiratory and immune health from these highly toxic compounds.

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