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Investigation of the Protective Effect of Betaine in an Experimental Pulmonary Fibrosis Model Induced by Intratracheal Administration of Bleomycin

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ABSTRACT

Objective: Pulmonary fibrosis is a progressive and fatal disease that is commonly seen in the general population, with an etiology and treatment methods that have not yet been fully elucidated. Intratracheal bleomycin (BLE) administration is one of the most common experimental models used to create pulmonary fibrosis. Betaine is a natural compound with proven antioxidant and anti-inflammatory properties. In the present study, the protective role of betaine against pulmonary fibrosis induced by intratracheal administration of bleomycin was investigated.

Materials and Methods: Four different groups were formed for the present study (n=7): Control, BLE, Betaine, and BLE+Betaine. BLE was given as a single dose on the first day of the experiment. The betaine application was given for 14 days, starting simultaneously with BLE, once a day. No application was made between days 14–21, and the experiment was completed on day 21. The lung tissues obtained were investigated using histopathological, immunohistochemical, and biochemical methods.

Results: BLE was found to trigger parenchymal fibrosis and the accompanying inflammatory response. Betaine tended to reduce these effects. Immunohistochemically evaluated α-SMA, MMP8, TGF-β, and IFN-ɣ increased due to BLE and tended to decrease in the BLE+Betaine group. There was no significant difference between the groups for IL-1β and IL-6. Galectin-3, hydroxyproline, and collagen type I, evaluated by ELISA, increased due to BLE and showed a decreasing trend in the BLE+Betaine group.

Conclusion: In conclusion, we believe that betaine tends to reduce the progression of bleomycin-induced fibrosis and that research should be continued to support current treatment strategies.

Keywords: Betaine, bleomycin, inflammatory markers, lung, pulmonary fibrosis.

INTRODUCTION

Idiopathic pulmonary fibrosis is a chronic, progressive, and life-threatening interstitial lung disease with an unknown cause.[1] It occurs with a frequency of over 50 per 100,000 people, and the average survival rate after diagnosis is 2–5 years.[2] The disease is characterized by abnormal lung structure and fibrosis due to excessive extracellular matrix accumulation, ultimately resulting in respiratory failure.[3]

Bleomycin (BLE) is an antibiotic with anticancer properties produced by Streptomyces verticillus.[4] BLE is used in the management of Hodgkin and non-Hodgkin lymphomas, testicular tumors, and ovarian and cervical cancers.[5]

BLE has some side effects in addition to its chemotherapeutic activity. Systemic administration of BLE, which has various administration routes, causes damage to endothelial cells, while intratracheal administration causes serious damage to parenchymal cells.[6] In the pneumonia caused by BLE, infiltration of inflammatory cells such as neutrophils, macrophages, and lymphocytes is observed. These inflammatory cells secrete inflammatory cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interferon gamma (IFN-ɣ).[7] Inflammatory cells also produce high levels of reactive oxygen species (ROS) and nitric oxide.[8] Subsequently, the existing pneumonia may progress toward pulmonary fibrosis. Fibrosis occurs due to the dense accumulation of collagen, fibrin, and fibronectin in the extracellular matrix, resulting in tissue hardening.[9] Chronic inflammation appears to play an important role in initiating the fibrosis process.[10]

Current treatments used to reduce bleomycin (BLE)-induced pulmonary fibrosis include anti-fibrotic and anti-inflammatory agents such as corticosteroids, pirfenidone, and nintedanib.[11] However, current treatments are still inadequate in both advancing the process and resolving the fibrosis that occurs. For this purpose, the effect of compounds with antioxidant properties, such as N-acetylcysteine, was investigated to protect alveolar epithelial cells from damage caused by BLE-induced ROS production.[12]

Betaine is a naturally occurring, non-toxic substance found in a variety of dietary sources.[13] It is also known that it can be synthesized endogenously in the body through choline metabolism.[14] Betaine plays an important role in transmethylation as a methyl group donor in the process of converting homocysteine to methionine.[15] Hyperhomocysteinemia is known to induce oxidative stress.[16] Betaine plays an important role in antioxidation by converting homocysteine to methionine.[17] To our knowledge, the present study is a pioneering study investigating the protective role of betaine on BLE-induced fibrosis.

KEY MESSAGES

- Peribronchial fibrosis occurred due to intratracheal bleomycin administration.
- An increase in cytokines such as α-SMA, MMP-8, TGF-β, IFN-γ, and galectin-3 was observed in response to bleomycin.
- Betaine partially prevented the progression to bleomycin-induced fibrosis.

In this context, in the present study, the effect of betaine on inflammatory and fibrosis-related processes against lung injury induced by intratracheally administered BLE was investigated using histopathological, immunohistochemical, and ELISA methods.

MATERIALS AND METHODS

Ethics and Subjects

Ethics committee approval for this study was obtained from the Erciyes University Local Ethics Committee for Animal Experiments on 06.03.2024, with the approval number 24/054. This study is a prospective *in vivo* experimental animal model conducted on rats over a period of 21 days. For the experiment, 28 adult female Wistar albino rats were used, and their weights ranged between 200 and 250 grams. All subjects were provided with access to food and water ad libitum under appropriate laboratory conditions (22±2 °C, 12 hours of light/darkness) throughout the experiment.

Drugs and Groups

Treatment doses for the groups to be given BLE were prepared using the commercial drug Bleomedac® (15 mg, Medac brand), containing bleomycin sulfate as the active ingredient. The active ingredient in powder form was dissolved in physiological serum and administered intratracheally at 5 mg/kg.[18] The anesthetized rats were placed on their backs on a flat surface and suspended from their front two teeth with the help of a rope, thus extending the head. The surface on which the rat was laid was made vertical at a 45-degree angle. Thus, the air route was brought to a more accessible position. The needle part of a sterile intravenous catheter was removed, and its plastic tip was advanced toward the trachea. To ensure access to the airway, the movement of a hair brought close to the outer end of the catheter was observed. BLE was administered at the calculated dose via an insulin syringe attached to the catheter (Fig. 1). Betaine (250 mg/kg)[19] was dissolved in physiological saline and administered orally using a gavage.

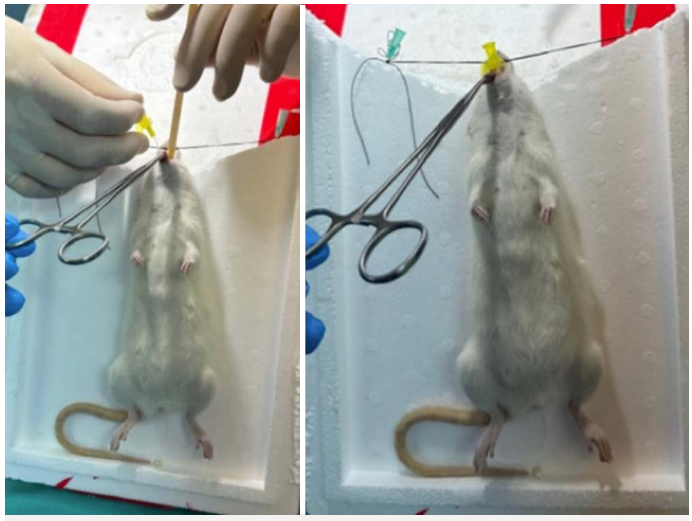

Figure 1. Creation of the experimental model. These photos were taken with a camera—endotracheal advancement of the plastic part of the vascular access catheter for BLE application.

The groups were formed by randomly dividing a total of twenty-eight rats into four groups (n=7): Control, BLE, Betaine, and BLE+Betaine. BLE was administered as a single dose on the first day of the experiment. Betaine administration was given once a day for 14 days, starting simultaneously with BLE. No application was made between days 14 and 21, and the experiment was completed on the 21st day. To complete the experiment, the rats were anesthetized, cervical dislocation was performed, and the lung tissues were quickly removed.

Histopathology

For histological evaluations, lung tissues taken from the subjects were quickly placed in 10% formaldehyde solution and kept in the solution for 72 hours for fixation. After fixation, the tissues were first washed in running tap water. Then, they were passed through a series of alcohols of increasing grades. After clearing with xylene, paraffin blocks were prepared by embedding in molten paraffin. 5 µm sections taken from paraffin blocks were placed on poly-L-lysine-coated slides. Hematoxylin and eosin (H&E) staining was performed for general histopathological evaluation, and Masson's trichrome (MT) staining was performed to evaluate the connective tissue.

The Ashcroft scale assesses fibrosis and is the most widely used lung injury scale in animals; it consists of a 9-step scoring system where 0 corresponds to normal lung and 8 corresponds to complete fibrotic obliteration of the lung. The Ashcroft scale, a semi-quantitative method, was used for histopathological analysis in MT-stained preparations.[20] According to this method, 5 different areas for each rat were scored according to the Ashcroft scale.

Immunohistochemical Analysis

For more detailed analyses of inflammation and fibrosis, staining was also performed with immunohistochemical methods. Primary antibodies used for immunohistochemistry were as follows: α-Smooth Muscle Actin (α-SMA) (1:200 dilution, bsm-33188M, Bioss), Matrix metalloproteinase 8 (MMP8) (1:500 dilution, Thermo Fisher Scientific), Transforming Growth Factor Beta (TGF-β) (1:200 dilution, bs-0086R, Bioss), Interferon gamma (IFN-γ) (1:400 dilution, bs-0480R, Bioss), Interleukin-1β (IL-1β) (1:200 dilution, bs-0812R, Bioss), and Interleukin-6 (IL-6) antibody (1:100 dilution, DF6087, Affinity Biosciences). For immunohistochemical staining, the streptavidin-biotin-peroxidase method was used. The Ultravision Polyvalent Horseradish Peroxidase Kit (125 ml, Thermo Fisher Scientific, USA) was preferred for this process. An Olympus BX51 microscope (Olympus, Tokyo, Japan) was used for photographs, and five different areas were randomly selected and photographed from each subject. ImageJ Software (version 1.45s) was used to evaluate immunoreactivity intensities, and the obtained intensity measurements were recorded.[21]

Enzyme-linked Immunosorbent Assay

One of the lung lobes was fixed in formaldehyde for histological processing. The other lobe was directly frozen and stored at -80°C for biochemical analyses. On the day of biochemical analysis, frozen lung tissues were removed from -80°C and allowed to thaw at +4°C. A 100 mg tissue piece was cut from each sample, weighed, and placed in Eppendorf tubes containing 1 mL of 0.015 M, pH 7.4 phosphate-buffered saline (PBS). For each sample, the homogenization process was carried out at 1000 rpm for 1 minute in containers filled with ice.

The obtained homogenates were centrifuged at 5000 rpm for 20 minutes at +4°C, and the supernatants were collected. Rat Galectin-3 (DZE201110054, Sun Red Biological Technology, China), Rat Hydroxyproline (DZE201110512, Sun Red Biological Technology, China), and Rat Collagen Type I (DZE201110009, Sun Red Biological Technology, China) levels were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's protocol. The sensitivity for collagen I was 5.127 ng/L, with a measurement range of 8–2000 ng/L; for galectin-3, the sensitivity was 0.07 ng/mL, with a measurement range of 0.1–20 ng/mL; and for hydroxyproline, the sensitivity was 25.152 ng/mL, with a measurement range of 30–9000 ng/mL. Correlation coefficients (R^2) of all calibration curves were determined to be >0.99.

Galectin-3 and Hydroxyproline levels were measured in ng/mL, while Collagen Type I levels were measured in ng/L. The measurement results were normalized by proportioning each sample to its weight value (mg). The results were expressed as ng/g for Galectin-3 and Collagen Type I levels,

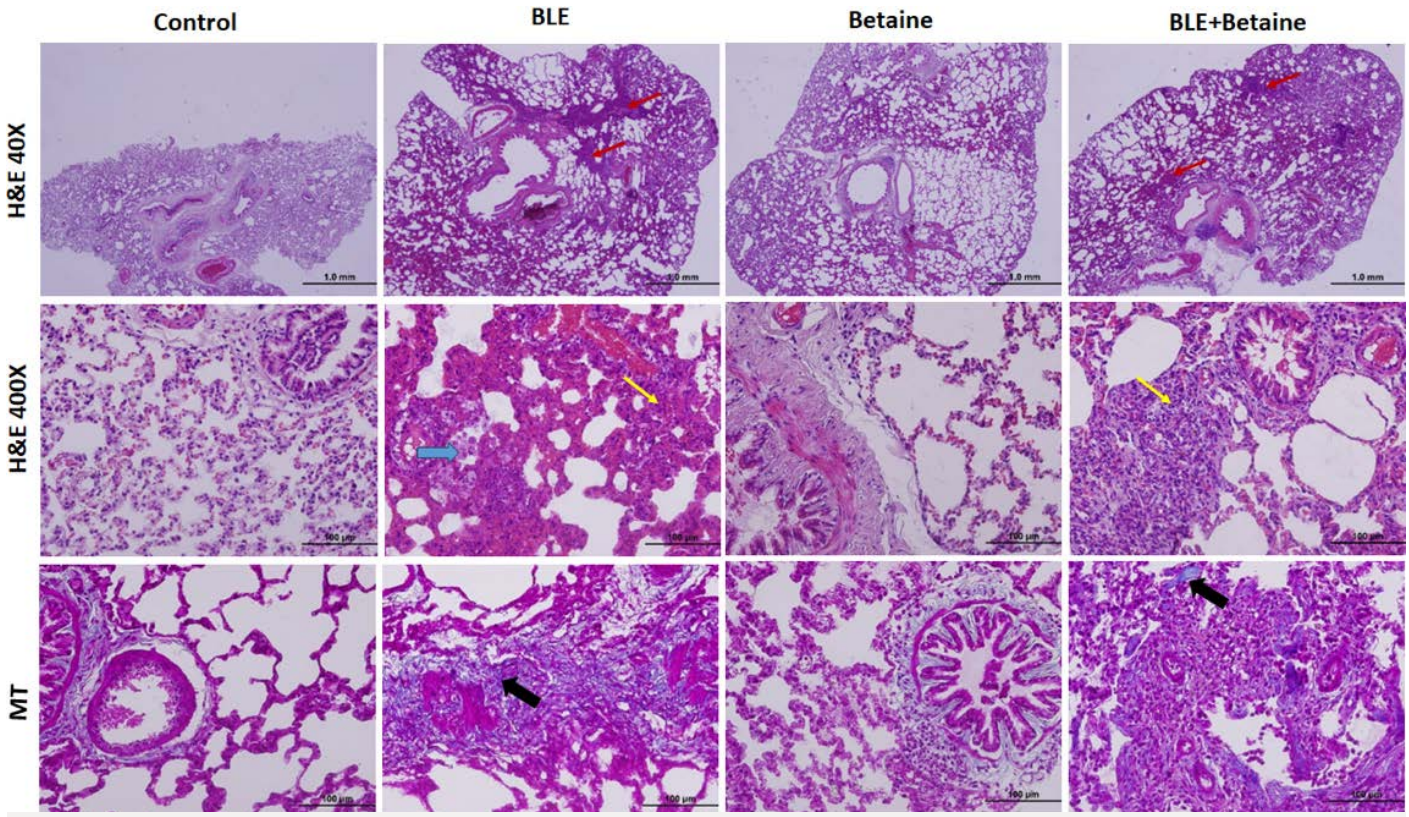

Figure 2. Photomicrographs of sections were stained with H&E and MT in the lung tissue in all groups. Pathological changes in lung tissue were assessed using H&E staining and collagen deposition, and fibrotic areas in lung tissue were determined by MT staining. Red arrow: fibrotic and remodeled areas, yellow arrow: inflammatory cell infiltration, blue arrow: alveolar macrophage, black arrow: collagen deposition.

BLE: Bleomycin; H&E: Hematoxylin and eosin; MT; Masson's Trikrom (Original magnification= 40x; scale bar=1.0 µm) (Original magnification=400x; scale bar=100 µm).

and ng/mg for Hydroxyproline levels, with the number of decimal places minimized.

Statistical Analysis

GraphPad Prism version 10 (GraphPad Software, La Jolla, CA) was used to perform statistical analyses. The D'Agostino-Pearson test was used to assess whether the data were normally distributed. One-way analysis of variance (ANOVA) was performed to compare normally distributed data. Tukey's post hoc test was then used. Results were presented as mean±standard deviation. A p-value of less than 0.05 was accepted for statistical significance.

RESULTS

Histopathological Findings

On day 21 following BLE administration, the lungs of the rats were histopathologically analyzed in terms of fibrosis and collagen content. Lung tissues of rats in the Control and Betaine groups had completely normal architecture. The alveolar septum and alveolar spaces were normal in structure, and no evidence of inflammatory cell infiltration or pulmonary fibrosis was observed (Fig. 2). In the BLE group, lung tissues were characterized by the fusion and destruction of alveolar structures, thickening of the alveolar septum, inflammatory cell infiltration within the tissue, especially in the peribronchial areas, and accumulation of alveolar macrophages (Fig. 2). The BLE group also exhibited increased collagen deposition, especially in the parenchyma of the peribronchial areas, and interstitial fibrosis formation with a remodeling process that disrupted the alveolar architecture (Fig. 2). Although pathological findings tended to decrease in the BLE + Betaine group compared to the BLE group, infiltrating inflammatory cells and alveolar macrophages were found in the parenchyma. Again, findings of destruction and fusion of alveolar structures, thickening of the interalveolar septum, and interstitial fibrosis continued to exist, although they tended to decrease (Fig. 2).

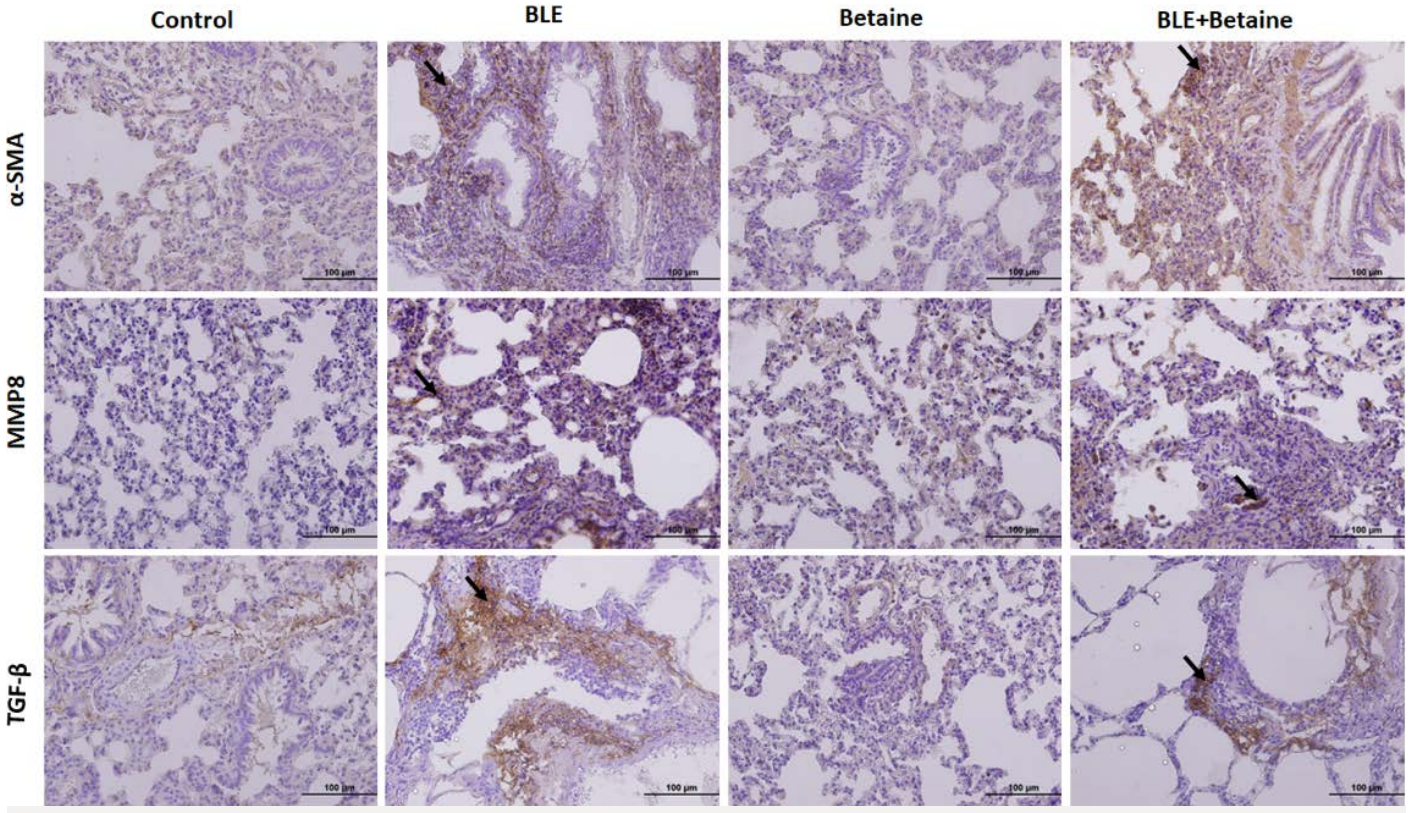

Figure 3. Photomicrographs of sections were stained with α-SMA, MMP8, and TGF-β antibodies in the lung tissue in all groups. Cells showing positive immunoreactivity were stained brown; black arrows indicate some.

BLE: Bleomycin; α-SMA: α-Smooth Muscle Actin; MMP8: Matrix metalloproteinase 8; TGF-β: Transforming Growth Factor Beta (Original magnification=400x; scale bar=100 μm).

Table 1. Statistical evaluation of the Ashcroft scale

	Control (n=7)	BLE (n=7)	Betaine (n=7)	BLE+Betaine (n=7)	p
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Ashcroft scale	0.46±0.59[a]	5.63±1.06[b]	0.53±0.59[a]	5.41±0.77[b]	0.0001

Data are expressed as mean±standard deviation. The letters (a, b) indicate the statistical differences between groups. P<0.05 was considered significant. BLE: Bleomycin; n: Number of subjects in each group.

MT staining was performed to observe collagen fiber accumulation. In the BLE group, normal alveolar structures were lost, and lung tissue remodeling was observed. Collagen fibers were abundant and accumulated in the blue remodeled lung tissue. The increasing degree of fibrosis was visible with distinct pathological changes (Fig. 2). Compared to the BLE group, the BLE + Betaine group showed a modest decrease in the destruction of alveolar structures and tissue fibrosis, and the accumulation of blue-stained collagen fibers continued to exist (Fig. 2). According to the statistical analysis of the Ashcroft scale, there was no significant difference between the Control and Betaine groups, while the BLE group was significantly higher compared to the Control group. Although the BLE+Betaine group showed a partial decrease compared to the BLE group, there was no significant difference (Table 1, Fig. 2).

Immunohistochemical Findings

The measurement results obtained from photographs of lung tissues stained with primary antibodies against α-SMA, MMP8, TGF-β, IFN-ɣ, IL-1β, and IL-6 were evaluated by statistical analysis, and the results are presented in Table 2. In general, we can say that immunoreactive areas, colored with DAB chromogen, are stained more intensely in areas that have undergone remodeling. However, when we evaluated

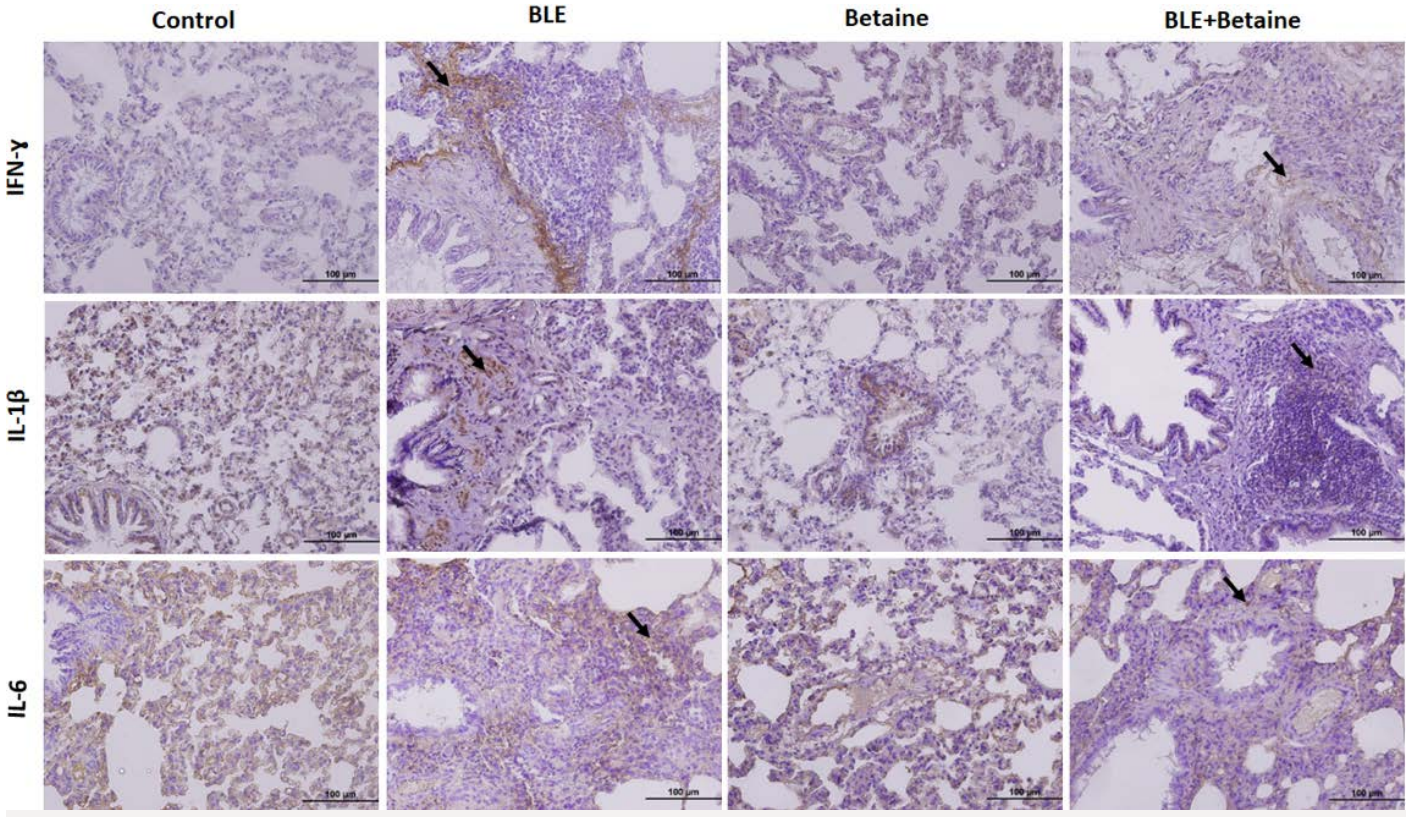

Figure 4. Photomicrographs of sections were stained with α-SMA, MMP8, and TGF-β antibodies in the lung tissue in all groups. Cells showing positive immunoreactivity were stained brown; black arrows indicate some.

BLE: Bleomycin; IFN-ɣ: Interferon gamma; IL-1β: Interleukin-1β; IL-6: Interleukin 6 (Original magnification=400x; scale bar=100 µm).

Table 2. Statistical results evaluating immunoreactivity intensities in the lung

	Control (n=7)	BLE (n=7)	Betaine (n=7)	BLE+Betaine (n=7)	p
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
α-SMA	76.11±2.53^{a}	78.65±2.87^{b}	76.58±3.06^{a}	77.28±2.59^{ab}	0.0001
MMP8	82.58±4.79^{ac}	85.72±4.89^{b}	80.88±5.11^{c}	84.28±4.75^{ab}	0.0001
TGF-β	78.18±4.42^{a}	80.64±5.05^{b}	78.71±4.12a^{b}	79.68±3.63^{ab}	0.0160
IFN-ɣ	80.21±3.03^{a}	82.59±4.26^{b}	79.09±3.88^{a}	79.62±4.03^{a}	0.0001
IL-1β	80.45±4.16^{a}	82.20±4.88^{a}	81.82±4.89^{a}	81.60±3.74^{a}	0.1641
IL-6	80.08±3.9^{a}	81.82±4.12^{a}	80.41±3.50^{a}	80.11±5.11^{a}	0.0852

Data are expressed as mean±standard deviation. The letters (a, b, c) indicate the statistical differences between groups. P<0.05 was considered significant. BLE: Bleomycin; α-SMA; α-Smooth Muscle Actin, MMP8: Matrix metalloproteinase 8; TGF-β: Transforming Growth Factor Beta; IFN-ɣ: Interferon gamma; IL-1β: Interleukin-1β; IL-6: Interleukin 6; n: Number of subjects in each group.

the results one by one, there was no significant difference between the Control and Betaine groups in terms of α-SMA and TGF-β immunoreactivity. The BLE group was significantly higher compared to the Control and Betaine groups. Although the density in the BLE+Betaine group tended to decrease compared to the BLE group, this decrease was not statistically significant (Fig. 3). MMP8 was significantly higher in the BLE group compared to the Control and Betaine groups. However, although the density in the BLE+Betaine group tended to decrease compared to the BLE group, this decrease was not statistically significant. The BLE+Betaine group was significantly higher than the Control and Betaine groups (Fig. 3). When we

Table 3. ELISA results of Galectin-3, Hydroxyproline, Collagen Type I levels are given in lung tissue samples

	Control (n=7)	BLE (n=7)	Betaine (n=7)	BLE+Betaine (n=7)	p
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Galectin-3 (ng/g)	18.97±4.22[a]	34.03±8.03[b]	23.40±4.78[ab]	29.18±15.89[ab]	0.0342
Hydroxyproline (ng/mg)	25.55±8.22[a]	33.09±8.56[a]	25.98±6.95[a]	30.57±17.40[a]	0.5277
Collagen type I (ng/g)	2.38±1.62[a]	4.73±0.96[a]	3.91±0.64[a]	4.13±2.53[a]	0.0763

Data are expressed as mean±standard deviation. The letters (a, b) indicate the statistical differences between groups. P<0.05 was considered significant. BLE: Bleomycin; n: Number of subjects in each group.

evaluated IFN-ɣ, we found that it was significantly higher in the BLE group compared to all other groups. IL-1β and IL-6 had the highest value in the BLE group, but there was no statistically significant difference between the groups (Fig. 4).

ELISA

Galectin-3, one of the ELISA markers we used to evaluate fibrosis in the parenchyma, was at the highest level in the BLE group, and the difference with the Control group was statistically significant. When we evaluated Hydroxyproline and Collagen Type I, the highest values were in the BLE group, but there was no significant difference between the groups (Table 3).

DISCUSSION

Pulmonary fibrosis is a serious and irreversible consequence of various lung diseases. Consequently, the need for antifibrotic strategies and treatments has become crucial.[22] Pirfenidone and nintedanib are FDA-approved drugs for the treatment of lung fibrosis,[23] but they still do not provide adequate treatment outcomes.

In this study, it was observed that BLE-induced pulmonary fibrosis could be partially prevented by betaine, which has been proven to have antioxidant and anti-inflammatory effects in previous studies.[24] However, this effect did not reach statistical significance according to histopathological, immunohistochemical, and ELISA evaluations. In our histopathological evaluation, fibrotic foci were primarily observed in the peribronchial areas, as BLE was administered intratracheally. The presence of alveolar macrophages and intense inflammatory cell infiltration in these fibrotic foci and parenchyma was notable. According to the Ashcroft scale, interstitial fibrosis was observed due to BLE. Although betaine partially reduced these findings, progression to fibrosis could not be completely prevented. Future studies could investigate the effect of betaine when administered before BLE exposure or explore different doses.

Immunohistochemical markers related to fibrosis, such as α-SMA, MMP8, and TGF-β, were induced by BLE and expressed at high levels, particularly in fibrotic areas. Although the expression of these markers was slightly reduced by betaine, the difference was not statistically significant. The experiment was terminated 21 days after BLE application, and it is known that the inflammatory phase decreases while the fibrotic phase begins during this period.[25] This may explain why some inflammatory cytokines, like IL-1β and IL-6, did not show significant differences between the groups. IFN-ɣ, however, showed a significant increase in the BLE group compared to the other groups.

ELISA measurements of Galectin-3, Hydroxyproline, and Collagen Type I in the parenchyma revealed higher levels in the BLE group, but only Galectin-3 showed a statistically significant difference. We believe this result may be influenced by the limited sample size.

BLE is a chemotherapy drug with serious toxic effects, such as inflammation and pulmonary fibrosis, particularly due to the lack of bleomycin hydrolase enzyme in the lungs.[26] This is a concern for patients receiving BLE treatment. In experimental animal models, agents such as BLE, silica, and radiation are commonly used to induce pulmonary fibrosis. However, the BLE model is the most widely used and best-characterized model for studying fibrosis.[27]

In the lung injury caused by BLE administration, inflammation precedes fibrosis.[28] The molecular mechanisms of this process involve excessive reactive oxygen species (ROS) production in lung cells, especially from alveolar epithelial cells and macrophages.[29] This may lead to mitochondrial DNA damage and apoptosis of alveolar epithelial cells, contributing to pulmonary fibrosis.[30] Another mechanism involves epithelial-mesenchymal transition and fibroblast-myofibroblast differentiation due to damage to alveolar epithelial cells and disruptions in tissue repair.[31]

Intratracheal administration of BLE can be performed using various methods. In the conventional surgical method, the trachea is exposed through an incision, and BLE is directly injected into the trachea.[32] Alternatively, transoral instillation is a non-invasive method where BLE is applied at the vocal

cord level using a micropipette.[32] However, both methods have limitations—surgical procedures are invasive, while the transoral method may result in drug loss. The method used in the present study is advantageous because it is non-invasive, easy to apply, and effective. In this method, rats under anesthesia were secured by a thread tied to their upper incisors, with the surface inclined at a 45-degree angle. The vascular access catheter was then inserted into the trachea, ensuring proper placement by observing the movement of a hair placed near the catheter's outer end.

The development and progression of BLE-induced lung injury occur in three phases. The inflammatory phase, which lasts from 1 to 7 days post-application, is characterized by inflammatory cell infiltration, increased vascular permeability, and elevated proinflammatory cytokines and chemokines due to epithelial damage.[33] Between 7 and 14 days, there is a transition from inflammation to active fibrosis, with fibroproliferation increasing and myofibroblast emergence beginning.[34]. After 14 days, the chronic fibrosis phase begins, and fibrosis in the intraalveolar and septal regions becomes evident.[33] In the current study, tissue examination was performed at the 21-day mark, which is at the start of the chronic fibrosis phase. This timing may explain the weak inflammatory response observed. Since the aim of this study was to investigate whether fibrosis progression could be prevented, the fibrosis peak period was selected for evaluation.

Collagen accumulation in lung tissue peaks 3-4 weeks after BLE application,[28] and total collagen and hydroxyproline analysis are recommended during this period to assess fibrosis.[28] Galectin-3 is a β-galactoside-binding lectin that is highly expressed in fibrotic tissues due to various etiologies. Inhibition of Galectin-3 is being explored as a new strategy for treating lung fibrosis.[35] MMPs, particularly MMP-8, play a role in promoting pulmonary fibrosis by increasing the activity of profibrotic mediators or reducing the levels of antifibrotic mediators.[36] The expression of most MMPs is regulated by cytokines such as TGF-β, TNF-α, and IL-1β.[36]

The inflammatory exudate after intratracheal BLE application consists mainly of mononuclear macrophages, lymphocytes, and neutrophils.[37] In this study, foci with intense inflammatory cell infiltration were observed in the parenchyma, and infiltration slightly decreased with betaine treatment. Following the BLE application, an increase in proinflammatory cytokines such as IL-1β, IL-16, and IFN-ɣ occurs, followed by an increase in profibrotic markers like TGF-β.[34] Fibroblasts are activated early in the BLE-induced lung injury process through cytokines like TGF-β.[38] α-SMA expression occurs in fibroblasts that differentiate into myofibroblasts.[39] Additionally, alveolar macrophages activated by BLE stimulate the synthesis of hyaluronan in fibrotic lungs.[40] The presence of alveolar macrophages in fibrotic foci in our study supports this mechanism.

In summary, BLE-induced fibrosis was assessed using histopathological, immunohistochemical, and biochemical methods. Although betaine showed a tendency to reduce fibrosis progression, its effect was not statistically significant within the timeframe of this study. Future studies exploring the timing of betaine administration and using larger sample sizes could provide more insight into its potential therapeutic benefits in BLE-induced pulmonary fibrosis.

CONCLUSION

The present study investigated the protective role of betaine against pulmonary fibrosis induced by intratracheally administered BLE, utilizing histopathological, immunohistochemical, and biochemical methods. Histopathological examination revealed that BLE caused inflammatory cell infiltration in the parenchyma and the formation of fibrotic foci. Histological scoring indicated that betaine was beneficial in reducing these pathological changes. Immunohistochemical analysis showed that certain inflammatory cytokines and fibrosis-related markers increased in response to BLE, whereas betaine tended to reduce these levels. Biochemical evaluation of fibrosis accumulation in the parenchyma demonstrated that BLE caused an increase in Galectin-3 levels, which was reduced by betaine.

Our findings suggest that betaine may help reduce the progression to fibrosis, but does not fully prevent it. To further support existing clinically approved treatments, we recommend that future studies focus on modifying the timing and duration of betaine administration for potential therapeutic benefits.

Ethics Committee Approval: The Erciyes University Clinical Research Ethics Committee granted approval for this study (date: 06.03.2024, number: 24/054).

Conflict of Interest: The authors have no conflict of interest to declare.

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Author Contributions: Concept – MÜ, BÜ, OT, ITT; Design – MÜ, BÜ, OT, HS; Supervision – MÜ, OT, SNK, ME; Resource – MÜ, ME, ITT; Materials – ME, SNL, MÜ; Data Collection and/or Processing – MÜ, ME, ITT, SNK; Analysis and/or Interpretation – MÜ, BÜ, HS; Literature Search – MÜ; Writing – MÜ, HS; Critical Reviews – MÜ, OT, BÜ.

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