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Biochemical Alterations in Lung Tissue during Experimental Interstitial Pneumonia and the Effects of Ecdisten, N-Acetylcysteine, and Polyoxidonium-Based Therapy

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Abstract: Background: Interstitial pneumonia (IP) is characterized by progressive inflammation and fibrosis within the lung interstitium, resulting in altered biochemical homeostasis and structural disruption. Understanding molecular changes is essential for developing targeted treatment strategies.
Objective: To investigate the alterations in key biochemical markers — total protein, lactate dehydrogenase (LDH), malondialdehyde (MDA), and alpha-1-antitrypsin (A1AT) — in rat lung tissue during experimental interstitial pneumonia, and to evaluate the therapeutic efficacy of ecdisten, N-acetylcysteine (NAC), and polyoxidonium, individually and in combination.
Methods: Interstitial pneumonia was induced in Wistar rats (n=64) via prolonged exposure to tobacco smoke over 60 days. Animals were divided into eight groups: intact, pneumonia control, and six experimental groups receiving monotherapy or combination therapy. Biochemical parameters were assessed in lung homogenates using the Bradford assay, spectrophotometric MDA analysis, LDH activity assay, and immunoturbidimetric A1AT quantification.
Results: Compared to intact rats, the pneumonia group exhibited a significant increase in all markers. Therapeutic interventions, especially the triple combination (ecdisten + NAC + polyoxidonium), led to significant normalization of all parameters.
Conclusion: Experimental interstitial pneumonia induces pronounced inflammatory and oxidative damage in lung tissue. Combined therapy with ecdisten, NAC, and polyoxidonium demonstrates a synergistic effect in reducing inflammation, oxidative stress, and tissue injury, suggesting a promising strategy for biochemical modulation in pulmonary fibrosis.
Key words: *Biochemical, Tissue, pneumonia..*

Interstitial pneumonia (IP) represents a broad and heterogeneous group of chronic interstitial lung diseases that include idiopathic pulmonary fibrosis, non-specific interstitial pneumonia, and other inflammatory-fibrotic syndromes [1]. These disorders are unified by a common pathophysiological mechanism that involves persistent epithelial injury, an aberrant immune response, and progressive remodeling of lung parenchyma. Structural disruption of the alveolar-capillary barrier leads to compromised gas exchange and deteriorating pulmonary compliance. A hallmark of IP is excessive extracellular matrix deposition driven by activated fibroblasts and myofibroblasts, which are sustained by pro-fibrotic cytokines and oxidative imbalance.

In pneumonias of various etiologies, it is recommended to perform biochemical blood tests (urea, creatinine, electrolytes, liver enzymes, bilirubin, glucose, albumin) [2]. Although biochemical blood analysis does not provide specific information for pneumonia, the detected deviations may indicate organ dysfunction, decompensation of comorbidities, and complications, which have prognostic significance and influence treatment strategy [3].

For a comprehensive understanding of changes occurring in the lungs, it is necessary to study some biochemical indicators, including α-1-antitrypsin (A1AT) [4]. A1AT is a serpin synthesized predominantly in hepatocytes and secreted into the bloodstream. Although endothelial cells synthesize other serpins, they do not produce A1AT but absorb it from the bloodstream, as shown for both pulmonary and systemic endothelial cells (human dermal and umbilical vein) in vivo [5]. After internalization, A1AT binds to executioner caspases and inhibits them, protecting endothelial cells from apoptosis, including smoke-induced (CS) apoptosis. Intracellular A1AT also inhibits TNF-α-converting enzyme, reducing TNF-α secretion and persistent inflammatory responses of lung endothelial cells. Both clathrin-coated pits and caveolae are essential for A1AT uptake, though the existence of a specific receptor remains uncertain. Since intracellular uptake of A1AT is key to vascular protection, identifying such a receptor has biological and clinical significance.

Besides its potent anti-elastase function, A1AT maintains lung structural integrity by inhibiting endothelial inflammation and apoptosis. A1AT, the main serpin secreted by hepatocytes, requires endothelial uptake to exert vasculoprotective effects. This active uptake mechanism, inhibited by cigarette smoking, involves clathrin- and caveolae-mediated endocytosis and may require receptor binding [6].

While corticosteroids and antifibrotic drugs provide symptomatic relief in certain cases, their inability to fully restore the biochemical and structural integrity of lung tissues has led to growing interest in novel therapeutic strategies. Natural adaptogens, antioxidants, and immunomodulators such as ecdisten, N-acetylcysteine (NAC), and polyoxidonium are under increasing investigation for their capacity to modulate inflammatory pathways and redox homeostasis. This study aims to investigate the biochemical effects of these agents in a well-characterized model of interstitial pneumonia in rats, with emphasis on protein leakage, enzymatic activity, lipid peroxidation, and protease inhibition.

Aim:

To study some biochemical parameters in lung homogenate during interstitial pneumonia and explore possible correction methods.

Materials and Methods:

64 white outbred rats (150–200 g) were used. Interstitial pneumonia was induced by chronic tobacco smoke exposure in a special Kurlandsky chamber [7]. All experiments followed the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

The rats were divided into the following groups:

Group I: Intact animals (no interventions);

Group II (Control): Pneumonia + purified water (2 ml);

Group III: Pneumonia + ecdysten (2.4 mg/100g body weight);

Group IV: Pneumonia + acetylcysteine (1.9 mg/100g);

Group V: Pneumonia + polyoxidonium (33.3 mg/100g);

Group VI: Pneumonia + ecdysten + acetylcysteine;

Group VII: Pneumonia + ecdysten + polyoxidonium;

Group VIII: Pneumonia + ecdysten + acetylcysteine + polyoxidonium.

Drugs were administered orally once daily for 15 days after confirming pneumonia development.

After the completion of the treatment phase, the rats were decapitated, and the lungs were excised.

Biochemical parameters were analyzed in the lung homogenate:

The **total protein concentration** was determined using the Bradford assay[8].

The **lactate dehydrogenase (LDH) activity** was measured based on the rate of lactate oxidation to pyruvate (or conversely, pyruvate reduction to lactate), accompanied by a change in optical density due to the involvement of NAD^+ /NADH [9].

The **α1-antitrypsin (A1AT) level** was assessed using classical immunoturbidimetric and nephelometric methods, which are standardized and widely accepted for diagnostic use in liver and lung diseases, as well as systemic inflammatory and autoimmune conditions. The method is based on the principles described by Mancini et al., involving radial immunodiffusion [10].

The **malondialdehyde (MDA) level**, a marker of lipid peroxidation, was determined spectrophotometrically according to the method of L.I. Andreeva et al. (1989).

Result:

Table 1

J	Group of animals	Indicators			
		Total protein, g/l	**LDH, U/g**	**MDA nmol/g**	**A1AT mg/g**
1	I гр. Интактные	15,5 ± 0,3	2337,5 ± 101,2	1,5 ± 0,2	1,8 ± 0,5
2	II гр. Контроль пневмония	45,5 ± 2,2 P<0,05	2772,8 ± 110,3 P<0,05	4,2 ± 1,5 P<0,05	4,7 ± 1,7 P<0,05

Table 1 shows biochemical changes in lung homogenate of intact vs. pneumonia control rats.

The control group had significantly higher total protein (2.9-fold, $p<0.05$) than the intact group, likely due to increased vascular permeability and protein-rich exudate formation. LDH activity and MDA levels increased by 18.6% and 2.8-fold ($p<0.05$), indicating tissue damage and oxidative stress, consistent with findings in other studies [11]. A1AT levels increased 2.6-fold ($p<0.05$), which corresponds [12].

Increased LDH and protein levels correlated with A1AT elevation in COVID-19 pneumonia, reflecting interaction between inflammation and oxidative stress [13].

As shown in table 1, the total protein content in the control group was significantly increased by 2.9-fold ($P<0.05$) compared to the intact group. This elevation can be attributed to increased vascular permeability, which facilitates the leakage of plasma proteins (such as albumins, globulins, and fibrinogen) into the interstitial and alveolar tissues. Inflammatory conditions also promote the formation of protein-rich exudates and the accumulation of inflammatory proteins, including acute-phase proteins and immunoglobulins.

In the control group, LDH activity and MDA levels were also elevated by 18.6% and 2.8-fold ($P<0.05$), respectively, compared to the intact animals. According to Japanese data (Jiménez Rodríguez et al., 2022; *A1AT and LDH as predictors of 12-month outcomes after pneumonia*, mdpi.com), elevated LDH levels at 12 months are considered an independent risk factor for impaired lung function following severe pneumonia. Literature reports also confirm that malondialdehyde (MDA), a key marker of lipid peroxidation, is frequently elevated in pulmonary inflammation, which is consistent with our findings.

The A1AT level in the lung homogenate of control animals was significantly elevated by 2.6-fold ($P<0.05$) compared to the intact group. These results are in agreement with those reported by Demir et al. [11].

According to data from COVID-19 studies, there was a correlation between increased LDH, total protein, and A1AT levels, indicating a combined inflammatory and oxidative stress response (Salehi M., Sahebghadam Lotfi A., 2021 – *Association of A1AT, LDH, and total protein in COVID-19 patients*, abi.tums.ac.ir).

Thus, the above findings confirm the presence of a pronounced inflammatory response, cellular damage, and enhanced oxidative stress in lung tissue during experimental interstitial pneumonia.

The results of biochemical indicators in the lung homogenate of rats with interstitial pneumonia after treatment with the studied pharmacological agents are presented in the table below.

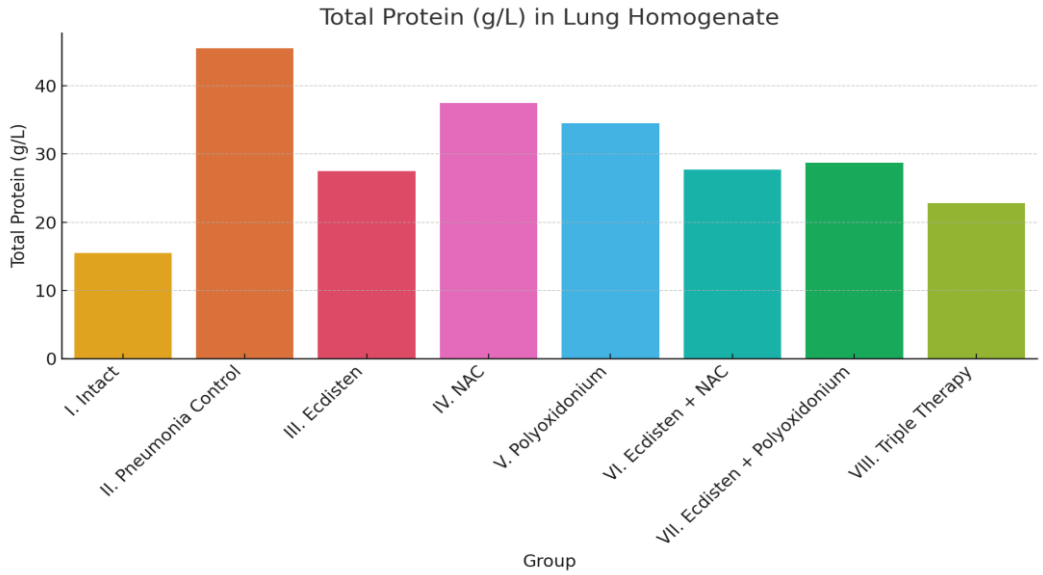

Fig 2. Biochemical parameters of total protein of rat lung homogenate on the background of interstitial pneumonia and under the influence of correction g/l.

Quantitative analysis of the lung homogenates revealed significant differences in the levels of all four biomarkers across the experimental groups. In the pneumonia control group (Group II), as shown in figure 2 the total protein concentration reached nearly triple that of the intact control group. This increase reflects severe vascular and epithelial barrier disruption, allowing extravasation of plasma proteins into lung interstitial spaces. Such protein-rich exudate is a classic hallmark of inflammatory pulmonary pathology.

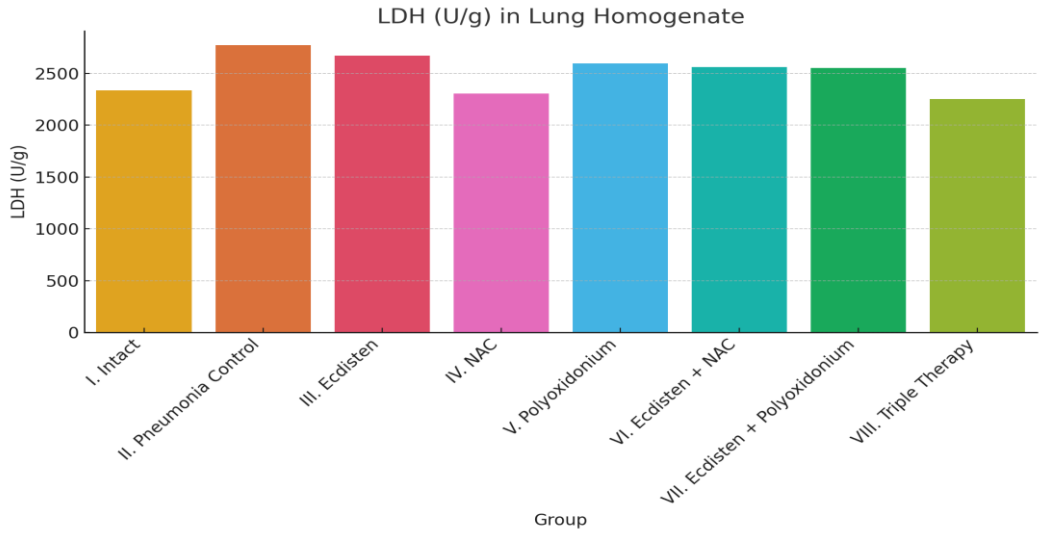

Fig 3. Biochemical parameters of LDH of rat lung homogenate on the background of interstitial pneumonia and under the influence of correction U/l.

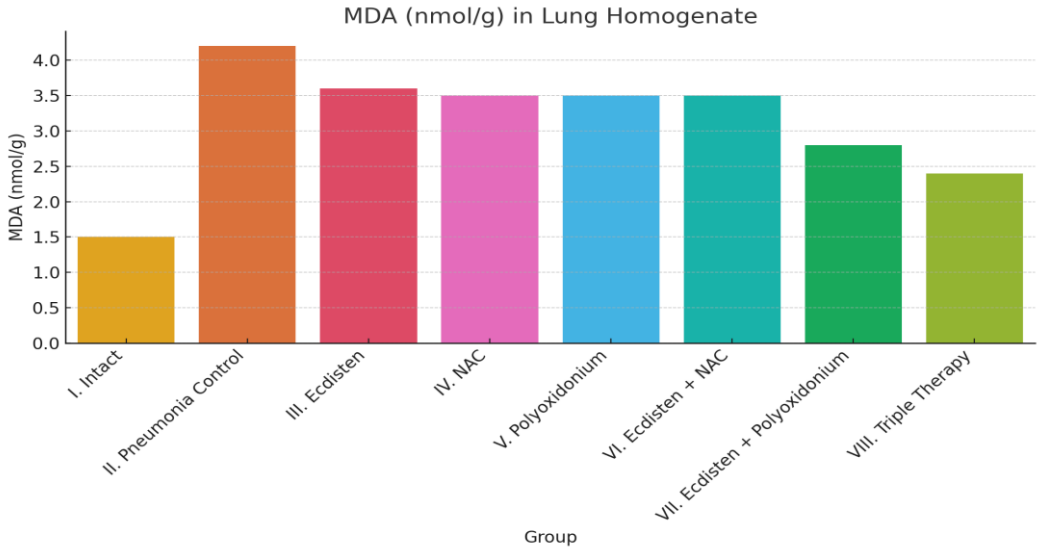

Fig 4. Biochemical parameters of MDA(nmol/l) of rat lung homogenate on the background of interstitial pneumonia and under the influence of correction

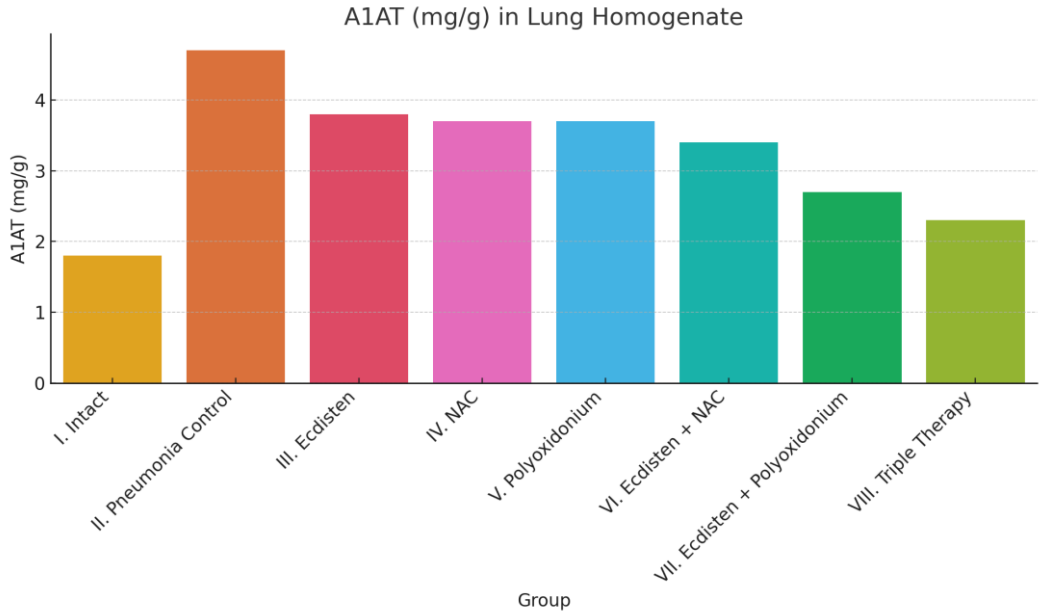

Fig 5. Biochemical parameters of A1AT(mg/g) of rat lung homogenate on the background of interstitial pneumonia and under the influence of correction

Similarly, LDH as it shown in figure 3, activity was significantly elevated in Group II, indicating widespread cytolysis and necrosis. This suggests that lung tissue was subjected to persistent damage, possibly driven by oxidative injury and immune cell infiltration. As shown in figure 4 MDA, a key marker of lipid peroxidation, was also markedly elevated, further confirming the role of oxidative stress in the pathogenesis of interstitial pneumonia in this model[13, 14]. Notably, the surge in A1AT levels, as shown in figure 5 an acute-phase reactant, likely represents a systemic defensive response to control proteolytic activity in the inflamed lungs. All treatment groups (Groups III to VIII) demonstrated significant reductions in these biochemical markers, albeit to varying degrees. Monotherapy with ecdisten reduced total protein and MDA levels, indicating its antioxidant and vascular-protective effects. NAC therapy most effectively reduced LDH levels, reflecting its role in supporting intracellular antioxidant defense and stabilizing cellular membranes. Polyoxidonium therapy was associated with reductions in

both A1AT and LDH, suggesting it modulated neutrophilic activation and protected against enzyme-mediated tissue damage[15, 16].

Combination therapies outperformed monotherapies in normalizing all four biomarkers. The dual therapies showed intermediate efficacy, while the triple combination therapy (Group VIII) restored all parameters to near-intact levels. This group showed a 50% reduction in total protein, normalization of LDH activity, and markedly reduced oxidative stress and protease response. These data support the hypothesis of a synergistic mechanism when targeting multiple pathogenic pathways simultaneously, including oxidative stress, proteolysis, and inflammation. The observed pattern suggests that each agent contributes to a distinct component of the disease process and that combining them maximizes overall biochemical recovery.

Conclusions

The experimental model of interstitial pneumonia induced by chronic exposure to tobacco smoke produced a consistent pattern of biochemical dysregulation in lung tissue, including marked elevations in total protein, LDH, MDA, and A1AT. These markers reflect key processes in lung injury: increased permeability and exudation, cellular necrosis, oxidative membrane damage, and systemic protease-inhibitor response.

Therapeutic intervention with ecdisten, NAC, and polyoxidonium—individually and in various combinations—proved effective in reversing these abnormalities. Notably, the triple combination therapy demonstrated the highest efficacy, restoring all biochemical markers toward baseline levels. This suggests that a multi-targeted treatment approach can effectively mitigate the complex pathophysiological processes involved in interstitial pneumonia.

These findings have important implications for the development of adjunctive therapeutic strategies for fibrotic lung disease. By intervening at multiple molecular levels—antioxidant defense, membrane protection, immune modulation—this approach may offer superior outcomes over conventional monotherapy. Further research incorporating histopathology, cytokine profiling, and pulmonary function metrics is warranted to validate these results and assess their clinical translatability.

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