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Changes in Matrix Metalloproteinase Activity and Their Inhibitor Levels in Experimental Interstitial Pneumonia and Pharmacological Correction Strategies

Tursunov D.Kh.
Tashkent State Medical University

Sabirova R.A.
Tashkent State Medical University

Alimbekova L.U.
Tashkent State Medical University

Abstract: Interstitial pneumonia (IP) is a group of diseases characterized by inflammation and fibrosis of the lung tissue. One of the key features of these processes is extracellular matrix (ECM) remodeling, in which matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) play a crucial role. A comprehensive pharmacological strategy involving three different mechanisms of action (hormone-like, antioxidant, and immunomodulatory) is proposed. For the first time, the effect of combined therapy on the expression of MMP-1, -7, -9 and TIMP-1 in two biological media (blood and lung tissue) in an experimental IP model has been demonstrated. Comparative quantitative data are presented confirming the efficacy of the treatment.

Key words: *interstitial pneumonia, matrix metalloproteinases, TIMP-1, combined therapy, lung tissue, inflammation.*

Introduction

Remodeling of the extracellular matrix (ECM) is a hallmark of interstitial lung diseases, with matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) playing a central role in the development of these pathological changes, as they are directly responsible for ECM degradation [6,7,18]. Notably, there is strong evidence linking altered levels of MMPs and TIMPs to the pathogenesis of interstitial pneumonia. Several studies have reported increased concentrations of these proteins — including MMP-1, MMP-2, MMP-7, MMP-9, MMP-12, and TIMP-1 — in blood samples from patients with interstitial pneumonia, highlighting MMP-7 as both a diagnostic and prognostic biomarker [2,3,14,15]. MMPs and TIMPs have also emerged as promising therapeutic targets for interstitial pneumonia [8,9,11,16]. However, to date, research on ECM remodeling biomarkers for the early and accurate diagnosis of connective tissue disease-associated interstitial lung disease (CTD-ILD) remains limited and insufficient for routine clinical use [4,12,13,17].

During normal wound healing and fibrosis, ECM proteases perform a critical role. The activity of specific members of this enzyme family depends on the stage of the fibrotic process: they are involved in

both profibrotic and antifibrotic reactions, contributing to tissue repair and matrix turnover [1]. Their activity is counterbalanced by tissue inhibitors of metalloproteinases (TIMPs) [5].

Matrix metalloproteinase-7 (MMP-7) is of particular importance in ECM remodeling and inflammatory responses in the lungs. In interstitial pneumonia, the activity and expression of MMP-7 are typically elevated, promoting matrix degradation, exacerbating inflammation, and contributing to tissue damage.

Aim of the Study: To investigate the levels of matrix metalloproteinases and the tissue inhibitor TIMP-1 in experimental interstitial pneumonia and evaluate their correction by ecdisten and polyoxidonium.

Materials and Methods

The study was conducted on 64 outbred white rats weighing 150–200 g. Interstitial pneumonia was induced by chronic exposure to tobacco smoke in a special chamber over a period of two months [10]. All procedures were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

The animals were divided into eight groups (8 rats per group):

Group I – Intact animals (no interventions);

Group II – Control group: rats with interstitial pneumonia received 2 ml of purified water;

Group III – Experimental group: rats received an aqueous solution of ecdisten at a dose of 2.4 mg per 100 g of body weight in the background of pneumonia;

Group IV – Experimental group: rats received N-acetylcysteine (ACC) at a dose of 1.9 mg per 100 g of body weight with pneumonia;

Group V – Experimental group: rats received polyoxidonium at a dose of 33.3 mg per 100 g of body weight with pneumonia;

Group VI – Experimental group: rats received combined therapy with ecdisten + N-acetylcysteine;

Group VII – Experimental group: rats received combined therapy with ecdisten + polyoxidonium;

Group VIII – Experimental group: rats received triple combination therapy with ecdisten + N-acetylcysteine + polyoxidonium.

After the treatment period, the rats were decapitated, and blood and lung tissue were collected. The levels of matrix metalloproteinases and TIMP-1 were determined in both serum and lung homogenates.

MMP-1, MMP-7, MMP-9, and TIMP-1 concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits from Quantikine, R&D Systems (USA). Lung homogenates were pre-processed by centrifugation to remove cellular debris. Specific antibodies were used to detect MMP-1, -7, and -9, with a colorimetric reaction visualized via enzyme conjugation (e.g., peroxidase) and substrate conversion. The intensity of color development was measured spectrophotometrically and was proportional to the concentration of each target protein.

Therapeutic agents were administered per os once daily for 15 days after the establishment of the pneumonia model.

Results and Discussion

Matrix metalloproteinases are key enzymes involved in the degradation of extracellular matrix components and play a critical role in inflammation and tissue remodeling in the lungs. Overexpression of these enzymes in interstitial pneumonia contributes to the destruction of alveolar structures, enhances inflammatory responses, and may be associated with the onset of pulmonary fibrosis.

In the intact group (Group I), the levels of MMP-1, MMP-7, and MMP-9 were 8.5 ± 0.27, 42.2 ± 7.2, and 22.5 ± 0.72 ng/ml, respectively, reflecting physiological enzyme activity in the absence of inflammation and tissue remodeling.

In the pneumonia control group (Group II), levels of MMP-1, MMP-7, and MMP-9 increased by 2.3-, 2-, and 2.6-fold, respectively ($P<0.05$), indicating active ECM degradation, progression of inflammation, and potential initiation of fibrotic changes in the lung tissue. These findings confirm the role of MMPs as important pathological markers in interstitial pneumonia (Figure 1).

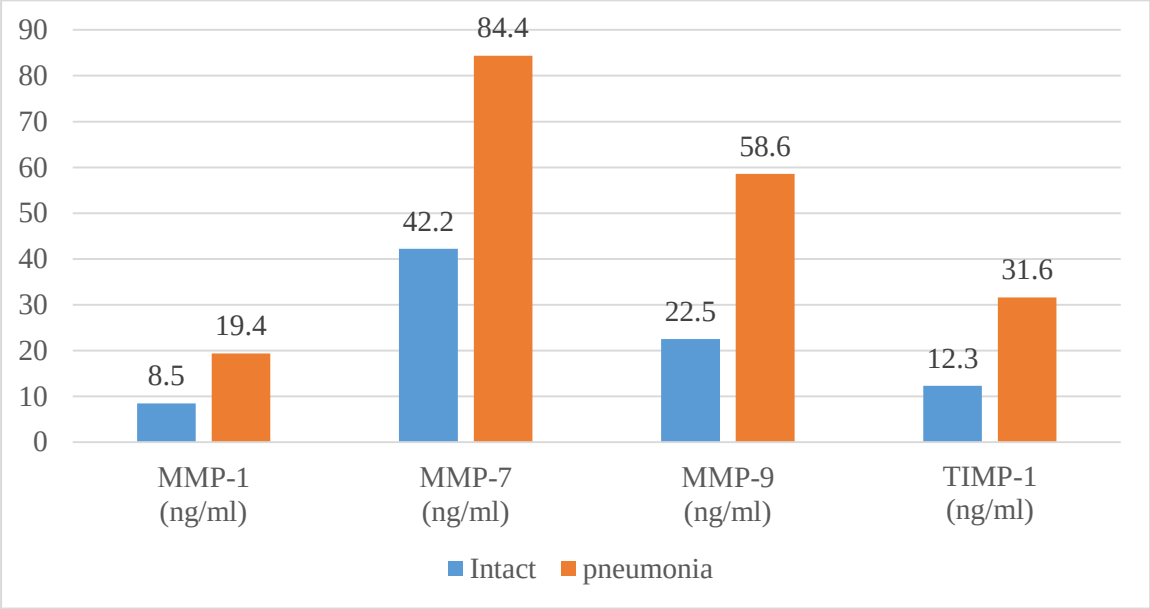

Figure 1. Levels of matrix metalloproteinases (MMP-1, -7, -9) and their inhibitor TIMP-1 in blood serum in interstitial pneumonia

The serum concentration of the tissue inhibitor TIMP-1 in intact rats was 12.3 ± 0.39 ng/ml, while in rats with interstitial pneumonia it significantly increased by 2.6-fold.

Thus, interstitial pneumonia is associated with elevated levels of all studied matrix metalloproteinases as well as their inhibitor TIMP-1, indicating the extent of the pathological process.

The results of the pharmacological intervention on serum levels of matrix metalloproteinases and their inhibitor in experimental interstitial pneumonia are presented in Table 1.

Table 1.

Serum levels of matrix metalloproteinases (MMP-1, -7, -9) and their inhibitor TIMP-1 in interstitial pneumonia and after pharmacological correction

№	Group of animals	MMP-1 (ng/ml)	MMP-7 (ng/ml)	MMP-9 (ng/ml)	TIMP-1 (ng/ml)
1	I gr. Intact	8.5±0.27	42,2 ± 7,2 P<0,05	22,5±0.72	12,3±0.39
2	II gr. Control, pneumonia	19.4±0.62	84,4 ± 8,2 P<0,05	58.6±1.86	31.6±1.01
3	III gr. ecdysten + pneumonia	14.3±0.46	72,8 ± 6,3 P<0,05	47.2±1.50	24.2±0.77
4	IV gr. AZC + pneumonia	16.2±0.52	73,5 ± 7,4 P<0,05	51.5±1.64	27.5±0.88
5	V gr. polyoxidonium + pneumonia	12.2±0.39	62,5 ± 5,2 P<0,05	39.8±1.27	21.1±0.67
6	VI gr. ecdystene + Acetylcysteine + pneumonia	10.6±0.39	58,7 ± 6,3 P<0,05	32.3±1.03	18.5±0.59
7	VII gr. ecdystene+ polyoxidonium +pneumonia	9.7±0.31	56,5 ± 6,5 P<0,05	30.6±0.97	17.3±0.55
8	VIII gr. ecdystene+	8.9±0.28	52,5 ± 5,5 P<0,05	25.8±0.82	14.0±0.45

	acetylcysteine+ polyoxidonium +pneumonia				

Treatment with ecdisten led to a reduction in serum levels of MMP-1, MMP-7, and MMP-9 by 26.3%, 13.8%, and 19.5%, respectively, compared to the control group. The most pronounced decrease was observed in MMP-1 levels. Additionally, this group showed a 23.5% decrease in TIMP-1 concentration.

Treatment with N-acetylcysteine (ACC) resulted in less pronounced reductions in the studied metalloproteinases and TIMP-1 compared to the ecdisten-treated group, amounting to 16.5%, 13%, 12.2%, and 12.9%, respectively, relative to the control group.

Polyoxidonium demonstrated a more significant and statistically reliable reduction in MMP-1, MMP-7, MMP-9, and TIMP-1 levels than both ecdisten and ACC, achieving decreases of 37.2%, 26%, 32.1%, and 33.7%, respectively.

These findings indicate a moderate suppression of metalloproteinase activity and confirm the therapeutic potential of each agent in mitigating inflammatory-destructive processes in the lungs.

Combined therapy showed the most substantial reduction in matrix metalloproteinases and TIMP-1 levels, especially in Group VIII, where the concentrations of enzymes and their inhibitor approached values seen in intact animals. This supports the hypothesis of a synergistic effect of the drugs, aimed at suppressing inflammation, stabilizing the extracellular matrix, and protecting lung tissue architecture from degradation.

Interstitial pneumonia (IP) is a group of diseases characterized by inflammation and fibrosis of the lung parenchyma. One of the key features of these processes is the remodeling of the extracellular matrix (ECM), where matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) play a pivotal role.

It is the imbalance between MMPs and TIMPs that leads to tissue destruction, exacerbation of inflammation, and the development of fibrosis.

Despite existing studies, data on the effectiveness of modern pharmacological strategies aimed at correcting MMP and TIMP activity in interstitial pneumonia remain insufficient, which served as the rationale for this study.

In view of the above, the current study examined agents with known immunotropic and antioxidant properties—ecdisten, N-acetylcysteine, and polyoxidonium—as potential contributors to an expanded therapeutic approach for interstitial pneumonia.

The results of the analysis of matrix metalloproteinase and TIMP-1 levels in lung homogenates are presented in Figure 2.

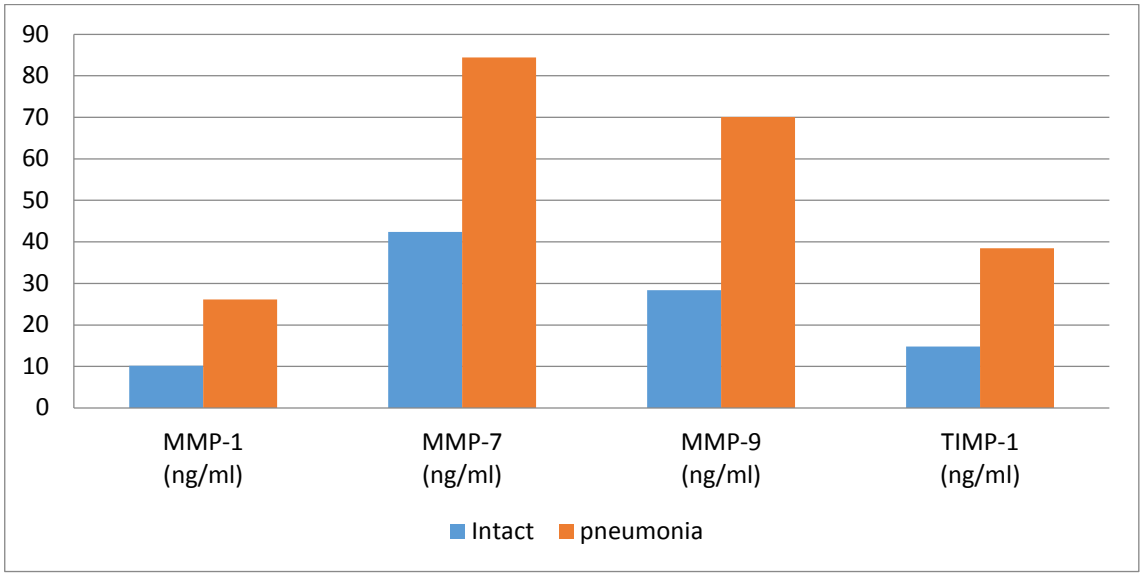

Figure 2. Levels of matrix metalloproteinases (MMP-1, -7, -9) and their inhibitor TIMP-1 in lung homogenate in interstitial pneumonia

As shown in Figure 2, the levels of MMP-1, MMP-7, and MMP-9 in lung homogenates were significantly elevated by 2.5-, 2-, and 2.5-fold, respectively, compared to the intact group. In contrast, the content of TIMP-1 in the lung homogenates of rats with interstitial pneumonia was reduced by 2.6-fold relative to intact animals. These findings are consistent with the serum data for MMP-1, MMP-7, MMP-9, and TIMP-1 in rats with interstitial pneumonia and reflect the extent of lung tissue destruction associated with this pathology.

The results of the pharmacological correction of matrix metalloproteinases and their inhibitor in lung homogenates under interstitial pneumonia are presented in Table 2.

Table 2.

Levels of matrix metalloproteinases (MMP-1, -7, -9) and their inhibitor TIMP-1 in lung homogenates in interstitial pneumonia and after pharmacological correction

№	Group of animals	MMP-1 (ng/ml)	MMP-7 (ng/ml)	MMP-9 (ng/ml)	TIMP-1 (ng/ml)
1	I gr. Intact	10.2±0.32	42,2 ± 7,2 P<0,05	28,4±0.90	14,8±0.47
2	II gr. Control, pneumonia	26.1±0.83	84,4 ± 8,2 P<0,05	70.1±2.23	38.5±1.23
3	III gr. ecdysten + pneumonia	20.5±0.65	72,8 ± 6,3 P<0,05	56.7±1.80	30.2±0.96
4	IV gr. AZC + pneumonia	23.0±0.65	73,5 ± 7,4 P<0,05	61.3±1.95	34.1±1.09
5	V gr. polyoxidonium + pneumonia	18.6±0.59	62,5 ± 5,2 P<0,05	49.6±1.58	27.4±0.87
6	VI gr. ecdystene + Acetylcysteine + pneumonia	15.7±0.50	58,7 ± 6,3 P<0,05	41.7±1.33	23.8±0.76
7	VII gr. ecdystene+ polyoxidonium +pneumonia	13.3±0.42	56,5 ± 6,5 P<0,05	39.2±1.25	21.6±0.69
8	VIII gr. ecdystene+	11.4±0.36	52,5 ± 5,5 P<0,05	32.8±1.04	17.2±0.55

	acetylcysteine+ polyoxidonium +pneumonia				

As shown in Table 2, treatment with ecdisten, ACC, and polyoxidonium reduced the activity of MMP-1, MMP-7, and MMP-9 in lung homogenates. Ecdisten reduced these enzymes by 21.5%, 13.8%, and 19.2%, respectively; ACC by 11.9%, 12.9%, and 12.6%; and polyoxidonium by 28.7%, 25.5%, and 29.3% compared to the control group. The TIMP-1 content in rats treated with ecdisten, ACC, and polyoxidonium was significantly decreased by 21.6%, 11.4%, and 71.2%, respectively.

Thus, the data indicate that among the monotherapy regimens for modulating MMP-1, -7, -9 and TIMP-1, polyoxidonium demonstrated the most pronounced therapeutic effect.

Combination therapies showed greater therapeutic efficacy in reducing matrix metalloproteinase levels and TIMP-1 content compared to monotherapy. The combination of ecdisten and ACC reduced the levels of MMP-1, -7, -9 and TIMP-1 by 39.85%, 30.45%, 59.5%, and 38.2%, respectively. Similarly, the combination of ecdisten and polyoxidonium resulted in reductions of 43.1%, 33.1%, 44.1%, and 43.9%, respectively.

The most significant effect was observed in Group VIII, where ecdisten, ACC, and polyoxidonium were administered together. In this group, the levels of MMP-1, -7, -9, and TIMP-1 were reduced by 56.3%, 37.8%, 53.2%, and 55.3%, respectively, compared to the control group. Notably, these levels approached those of the intact animals, confirming the synergistic effect of the combined therapy.

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