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Original Scientific Paper

THE EFFECT OF ARTESUNATE ON BLEOMYCIN--INDUCED PULMONARY FIBROSIS

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SUMMARY — Despite new drug alternatives, no curative treatment for idiopathic pulmonary fibrosis (IPF) currently exists. This study aimed to evaluate the effects of artesunate on fibrosis, pulmonary hypertension, and inflammation-related changes in IPF. We divided a total of 32 male Wistar albino rats into four groups: a control group (group A, n=7), a group receiving artesunate (group B, n=7), a group receiving bleomycin (group C, n=9), and a group receiving both bleomycin and artesunate (group D, n=9). Groups A and B received intratracheal saline (0.1 mL), and groups C and D received intratracheal bleomycin (2.5 mg/kg). Groups A and C received intraperitoneal (i.p.) saline (0.1 mL/day), and groups B and D received i.p. artesunate (30 mg/kg/day) for 21 days. We measured the rats' exercise capacity by using a treadmill. We also examined heart and pulmonary tissues for right ventricular hypertrophy (RVH) and pulmonary arteriolar wall thickness for fibrosis, respectively. Finally, for immunohistochemistry, we performed Masson's trichrome stain and a macrophage marker antibody. The rats' measured exercise capacity was 1665 ± 145 m in the control group, 1142 ± 280 m in the group receiving bleomycin, 1490 ± 185 m in the group receiving artesunate, and 1207 ± 231 m in the group receiving both bleomycin and artesunate. The intergroup difference was statistically significant (p=0.001), but the difference between the bleomycin + artesunate and bleomycin-only groups was not statistically significant (p=0.95). RVH was common in the bleomycin group (0.44 ± 0.02). The difference between the bleomycin + artesunate

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and bleomycin groups was significant (0.37 ± 0.03). The medial wall of the pulmonary arterioles was thicker in bleomycin recipients than in artesunate recipients and controls, whereas it was thinner in bleomycin + artesunate recipients ($p<0.001$, $p=0.026$, respectively). Fibrosis and inflammatory changes improved in the bleomycin + artesunate group ($p<0.001$). The authors conclude that artesunate improved fibrosis, inflammatory changes, medial layer thickness of the pulmonary arterioles, and RVH in rats with bleomycin-induced pulmonary fibrosis.

Keywords: *Idiopathic pulmonary fibrosis*; *Bleomycin*; *Artesunate*; *Right ventricular hypertrophy*

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive fibrotic lung disease of unknown etiology. The estimated prevalence of the disease is 2-29/100,000, and its incidence increases with age[1].

The most frequently encountered symptoms and findings in patients with IPF are shortness of breath, dry cough, bibasilar inspiratory rales, and clubbed fingers. The disease typically manifests in the sixth and seventh decades of life, and it rarely occurs in patients younger than 50 years of age. Dyspnea, the most common symptom causing loss of function, is usually progressive and has been observed in most patients 6 months prior to diagnosis[1].

The clinical course of IPF is highly variable. Some patients exhibit rapid deterioration, whereas others may exhibit a slower course. In some patients, acute attacks may develop despite a stable course. The mortality rate of IPF is very high: only 20% to 30% of patients survive for 5 years after diagnosis, with an average survival of between 2 and 5 years. Research reports the mortality rate as 13.36/100,000[1,2].

Pulmonary hypertension (PH) is a progressive pathology involving the small vessels of the pulmonary vascular bed. PH is closely associated with a poor prognosis and high mortality in IPF[3]. Researchers have reported that the overall prevalence of PH in patients with IPF ranges from 36% to 86%. The development of PH in IPF is associated with hypoxemic vasoconstriction and destruction resulting from progressive fibrosis of the vascular bed[4].

In recent years, two new antifibrotic drugs, pirfenidone and nintedanib, have been used to treat IPF and inhibit the progression of fibrosis in the lung parenchyma[5,6]. These two drugs offer a new treatment method, delay disease progression, and partially extend survival time by slowing functional loss[7]. Despite these new developments, curative options remain unavailable, and the search for new treatment alternatives for IPF is ongoing.

Artesunate is a semi-synthetic derivative of artemisinin used in cases of malaria that are moderately resistant or severe. Recent studies have demonstrated its positive anticancer, antiarrhythmic, antiallergic, and antiviral effects[8-11].

Preclinical studies have examined the effects of artesunate on fibrosis. In some cell cultures and animal studies, artesunate has been reported to have beneficial effects on hepatic, renal, and pulmonary fibroses[12-16].

The objective of this study was to evaluate the effects of the antimalarial drug artesunate on fibrosis, exercise capacity, PH, and inflammatory changes in a rat model of bleomycin-induced pulmonary fibrosis.

Materials and Methods

Animals and experimental design

This study utilized 32 healthy, male, 10-week-old Wistar albino rats. The Kocaeli University (Kocaeli, Turkey) Animal Reproduction Center provided all the experimental animals, which were housed in the Experimental Animal Laboratory of Kocaeli University. The rats were housed in cages under controlled temperature and humidity conditions with a 12/12-hour light/dark cycle. The rats were able to feed *ad libitum*. Before initiating the experiment, all rats were acclimated to the environment for 2 weeks. This study conformed to all aspects of the 1996 National Academy of Sciences

Guide for the Care and Use of Laboratory Animals. The Kocaeli University Committee on the Use and Care of Animals approved the experiments (KOU HADYEK 4/2-2017).

The rats were divided into four groups: a control group (group A, n=7), a group receiving artesunate (group B, n=7), a group receiving bleomycin (group C, n=9), and a group receiving both bleomycin and artesunate (group D, n=9). Intratracheal saline (0.1 mL) was administered to groups A and B, and intratracheal bleomycin (2.5 mg/kg) to groups C and D. Then, intraperitoneal (i.p.) saline (0.1 mL/day i.p.) was administered to groups A and C, and artesunate (30 mg/kg/day, i.p.) was administered to groups B and D for 21 days. On day 21, exercise capacity was measured using a treadmill. The subjects were sacrificed under anesthesia, and the lungs and hearts were excised.

Drug application

Bleomycin (15 mg/1 vial; Onko Pharmaceuticals, Istanbul, Turkey) was used for bleomycin delivery, and Falcigo (60 mg/1 vial; Zydus Cadila Healthcare Ltd, Ahmedabad, India) for artesunate delivery.

Anesthesia was achieved with an i.p. dose of 90 mg/kg ketamine hydrochloride (Ketalar; Pfizer, Inc., New York, USA) and 12 mg/kg xylazine hydrochloride (Rompun; Bayer AG, Leverkusen, Germany).

Intratracheal bleomycin was administered to groups C and D after shaving the midline neck hair, and the trachea was exposed by cutting the skin and subcutaneous layer on the midline. Saline (0.1 mL) or bleomycin (2.5 mg/kg) was administered into the trachea using a 24-gauge syringe. The skin was then closed with surgical sutures[17].

Artesunate (30 mg/kg/day, i.p.) was administered to groups B and D, and saline (0.1 mL) was administered to groups A and C for 21 days, beginning on the day of the procedure.

Exercise test

For two days prior to testing, the rats were placed on a treadmill for 10 min/day at a speed of 10 m/min to adapt to the device. To measure exercise capacity, an exercise test was performed on the 21st day of the study to determine the distance covered by the rats on the treadmill[16]. On the day of the test, the acute exhaustive exercise model was applied, and the rats were placed on the treadmill at a speed of 20 m/min until they demonstrated exhaustion.

Electric stimulation (20-40 volts) was applied with a stimulator to induce the rats to continue running[18]. Exhaustion was determined when the rats were so tired or immobilized that they did not demonstrate any activity in response to physical or electrical stimuli. The treadmill device was stopped, and the test was terminated.

Assessment of right ventricular hypertrophy

Heart tissue was assessed using Fulton's index for right ventricular hypertrophy. The atria were carefully separated from the ventricles. The free wall of the right ventricle was dissected from the interventricular septum. The right ventricle, left ventricle, and the interventricular septum were weighed. The Fulton index value was calculated as the weight ratio of the right ventricle to the sum of the left ventricle and septum (RV/LV + S)[19].

Histopathological examination of the lung tissue

Lung tissues were fixed with 4% paraformaldehyde. Following fixation, a routine series of paraffin tissue tests was performed. Five-µm sections were obtained from the paraffin blocks. Immunohistochemical staining was performed using Masson's trichrome stain and the macrophage marker antibody MAC387 (1:200 dilution, sc-66204; Santa Cruz Biotechnology, Inc., Dallas, Texas, USA).

The ImmunoCruz mouse ABC Staining System (sc-2017; Santa Cruz Biotechnology, Inc., Dallas, Texas, USA) was used for immunohistochemical staining. Tissue sections were immersed in xylene to remove paraffin and then passed through an alcohol series. Next, they were treated with 0.3% hydrogen peroxide to inhibit endogenous peroxidase activity. The sections were placed in 10% normal serum and then incubated overnight at 4°C. The next step was incubation with biotinylated anti-rabbit immunoglobulin G and avidin D horseradish peroxidase for 30 min at room temperature. The sections were then treated with 3,3'-diaminobenzidine chromogen and covered with a coating medium. Negative control sections were subjected to the aforementioned procedures without the application of the primary antibody.

The sections were stained and then recorded in the tagged image file format using a Leica DM2500

microscope and Leica DFC295 HD camera (Leica Microsystems GmbH, Wetzlar, Germany). The photographs were analyzed using pulmonary arteriolar morphometry to determine the extent of fibrosis, and inflammation was examined using the ImageJ analysis program and MAC387 staining intensity.

Twelve vessels with diameters ranging from 30 to 100 μm were retrieved from the right lung tissue of the animals in each group (n=3). The formula for the thickness of the media layer divided by the thickness of the vascular wall (the sum of the intima, media, and adventitia layers) was used to determine the morphometric measurement of the pulmonary arterioles.

In Masson's trichrome-stained sections, the increase in the amount of collagen was assessed by calculating the lung tissue area[20]. Inflammation in the lung tissue was measured by the intensity of staining using the ImageJ analysis program, with MAC387 immunohistochemical staining, to determine changes in the number of macrophages.

Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, Illinois, USA). Differences between the groups were assessed using one-way analysis of variance and a post-hoc Tukey test. The statistical significance level was set at $p<0.05$.

Results

Assessment of exercise capacity using a treadmill on the 21st day of the study yielded the following running distances: 1665 ± 145 m in the control group, 1490 ± 185 m in the artesunate control group, 1142 ± 280 m in the bleomycin group, and 1207 ± 231 m in the bleomycin + artesunate group. There was a statistically significant difference between the groups ($p<0.001$); however, no statistically significant difference was found between the bleomycin + artesunate group and the bleomycin group ($p=0.95$).

A statistically significant intergroup difference was observed in the evaluation of right ventricular hypertrophy ($p<0.001$); the value was highest in the bleomycin group (0.44 ± 0.02). The assessment in the bleomycin + artesunate group (0.37 ± 0.03) was significantly lower than that of the bleomycin group ($p<0.001$). The hypertrophy measurement in the control group was 0.31 ± 0.02, while it was 0.30 ± 0.01 in the artesunate group.

Histopathological evaluation of the lung tissue revealed normal alveolar structure and pulmonary arteriolar morphometry in the control and artesunate control groups. There was no significant difference between the two groups in terms of pulmonary tissue area, percentage of pulmonary arteriolar wall thickness, or MAC387 immunohistochemistry. Alveolar wall thickening, pulmonary edema, hemorrhage, interstitial fibrosis, and inflammatory changes were observed in the bleomycin group. Extremely vacuolated and foamy macrophages were observed in the alveolar areas. In the bleomycin group, vasoconstricted pulmonary arterioles with hypertrophied endothelial cells were detected, squeezed between the folds of the lamina elastica interna, with vacuolization observed in the lumen (Figures 1, 2, and 3).

A marked increase was observed in the medial layer of the pulmonary arteriolar wall in the bleomycin group when compared with the control and artesunate control groups. The media layer thickness was significantly lower in the bleomycin + artesunate group than in the bleomycin group ($p=0.026$) (Table 1).

The bleomycin group exhibited the greatest measure of fibrosis and inflammation, whereas significant improvement was detected in the bleomycin + artesunate group ($p <0.001$ and $p <0.001$, respectively) (Table 1).

Discussion

IPF is a fibrotic pulmonary disease with a poor prognosis and high mortality[1,21,22]. Excessive fibroblast proliferation after alveolar injury, abnormal re-epithelialization, and dysregulation of extracellular matrix accumulation are associated with the pathogenesis of IPF[23]. Although the etiology of IPF is unknown, it is believed to develop as a result of inflammatory changes following an unknown infection or injury[24]. In recent years, two antifibrotic drugs have been introduced for the treatment of IPF to prevent the progression of fibrosis in the lung parenchyma. Although the two new targeted drugs, pirfenidone and nintedanib, have

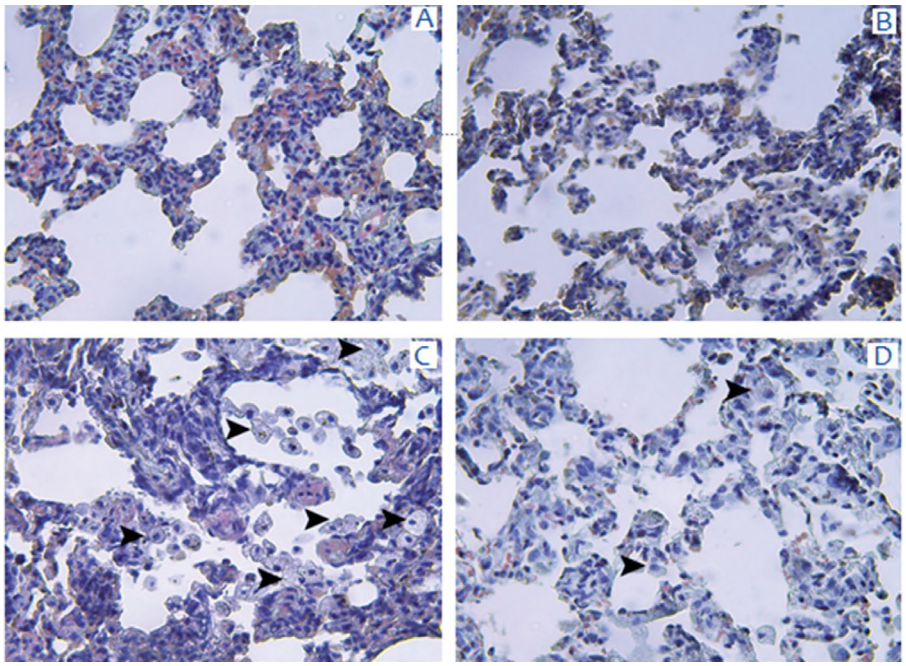

Fig. 1. General morphological appearance of the control, artesunate control, bleomycin, and bleomycin + artesunate groups (1A-D, Masson trichrome staining). In the bleomycin group, clusters of hypertrophic macrophages were visible in the alveolar areas, alveolar walls, and interstitial regions (arrowheads, 1C). In the bleomycin + artesunate group, a small number of hypertrophic macrophages were visible in the alveolar areas (arrowheads, 1D).

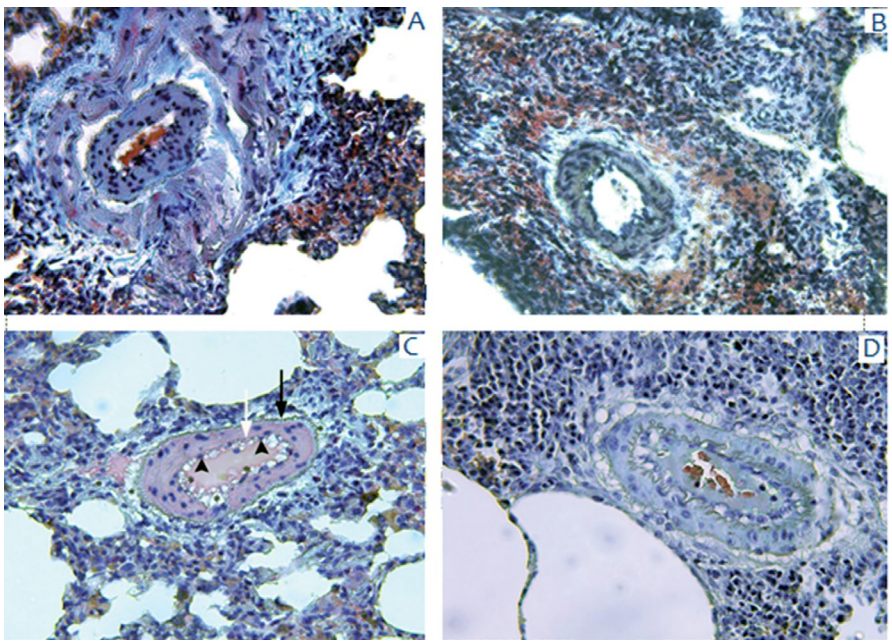

Fig. 2. Morphometry of pulmonary arterioles in the control, artesunate control, bleomycin, and bleomycin + artesunate groups (2 A-D, Masson trichrome staining). In the bleomycin group, hypertrophied endothelial cells squeezed between folds of lamina elastica interna (white arrow), and the resulting vacuolization in the lumen (asterisks) is visible (2C).

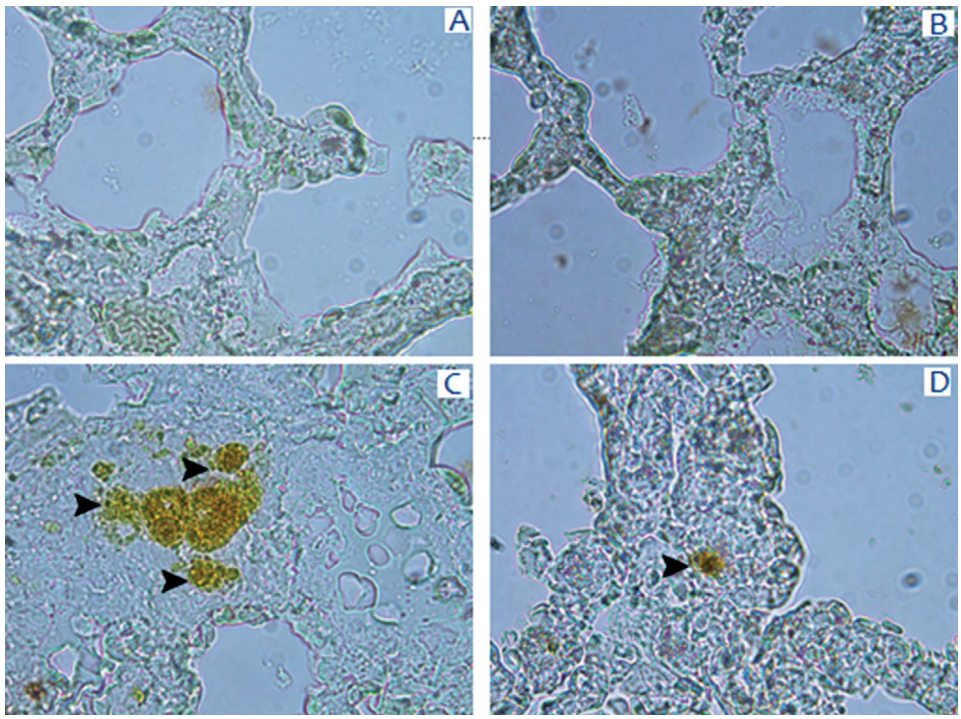

Fig. 3. Macrophages labeled with MAC387 immunohistochemical staining for the analysis of inflammation in pulmonary tissue (3A-D). In the bleomycin group, clusters of macrophages were stained brown with 3,3'-Diaminobenzidine (DAB) chromogen dye (arrowheads, 3C). In the bleomycin + artesunate group, a macrophage stained brown in the lung parenchyma (arrowhead, 3D).

Table 1. Results of the histopathological examination of the pulmonary tissuea

	Control	**Artesunate control**	**Bleomycin**	**Bleomycin + Artesunate**	**p**
Measurement of fibrotic area in the lung parenchyma	14.520 ± 1.687	13.591 ± 3.443	19.198 ± 1.038	13.828 ± 1.670	< .001[b]
Medial wall thickness of the pulmonary arteriole	0.280 ± 0.029	0.346 ± 0.336	0.776 ± 0.146	0.539 ± 0.140	< .001[b]
MAC387 staining intensity (inflammation)	180.315 ± 7.313	171.909 ± 5.167	228.925 ± 5.353	189.586 ± 10.476	< .001[b]

The data are presented as mean value ± standard deviation.

[a] For the analysis of fibrosis, areas of fibrotic pulmonary tissue were estimated in histochemical tissue sections stained with Masson's trichrome. The ratio of medial layer thickness to vascular wall thickness measurements was used to perform a morphometric analysis of the pulmonary arterioles. Pulmonary tissue inflammation was analyzed by measuring the staining intensity in immunohistochemical sections stained with MAC387 to label macrophages.

[b] Statistically significant intergroup differences were observed in the fibrosis measurements in pulmonary tissue, the thickness of the medial wall of the pulmonary arteriole, and the inflammation analysis values ($p<0.001$). In the fibrosis analysis, a statistically significant difference was observed between the bleomycin and the bleomycin + artesunate groups ($p<0.001$). A statistically significant difference was also observed between the bleomycin and bleomycin + artesunate groups in the morphometric analysis of pulmonary arterioles ($p=0.026$). Furthermore, a statistically significant difference was observed between the bleomycin and bleomycin + artesunate groups in the analysis of lung tissue inflammation ($p<0.001$).

provided new treatment options, they are only able to slow down functional loss, delay disease worsening, and partially extend survival time rather than treating the disease[7,25,26]. The search for a curative treatment for IPF is ongoing. Bleomycin is widely used in animal models to induce pulmonary fibrosis. As an antineoplastic agent, the mechanism of action involves the reduction of the molecular oxygen present in the bleomycin-iron complex to superoxide and hydroxyl radicals, resulting in the breakdown of the DNA strands[27,28]. A bleomycin-induced pulmonary fibrosis model leads to a fibrotic histological appearance similar to that observed in humans. Bleomycin increases the expression of a group of genes generated by the extracellular matrix components of proinflammatory mediators and transforming growth factor beta (TGF-β). Pulmonary fibrosis mimicking IPF 14 to 21 days after intratracheal administration of bleomycin is well established[29-31].

In our study, the antimalarial agent artesunate was found to improve fibrotic changes, inflammatory changes, pulmonary arteriolar medial layer thickness, and right ventricular hypertrophy in bleomycin-induced pulmonary fibrosis without any significant change in exercise capacity.

Artesunate, a derivative of artemisinin, has been used as an antimalarial agent since the 1970s. In recent years, studies have been conducted on the antimalarial effects of artemisinin, as well as its antiviral, antiparasitic, antiprotozoal, antifungal, anticystosomal, antiallergic, anti-inflammatory, and antitumoral effects[10].

Several recent studies have demonstrated the antifibrotic properties of artesunate in many different tissues. Xu *et al.*[13] found that artesunate reduced the expression of matrix metalloproteinases (MMP) 2 and 9, alpha-smooth muscle actin (α-SMA), and type I collagen in a rat hepatic fibrosis model, and increased MMP-13 levels in rats. Lai *et al.*[14] reported that artesunate decreased the accumulation of inflammatory infiltrates and the extracellular matrix in the liver and reduced the levels of tumor necrosis factor alpha and interleukin 6 in a hepatic fibrosis model. Additionally, it downregulated the expression of α-SMA, toll-like receptor 4, myeloid differentiation factor 88, and TGF-β1, and inhibited the translocation of the nuclear translocation factor kappa B p65. Cao *et al.*[15] reported that artesunate improved the accumulation of interstitial collagen and renal function, upregulated bone morphogenetic protein 7 and E-cadherin, and downregulated α-SMA and uterine sensitization-associated gene 1 in a renal fibrosis model of urinary obstruction.

In addition to its effects on fibrosis, Reid *et al.*[32] evaluated the cardiac effects of artesunate and found that artesunate inhibited the development of left ventricular hypertrophy and worsened cardiac function in transverse aortic constriction-induced mice.

Furthermore, *in vitro* and *in vivo* studies have evaluated the effects of artesunate on lung fibrosis. Zeng *et al.*[12] studied the effect of artesunate on human embryonic lung fibroblasts inoculated with human cytomegalovirus (CMV) *in vitro* and found that artesunate inhibited the proliferation of infected lung fibroblasts. It has been suggested that this approach may be a treatment option for preventing fibrosis in humans with CMV pneumonia.

In a study by Li *et al.*[33], artesunate was reported to block the mitogen-activated protein kinase cell-signaling pathway through the protein, mothers against decapentaplegic homolog (Smad) 7, which might be effective in IPF in human lung fibroblast cell cultures. Liu *et al.*[34] assessed the *in vivo* and *in vitro* effects of artesunate on pulmonary fibrosis and found that artesunate inhibited TGF-β1-induced fibroblast and myofibroblast transformation, thereby improving bleomycin-induced pulmonary fibrosis.

Wang *et al.*[16] assessed collagen-IV, matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), tissue inhibitor matrix metalloproteinase (TIMP) 1 and 2 protein levels in a bleomycin-induced pulmonary fibrosis model and reported that artesunate histopathologically improved these protein levels, as well as alveolitis and fibrosis.

In another study, artesunate was found to reduce histopathological fibrosis and decrease levels of TGF-β1, Smad3, heat-shock protein 47, α-SMA, and collagen type I in a bleomycin-induced pulmonary fibrosis model[36]. In our study, we also demonstrated histopathologically that artesunate improved lung fibrosis, as reported in the results of the 3 animal studies cited above.

Different daily doses have been used in various studies to evaluate the effects of artesunate on lung and other organ fibrosis. Antifibrotic effects have been observed at doses of 3.2 mg/kg/day to 100 mg/kg/day[13-15,33-35]. In studies on pulmonary fibrosis, artesunate was

more frequently used at a daily dose of 100 mg/kg. In the present study, a moderate dose of artesunate (30 mg/kg/day) was used, as in other fibrosis models, and favorable effects on pulmonary fibrosis were observed.

In contrast to other studies, we assessed not only the effects of artesunate on fibrosis but also its effects on the medial wall thickness of pulmonary arterioles, right ventricular hypertrophy, inflammation, and exercise capacity.

PH is a progressive pathology involving the small vessels of the pulmonary vascular bed and is associated with poor prognosis in IPF. The development of PH in IPF is associated with hypoxemic vasoconstriction and destruction resulting from progressive fibrosis of the vascular bed[4]. In our study, the wall thickness of the pulmonary arterioles and right ventricular hypertrophy, indicative of PH, were significantly improved by artesunate treatment.

Most patients with IPF (or intestinal lung disease) develop significant PH after exercise[36]. PH is significantly correlated with hypoxemia during exercise; thus, hypoxemia plays an important role in exercise limitation in IPF patients. It has been suggested that gas exchange abnormalities (secondary to this circulatory pathophysiology) may be the most important factor in exercise limitation in patients with IPF[37].

In patients with IPF, measuring exercise capacity after treatment is an important method for evaluating treatment response. Therefore, the 6-minute walking test, a simple and easily accessible method, is usually performed in clinics. Although the literature describes several methods for measuring exercise capacity in experimental animals, a treadmill is often preferred as a test device[18].

When we examined the running distance of the experimental groups in our study, the exercise capacity of the bleomycin group was significantly lower than that of the control and artesunate control groups, in which bleomycin was not used ($p<0.001$ and $p=0.041$, respectively).

The exercise capacity of our experimental group, treated with artesunate after bleomycin administration, was greater than that of the group treated with bleomycin alone. However, no significant intergroup difference was detected.

Considering the improved pulmonary arteriolar wall thickness and right ventricular hypertrophy in the artesunate + bleomycin group, the lack of a significant difference in exercise capacity in this group remains unexplained.

Exercise capacity (e.g., duration of exercise, running distance) measured using a consumer-type test is closely related to aerobic capacity, and maximal aerobic capacity (VO_2max) relies greatly on the effective integration of the cardiac and pulmonary systems as it pertains to the movement, exchange, and circulation of gases (e.g., O_2 and CO_2) into and through the body[38].

Based on the Fick equation, VO_2max is defined as the body's ability to transport oxygen (i.e., cardiac output) and utilize it (i.e., a-$vO_{2\ diff}$). Both components can be influenced by age, gender, regular training, detraining, different environments (e.g., hypobaria, microgravity), the use of certain medications, and illness[38].

Briefly, the administration of artesunate in our study treated the pathological effects of bleomycin on the respiratory system and the right heart; however, other systems and metabolic processes that determine exercise capacity might have caused some limitations. In other words, there may always be a linear relationship between the oxygen intake of the respiratory system and its use at the musculoskeletal level.

Additionally, we can say that the individual characteristics of experimental animals may determine their performance on the exercise test and affect the study outcomes. If the exercise capacity of each group had been measured before and after the treatment protocol, our study would have been more statistically robust. This is a limitation of the present study.

In conclusion, artesunate therapy improved fibrotic and inflammatory changes, pulmonary arterial wall thickness, and right ventricular hypertrophy in a bleomycin-induced pulmonary fibrosis model. Although it provided minimal improvement in exercise capacity, a significant intergroup difference was not observed. Artesunate is an easily accessible and inexpensive drug that is currently used for other indications. It may be a new treatment alternative for IPF, which currently has no cure. Further investigations in humans are required to assess the efficacy and safety of long-term artesunate use.

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Sažetak

UČINAK ARTESUNATA NA BLEOMICINOM INDUCIRANU PLUĆNU FIBROZU

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Unatoč novim lijekovima, još uvijek ne postoji kurativno liječenje idiopatske plućne fibroze (IPF). Cilj je ove studije bio istražiti učinke artesunata na fibrozu, plućnu hipertenziju i s upalom povezane promjene kod IPF-a. Podijelili smo ukupno 32 mužjaka Wistar albino štakora u četiri skupine: kontrolnu skupinu (skupina A, n=7), skupinu koja je primila artesunat (skupina B, n=7), skupinu koja je primila bleomicin (skupina C, n=9) i skupinu koja je primila i bleomicin i artesunat (skupina D, n=9). Skupine A i B primile su intratrahealnu fiziološku otopinu (0.1 mL), a skupine C i D primile su intratrahealni bleomicin (2.5 mg/kg). Skupine A i C primile su intraperitonealnu (i.p.) fiziološku otopinu (0.1 mL/dan), a skupine B i D primile su i.p. artesunat (30 mg/kg/dan) tijekom 21 dana. Kapacitet vježbanja mjerili smo pomoću trake za trčanje. Također smo pregledali tkiva srca i pluća za hipertrofiju desne klijetke (HDK) te debljinu stijenke plućnih arteriola za fibrozu. Konačno, za imunohistokemiju izveli smo bojanje Massonovim trikromom te protutijelo za biljeg makrofaga. Izmjereni kapacitet vježbanja štakora bio je 1665 ± 145 m u kontrolnoj skupini, 1142 ± 280 m u skupini koja je primila bleomicin, 1490 ± 185 m u skupini koja je primila artesunat te 1207 ± 231 m u skupini koja je primila i bleomicin i artesunat. Razlika između skupina bila je statistički značajna (p=0.001), no razlika između skupine koja je primila bleomicin + artesunat i skupine koja je primila samo bleomicin nije bila statistički značajna (p=0.95). HDK je bila česta u bleomicinskoj skupini (0.44 ± 0.02). Razlika između skupina bleomicin + artesunat i bleomicin bila je značajna (0.37 ± 0.03). Medijalna stijenka plućnih arteriola bila je deblja u štakora koji su primali bleomicin u usporedbi s onima koji su primali artesunat i kontrolnoj skupini, dok je bila tanja u štakora koji su primali bleomicin + artesunat (p<0.001, odnosno p=0.026). Fibroza i upalne promjene poboljšale su se u skupini bleomicin + artesunat (p<0.001). Autori zaključuju da je artesunat poboljšao fibrozu, upalne promjene, debljinu medijalnog sloja plućnih arteriola i HDK u štakora s bleomicinom induciranom plućnom fibrozom.

Ključne riječi: *Idiopatska plućna fibroza*; *Bleomicin*; *Artesunat*; *Hipertrofija desne klijetke*