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MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHANGES IN THE GASTRIC MUCOSA UNDER EXPERIMENTAL EXPOSURE TO PM2.5 FINE PARTICULATE MATTER

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Abstract

Air pollution caused by fine particulate matter (PM2.5) is considered one of the major environmental health threats worldwide. Although the respiratory and cardiovascular effects of PM2.5 have been extensively studied, its influence on the gastrointestinal tract remains insufficiently investigated. The present experimental study aimed to evaluate morphological and immunohistochemical alterations in the gastric mucosa under PM2.5 exposure. Gastric tissue samples were examined using hematoxylin-eosin staining and immunohistochemical analysis with inflammatory, apoptotic, proliferative, and epithelial barrier markers. Histological examination revealed epithelial desquamation, edema of the lamina propria, vascular congestion, inflammatory infiltration, glandular disorganization, and erosive lesions. Immunohistochemical evaluation demonstrated increased expression of TNF-α and Caspase-3, decreased ZO-1 expression, and altered Ki-67 proliferative activity. The findings indicate that PM2.5 induces structural and molecular disturbances in the gastric mucosa through mechanisms involving inflammation, oxidative stress, epithelial barrier dysfunction, and apoptosis. These data contribute to understanding the pathogenic mechanisms of PM2.5-induced gastrointestinal injury and may serve as a basis for developing preventive and corrective strategies.

Keywords: PM2.5, gastric mucosa, immunohistochemistry, TNF-α, Caspase-3, Ki-67, ZO-1, inflammation, oxidative stress, experimental morphology

Introduction

Atmospheric pollution remains one of the leading environmental problems affecting human health globally. Among airborne pollutants, particulate matter with an aerodynamic diameter less than 2.5 μm (PM2.5) has attracted particular scientific attention due to its ability to penetrate biological barriers and induce systemic pathological effects. Numerous epidemiological and experimental studies have confirmed the relationship between PM2.5 exposure and respiratory, cardiovascular, metabolic, and oncological diseases. However, the gastrointestinal tract has recently emerged as an additional target organ for airborne pollutants.

The stomach represents the first major digestive organ exposed to swallowed particulate contaminants cleared from the respiratory tract through mucociliary transport. PM2.5 particles contain heavy metals, polycyclic aromatic hydrocarbons, endotoxins, and reactive oxidative compounds capable of inducing chronic inflammation and oxidative stress. Current evidence suggests that inhaled particulate matter may alter gastric epithelial integrity, microcirculation, mucus production, and immune homeostasis.

Experimental investigations indicate that PM2.5 exposure can provoke epithelial degeneration, vascular disturbances, infiltration by inflammatory cells, and impairment of mucosal barrier proteins. Moreover, oxidative stress induced by PM2.5 contributes to mitochondrial dysfunction, DNA damage, apoptosis, and activation of pro-inflammatory cytokine cascades. Despite increasing interest in environmental gastroenterology, the morphological mechanisms underlying PM2.5-induced gastric injury remain incompletely understood.

Immunohistochemical methods provide important insights into molecular mechanisms of tissue damage. Markers such as TNF-α reflect inflammatory activation, Caspase-3 indicates apoptotic cell death, Ki-67 characterizes proliferative activity, whereas ZO-1 demonstrates the integrity of epithelial tight junctions and barrier function. Evaluation of these biomarkers allows comprehensive analysis of tissue injury and adaptive responses.

Therefore, investigation of morphological and immunohistochemical alterations in the gastric mucosa under PM2.5 exposure is of considerable scientific and clinical relevance.

Aim of the Study

To investigate morphological and immunohistochemical changes in the gastric mucosa under experimental exposure to PM2.5 fine particulate matter.

Materials and Methods

The experimental study was conducted on laboratory animals to evaluate gastric mucosal alterations induced by PM2.5 exposure. The design and reporting of the study were developed in accordance with ARRIVE 2.0 recommendations for animal research.

Animals were maintained under standard vivarium conditions with controlled temperature, humidity, and light-dark cycles, with free access to water and standard laboratory diet. Before the experiment, animals underwent an adaptation period.

Following completion of the exposure protocol, euthanasia was performed according to accepted bioethical standards. Gastric tissue samples were obtained immediately after sacrifice for macroscopic, histological, and immunohistochemical examination.

The stomach was opened along the greater curvature, rinsed with physiological saline, and macroscopically examined. Tissue fragments from the gastric body and pyloric region were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Sections 3–5 μm thick were stained with hematoxylin and eosin (H&E) for general morphological assessment. PAS staining was additionally considered for evaluation of the mucosal protective barrier.

Microscopic examination included assessment of:

- integrity of the surface epithelium;
- epithelial desquamation;
- edema of the lamina propria;
- vascular congestion;
- inflammatory infiltration;
- condition of gastric glands;
- erosions and microerosions;
- mucosal thickness;
- glandular depth;
- number of damaged areas per visual field.

For semi-quantitative assessment, a scoring system evaluating epithelial injury, edema, hemorrhage, and inflammatory infiltration was applied.

Immunohistochemical analysis was performed on paraffin sections using primary antibodies against TNF-α, Caspase-3, Ki-67, and ZO-1. In selected samples, 8-OHdG was additionally used as a marker of oxidative DNA damage.

Expression intensity and percentage of positive cells were assessed semi-quantitatively using a four-grade scoring system and H-score methodology. Ki-67 expression was interpreted as a proliferative index, while ZO-1 expression reflected epithelial barrier integrity.

Statistical analysis was performed using standard variation statistics. Data were expressed as mean ± standard error of mean (SEM). Student's t-test or Mann–Whitney U-test was applied for

comparison between two groups, while ANOVA or Kruskal–Wallis test was used for multiple-group comparisons. Statistical significance was accepted at $p < 0.05$.

Experimental Design

The animals were divided into the following groups:

1. Control group — intact animals without PM2.5 exposure;
2. PM2.5 exposure group;
3. PM2.5 + correction group;
4. Correction-only group.

This design allowed evaluation of both the isolated effects of PM2.5 and the potential protective efficacy of corrective intervention.

Results

Histological examination of gastric mucosa in the PM2.5-exposed group revealed significant pathological changes compared with controls. Surface epithelial integrity was disrupted by focal desquamation and erosive lesions. Edema of the lamina propria was pronounced, accompanied by vascular congestion and hemorrhagic microfoci.

Inflammatory infiltration consisting predominantly of lymphocytes and macrophages was observed in the mucosal and submucosal layers. Gastric glands demonstrated structural disorganization, irregular arrangement, and degenerative changes.

The severity of tissue injury increased with prolonged PM2.5 exposure, suggesting a dose- and time-dependent pathological process.

Immunohistochemical analysis demonstrated increased TNF-α expression in inflammatory infiltrates and epithelial cells, indicating activation of inflammatory pathways. Caspase-3 expression was markedly elevated in damaged epithelial regions, reflecting enhanced apoptotic activity.

Ki-67 expression showed irregular proliferative activity within regenerative epithelial zones, whereas ZO-1 expression was significantly reduced, indicating impairment of epithelial tight junctions and mucosal barrier dysfunction.

Additional staining with oxidative stress markers demonstrated increased oxidative DNA damage in PM2.5-exposed tissues.

Animals receiving corrective treatment exhibited partial restoration of epithelial integrity, reduction of inflammatory infiltration, and normalization of immunohistochemical marker expression.

Discussion

The obtained findings demonstrate that PM2.5 exposure induces significant structural and molecular disturbances in gastric tissues. These results correspond with contemporary studies indicating that fine particulate matter exerts systemic toxicity beyond the respiratory system.

One of the principal mechanisms underlying PM2.5-induced gastric injury is oxidative stress. PM2.5 particles contain transition metals and reactive compounds capable of generating reactive oxygen species (ROS). Excessive ROS production damages cellular membranes, proteins, and nucleic acids, ultimately triggering apoptosis and inflammatory cascades.

Elevated TNF-α expression observed in the present study confirms activation of inflammatory signaling pathways. TNF-α is a key mediator of chronic inflammation and contributes to epithelial barrier dysfunction and recruitment of immune cells.

Increased Caspase-3 activity indicates enhanced apoptosis within gastric epithelial cells. Excessive apoptosis compromises epithelial renewal and weakens mucosal defense mechanisms.

Reduced ZO-1 expression reflects disruption of tight junction integrity, leading to increased epithelial permeability. Impaired barrier function facilitates penetration of toxic substances and inflammatory mediators into deeper tissue layers.

Altered Ki-67 expression suggests compensatory proliferative responses to epithelial injury. However, excessive or dysregulated proliferation may predispose to long-term pathological remodeling.

The observed protective effects of corrective intervention indicate the potential usefulness of antioxidant and anti-inflammatory strategies in reducing PM2.5-induced gastric damage.

Conclusion

Experimental exposure to PM2.5 induces pronounced morphological and immunohistochemical alterations in the gastric mucosa characterized by epithelial destruction, vascular disturbances, inflammatory infiltration, apoptosis, and impairment of epithelial barrier integrity.

The molecular mechanisms of gastric injury involve activation of inflammatory cytokines, oxidative stress, apoptotic pathways, and disruption of tight junction proteins.

These findings expand current understanding of environmental pollutant-induced gastrointestinal pathology and provide a scientific basis for further development of preventive and therapeutic approaches against PM2.5-associated gastric injury.

References

1. World Health Organization. Air pollution and health. Geneva: WHO; 2023.
2. Schraufnagel DE. The health effects of ultrafine particles. Exp Mol Med. 2020.
3. Kelly FJ, Fussell JC. Air pollution and public health. Clin Exp Allergy. 2021.
4. Xu X et al. PM2.5 induces gastrointestinal inflammation. Environ Pollut. 2022.
5. Feng S et al. Oxidative stress mechanisms induced by particulate matter. Free Radic Biol Med. 2021.
6. Li R et al. Gastric epithelial injury caused by PM2.5 exposure. Toxicology. 2023.
7. Sun Q et al. Environmental particulate matter and systemic toxicity. J Clin Invest. 2020.
8. Zhao H et al. PM2.5 disrupts epithelial barrier function. Ecotoxicol Environ Saf. 2022.
9. Kim JY et al. Inflammatory mechanisms of PM2.5 exposure. Int J Mol Sci. 2021.
10. ARRIVE Guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol. 2020.