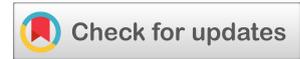Check for updates

(REVIEW ARTICLE)

Pulmonary fibrosing diseases: A short review and a therapeutic alternative

Luana Oliveira Prata [1, 2], Celso Martins Queiroz-Junior [3], Carolina Rego Rodrigues [1], Fabrício Marcus Silva Oliveira [1], Anderson José Ferreira [3], Maria da Glória Rodrigues-Machado [4] and Marcelo Vidigal Caliari [1, *]

[1] Department of General Pathology, Institute of Biological Sciences, Federal University of Minas Gerais. Av. Antônio Carlos 6627, Belo Horizonte, Minas Gerais, Brazil.
[2] Postgraduate Program in Pathology, Institute of Biological Sciences, Federal University of Minas Gerais. Av. Antônio Carlos 6627, Belo Horizonte, Minas Gerais, Brazil.
[3] Department of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais. Av. Antônio Carlos 6627, Belo Horizonte, Minas Gerais, Brazil.
[4] Medical Sciences Faculty of Minas Gerais, Alameda Ezequiel Dias 275, Belo Horizonte, Minas Gerais, Brazil.

Abstract

Pulmonary interstitial diseases are characterized by a wide spectrum of alterations, with idiopathic pulmonary fibrosis (IPF) being one of the most important. Recent studies have shown that COVID-19 can also progress to pulmonary fibrosis. IPF affects elderly individuals, its etiological agent is unknown and its prognosis is poor. Studies have shown an increased incidence and prevalence of IPF, especially in males. Unordered and excessive extracellular matrix deposition is the main lesion of IPF, leading to loss of normal alveolar architecture, decreased pulmonary compliance, and reduced gas exchange. Clinical trials of conventional treatments have not shown significant improvement in patients with IPF, proving the need of more effective alternatives.

Studies have shown the association of angiotensin-converting enzyme (ACE)/Angiotensin (Ang)II/AT1 receptor axis with the development of pulmonary fibrosis and hypertension. On the other hand, it was observed that angiotensin 2 converting enzyme (ACE 2)/Angiotensin1-7[Ang-(1-7)]/Mas receptor axis plays an important role in the balance of the ACE/AngII/AT1 axis. In this sense, drugs that increase the activity of the ACE 2/Ang-(1-7)/Mas receptor axis could present therapeutic potential for the treatment of IPF. In addition, when the effects of ACE 2 pharmacological treatment associated with a swimming protocol were analyzed in an experimental model of bleomycin-induced lung lesions, a potent reduction of pulmonary fibrosis and an increase in endurance capacity of animals were observed. Even without fully understanding the mechanisms involved, the results of this study showed that the combination of these two treatment methods might contribute to the treatment of fibrosing interstitial lung diseases.

Keywords: Pulmonary Fibrosis; Physical Training; Angiotensin Converting Enzyme 2 (ACE 2); Diminazene Aceturate (DIZE).

1. Introduction

Interstitial lung diseases (ILD) encompass a variety of pathological processes, ranging from acute inflammatory diseases to diseases that present progressive and irreversible fibrosis [1]. ILD mechanisms are not completely understood and may be related to genetic alterations, connective tissue diseases, environmental and infectious agents, as well as to unknown etiologic agents, as verified in Idiopathic Pulmonary Fibrosis (IPF). The main complaint of ILD patients is

* Corresponding author: Marcelo Vidigal Caliari
Department of General Pathology, Institute of Biological Sciences, Federal University of Minas Gerais. Av. Antônio Carlos 6627, Belo Horizonte, Minas Gerais, Brazil.

dyspnea, which is aggravated by physical activities. Crackles in the pulmonary auscultation may indicate the development of advanced fibrotic disease [2]. Preliminary studies have shown that patients with Coronavirus Disease 2019 (COVID-19) may progress to pulmonary fibrosis [3]. On March 11, 2020, WHO declared that the spread of the new coronavirus, denominated Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2), in the world reached the levels of a pandemic [4]. As of December 22, 2020, WHO reported that there were over 75 million confirmed cases of Coronavirus Disease 2019 (COVID-19) and over 1.6 million deaths worldwide since the start of the pandemic [5]. There is still a great need for studies to assess the pathogenesis of fibrosis observed in COVID-19, as well as its evolution and relevance.

Epithelial lesion is considered the main triggering event of important pulmonary diseases, such as Acute Respiratory Distress Syndrome (ARDS) [6], Cystic Fibrosis [7] and IPF [8]. IPF is a distinct type of ILD, which is characterized by different degrees of fibrosis and thickening of the alveolar walls and septa, accumulation of type II pneumocytes (PII) and myofibroblasts, as well as narrowing of the airways and gas exchange alteration [8].

The incidence and prevalence of IPF increase with age, mainly in individuals over 50 years of age, and predominantly in males [9]. A study analyzed the prevalence of IPF in patients over 65 years old and found an increase from 202.2 cases/100,000 inhabitants per year in 2001 to 494.5 cases/100,000 inhabitants per year in 2011 [10]. In Europe and North America studies the prevalence ranges from 2.8 to 19 cases/100,000 inhabitants per year, and most diagnoses are performed in the sixth and seventh decades of life [11]. In Japan, the prevalence and incidence of IPF was 10.0 and 2.23 for every 100,000 people [12].

Little is known about this disease in Brazil, but a study carried out in the Department of Informatics of the Health Unique System (DATASUS), showed a progressive increase in incidence and mortality between the period of 1996 and 2010. When applying the indexes of the American studies (classified according to age and gender) in Brazil, it was verified that the annual incidence of IPF cases could be of 3,5 a 5,1 cases/100,000 inhabitants [13]. Analyzing only individuals with 55 years or older, the prevalence ranged from 5,1 to 8,3 cases/100,000 inhabitants [13]. Recently, an increase from 0.24 cases/100,000 inhabitants to 1.10/100,000 inhabitants has been shown, which means that between 1979 and 2014 there was a 4.6-fold increase in mortality [14]. The 68% increase in the Brazilian population over the age of 50 may have contributed to this rise in mortality.

Diagnosis by lung biopsy in IPF patients usually demonstrates a slight inflammation in fibrotic areas indicated in examinations of high-resolution imaging. However, surgical lung biopsy is performed in few patients due to the risk associated with the procedure, especially in elderly individuals [10]. In high-resolution computed tomography, bilateral honeycomb changes are observed predominantly in the sub pleural and basal regions [10].

IPF etiology is unknown, but risk factors are considered to be smoking, environmental exposures to certain chemicals such as metal and wood dust and gases emitted by the combustion of fossil fuels, infectious agents and autoimmunity [8]. The mechanism for IPF development has not yet been fully elucidated. Pulmonary fibroblasts, their differentiation in myofibroblasts and alveolar epithelial cells play an important role in the pulmonary fibrosis pathogenesis by producing pro-fibrotic mediators and depositing collagen in the parenchyma. In pulmonary fibrosis (PF), after epidermal alveolar injury, re-epithelialization occurs due to hyperplasia of type II pneumocytes (PII) and undifferentiated cells that regenerate the epithelium [15]. PII comprise about 15% of the alveolar epithelial cells and are able to repair the alveolar wall [16]. Such proliferative capacity is described in different types of acute pulmonary lesions, as well as in interstitial lung diseases (ILD) [17].

The alveolar surface tension is reduced by the action of the surfactant substance produced by PII [18]. During inspiration, the surfactant promotes uniform alveolar recruitment, reducing the pressure gradient between the interstitium and the alveolus, thus decreasing the formation of alveolar edema. Surfactant protein A (SP-A) expression is a specific marker of pulmonary diseases and its concentration in pulmonary fibrosis is reduced in Bronchoalveolar Lavage (BAL) and elevated in plasma when compared to healthy individuals. The mechanisms involved in the SP-A appearance in plasma have not yet been established and several hypotheses have been proposed; 1) increased permeability of pulmonary vessels, 2) destruction of the alveolar-capillary barrier and 3) decreased clearance rates [19].

In addition to the surfactant, PII also produce cytokines and growth factors, such as transforming growth factor-β (TGF-β), interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1). Increased expression of active TGF-β induces the proliferation of fibroblasts with production of type I collagen, which is the main structural protein of the pulmonary interstitium [20,21]. Released cytokines may also promote the epithelial-mesenchymal transition (EMT) phenomenon, that transdifferentiates epithelial cells into fibroblasts and myofibroblasts [22]. The retraction and deformity of the

alveolar septa observed in fibrosis appear to be responsible for the contraction of cytoplasmic α-actin filaments of myofibroblasts [23]. Alveolar epithelial injury sites are commonly replaced by foci of fibroblastic proliferation and myofibroblasts differentiation, with exuberant deposition of extracellular matrix, which causes destruction of the alveoli-capillary units and decrease or loss of organ function [8].

The excessive and disordered collagen I deposition promotes loss of normal alveolar architecture and contributes to the reduction of lung compliance, reduction of gas exchange and other pulmonary functional alterations [24]. In an attempt to repair previous alveolar-capillary damage, the fibroproliferative response seems to act almost immediately after the onset of injury. As the repair proceeds, fibroblasts synthesize and deposit increased amounts of extracellular matrix, which is enhanced by the release and action of various growth factors and cytokines [8].

In human and experimental lung fibrosis, TGF-β is expressed by PII and alveolar macrophages, induces the differentiation of myofibroblasts that appear after the end of the initial inflammatory phenomena, stimulates the production of extracellular matrix by mesenchymal cells and inhibits matrix degradation [25].

Despite its great diversity, the main constituents of the extracellular matrix are collagen fibers. In connective tissue, the main fibers are types I, II and III (fibrillar), and types IV, V and VI (non-fibrillary or amorphous). Collagen I, the main structural protein of the pulmonary interstitium, is produced in large quantities during fibrotic reactions and its deposition as a substitute for the type III collagen causes loss of normal alveolar architecture and contributes to a decrease in pulmonary compliance, reduction of gas exchange and other pulmonary functional alterations [26]. However, the total amount of deposited cell matrix depends not only on the intensity of its synthesis but also on its degradation.

There are two main mechanisms involved in collagen degradation: phagocytosis and metalloproteinase (MMP)-mediated degradation [27]. When activated under normal conditions, MMPs are rapidly blocked by MMP-specific tissue inhibitors, since their increased activity may destroy the architecture of an organ, including the lung. Collagenases are metalloproteinases-1 (MMP-1), metalloproteinases-8 (MMP-8), metalloproteinases-13 (MMP-13) [28] and are responsible for the cleavage of collagens I, II and III. The resulting fragments are more susceptible to digestion by gelatinases (MMP-2 and MMP-9), facilitating their removal from tissue [28,29]. MMP-2 or gelatinase A is synthesized by several cells, including fibroblasts, endothelial cells and alveolar epithelial cells, whereas MMP-9 or gelatinase B is produced by macrophages, lymphocytes, neutrophils and, in certain situations, by alveolar epithelial cells [30]. MMP-2 degrades collagen IV, the major constituent of the basement membrane and it is increased in BAL and inflammatory cells of the pulmonary parenchyma, suggesting an important role of this MMP in pulmonary remodeling [31].

The presence of fibrosis with alveolar airspace size reduction decreases pulmonary distensibility and the deviation of the pressure-volume curve to the right, which causes the reduction of compliance and the increase of lung elastic recoil. Additionally, due to the loss of functional units and/or decreased expansibility of the damaged alveoli, there is a decrease in total lung capacity (TLC), forced vital capacity (FVC) and residual volume (RV). According to Mura et al. (2006), the extent of pulmonary fibrosis (% of total lung volume) negatively correlates with residual volume (% of predicted residual volume) [32].

Due to the increase in the force of elastic recoil of the lungs and the increase of the bronchiolar lumen size by retraction of the fibrotic areas, the pulmonary flows are increased when compared to the pulmonary volumes. Thus, the relationship between forced expiratory volume in one second (FEV1) and FVC is normal or elevated. This change in lung function is called restrictive ventilatory dysfunction [24]. Gas exchange changes are the earliest, mainly due to the disturbance of the ventilation/perfusion ratio. The reduction of arterial partial pressure of oxygen (PaO_2) is progressive and more pronounced in exercise. The partial pressure of carbon dioxide in the arterial blood ($PaCO_2$) is generally reduced due to the increase in alveolar ventilation imposed by the increased respiratory rate.

The diffusion measurement evaluated by the diffusion of carbon monoxide (DLCO) is considered an early marker of the disease and it was inversely correlated with dyspnea [32]. This change is secondary to the reduction of the cross-sectional area and thickening of the alveolar-capillary membrane [24].

Recent advances in understanding the pathogenesis of IPF suggest that inhibitors of pro-fibrogenic activities may be useful therapeutic agents in the control of fibrosing lung diseases, interfering or modulating the progression of pulmonary fibrosis and potentially improving respiratory function. Two antifibrotic drugs were able to reduce the rate of decline in lung function, the risk of acute worsening of respiratory function and the mortality of patients [33,34]. The first is perfenidone, a pyridine with anti-oxidant and anti-inflammatory properties, capable of inhibiting pro-inflammatory cytokines such as TNFα [35]. In addition, perfenidone is able to inhibit TGF-β, blocking the proliferation

and activation of fibroblasts, as well as collagen synthesis, being an effective anti-fibrotic [36]. The second drug is nintedanib, a tyrosine kinase inhibitor capable of blocking receptors for TGFβ, FGF, PDGF and VEGF, which are growth factors directly involved in the development of granulation tissue and fibrosis [37,38]. Therefore it inhibits the proliferation, migration and differentiation of fibroblasts as well as extracellular matrix deposition [38,39]. However, despite nintedanib and perfenidone benefits, both drugs are capable of producing side effects such as hepatotoxicity, increased plasma liver enzymes, nausea, abdominal pain, vomiting, diarrhea and weight loss, which can negatively affect the patient adherence to medication regimen [40].

2. Bleomycin and Interstitial Lung Disease

Rodents have been used as a model to study the pathophysiology of pulmonary fibrosis. The administration of bleomycin in rodents is the most used experimental method to study pulmonary fibrosis [41] and it is based on the observation that pulmonary fibrosis is one of bleomycin side effects when this drug is used for the treatment of some human cancers [42]. Bleomycin sulphate is a chemotherapeutic agent derived from water-soluble cytotoxic glycopeptide antibiotics and isolated from Streptomyces verticillus. Administration of bleomycin may produce pulmonary fibrosis, hypersensitivity pneumonitis and pulmonary nodules [43]. Evidences indicate that bleomycin inhibits the synthesis of deoxyribonucleic acid (DNA) and partially blocks the synthesis of ribonucleic acid (RNA) and proteins. It has a potent antitumor activity and has been used in the treatment of lymphomas, germ cell tumors and squamous cell carcinomas [44]. Bleomycin treatment in patients with lymphomas leads to a frequency of pneumonitis of around 18% and can cause 24% of mortality in these cases [45]. It induces epithelial damage and alveolar inflammation, initiated by a super-expression of reactive oxygen species, followed by a fibroproliferative process, fibrosis and simultaneous depletion of antioxidants. The pneumonitis may progress gradually during treatment or develop months after its completion.

The factors that contribute to the increased bleomycin-induced toxicity are: advanced age, dose, use of supplemental O2, radiotherapy, renal insufficiency, occurrence of pre-existing lung diseases, smoking, and concomitant use of granulocyte colony-stimulating factor (G-CSF – Granulocyte Colony-Stimulating Factor). This bleomycin-induced pulmonary toxicity is clinically associated with the development of cough, dyspnea, fever, cyanosis and decreased pulmonary function parameters [46].

3. Treatment of Pulmonary Interstitial Disease by diminazene aceturate (DIZE)

The renin-angiotensin system (RAS) is composed of several biologically active peptides that act in the regulation of arterial pressure, hydroelectrolytic homeostasis, vascular tone and cellular function. Imbalances in this system, in addition to being related to various cardiovascular and renal diseases such as arterial hypertension, heart failure, cardiac remodeling, ventricular hypertrophy and chronic renal failure, may also be involved in pulmonary diseases [47]. The functional balance of RAS depends on the balance of activities of the two axes that form this system angiotensin converting enzyme (ACE)/Angiotensin(Ang)II/AT1 receptor and angiotensin converting enzyme 2 (ACE 2/Angiotensin-(1-7)/receptor Mas [47]. The ACE/AngII/AT1 axis has inflammatory, fibrotic, vasoconstricting, proliferative and oxidative stress inducing actions and, thus is associated with the pathophysiology of different diseases [48–53]. In addition, several studies have demonstrated this axis participation in the development of fibrosis and pulmonary hypertension [47,54]. On the other hand, the axis composed of ACE 2, Ang-(1-7) and Mas has an important role in the ACE/AngII/AT1 axis balance. ACE 2, an enzyme homologous to ACE, catalyzes the hydrolysis of the C-terminal residue of Ang II, producing the protective Ang-(1-7) peptide, which is the specific binding of Mas receptor [55,56]. Thus, drugs that increase ACE 2/Ang-(1-7)/Mas axis activity may have therapeutic potential for the treatment of IPF.

Studies have shown that diminazene aceturate (DIZE), by activating ACE 2, is able to reduce mean arterial pressure, heart weight to body weight ratio and myocardial fibrosis, as well as attenuate pulmonary fibrosis induced by bleomycin and remodel cardiac tissue. It also lowers right ventricular systolic pressure and performs sympathetic modulation [57].

DIZE is the main synthetic compound used in the treatment of animal trypanosomiasis [58]. It has been used for over six decades and its synthesis was described by Brodersen (1958) from the modification of diminazeno, which was developed by Hochst (1954) [59, 60]. DIZE pharmaceutical formulation contains two aceturate salt compounds associated with diminazene. It is also known as diminazine aceturate, diminazene aceturate, diminazine or 4-[2-(4-carbamimidoylphenyl) iminohydrazinyl] benzenecarboximidamide (IUPAC name). Diminazene, an aromatic diamidine with two triazene bridge-linked amidinophenyl groups, has instability and insolubility characteristics in aqueous solutions that favor the chemical synthesis of DIZE (Figure 1). Such synthesis is dicathonic in nature and occurs by the reaction between diminazene and aceturate salt diluted in methanol. The resulting compound has molecular formula

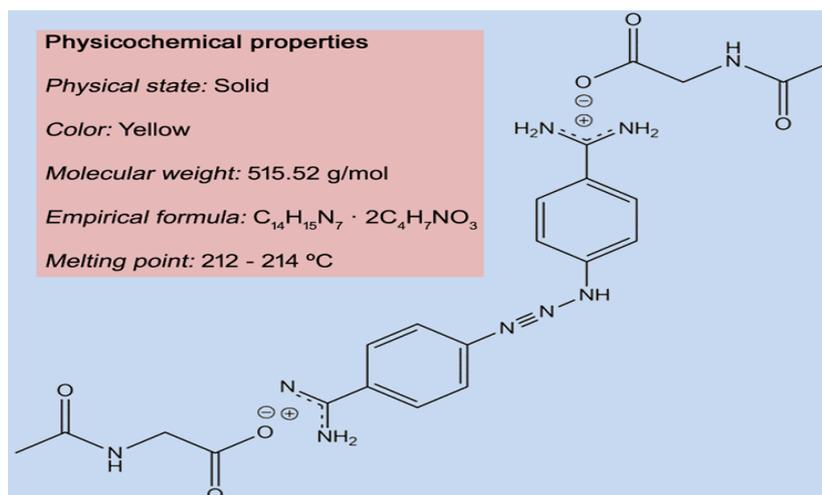C22H29N9O6, molecular weight 515.52 g / mol, melting point between 202°C and yellow color (PubChem - U.S. National Library of Medicine https://pubchem.ncbi.nlm.nih.gov/compound/2354).

Figure 1 Physicochemical properties of diminazene aceturate**.**

DIZE is used mainly in the treatment of diseases caused by Trypanosoma flagellate protozoa (*T. congolense*, *T. brucei*, *T. evansi* and *T. vivax*), which is responsible for several infections in animals such as horses, dogs, cats, cattle and even humans. In addition to trypanocidal activity, DIZE is also used to treat protozoan infections of the Babesia genus (*B. canis*, *B. vogeli* and *B. gibsoni*) [61,62]. The main route of administration is single or repeated intramuscular (IM) doses, which allow the circulation of high concentrations of the compound. Nevertheless, pharmacokinetic studies in cattle, rabbits, goats and sheep indicate that absorption also occurs immediately after oral administration, leading to rapid plasma IC50 [63]. The main route of elimination seems to be the renal excretion, although metabolites are not completely known [64]. Marketed under the brand names Azidine®, Berenil®, Ganaseg®, Ganasegur®, and Veriben®, DIZE trypanocidal mechanism involves the connection of the aromatic diamidine to the non-interchangeable trypanosomal kinetoplast DNA (kDNA) through specific interaction with adenine thymine rich sites [65]. Therefore, DIZE induces heterochromatin condensation during the G2 phase of the cell cycle, what causes the DNA to become fully unfolded and modifies DNA conformation by interfering with the binding of DNA topoisomerases [66, 67] . These mechanisms make this long-marketed compound retain its relevance as an important trypanocidal agent because of its broad therapeutic efficiency over other compounds used in the treatment of trypanosomiasis in dogs, cattle, sheep and goats.

Moreover, the direct anti-parasitic effects arising from DIZE biochemical properties appears to have effects on animals' immune systems. Administration of DIZE to T. congolense infected mice, for example, induces increased plasma levels of IgG2a and IgG3, associated with parasitemia control [68]. In addition, a hyperactivation of T-cells and macrophages and a reduction of serum levels of proinflammatory cytokines such as IL-6, IL-12, TNF and IFN-γ are observed by directly altering the production of these mediators by splenic and hepatic macrophages [69]. Recently, it has also been described that DIZE may activate the ACE 2, which converts Angiotensin II into Ang-(1-7) [70]. This activation seems to influence several physiological and pathological conditions related to the ACE-Ang II and ACE 2-Ang-(1-7) pathways. DIZE chronic administration prevents and reduces pulmonary hypertension in experimental models of bleomycin and monocrotaline-induced lung injury [71]. Similarly, activation of ECA 2 by DIZE also induced protection against experimental ischemic stroke and glaucoma by inhibiting the Ang-(1-7)/Mas [72,73]. Evidence such as this indicates that DIZE, in addition to its tripanolytic effects, can also modulate the immune system and physiological enzymatic pathways.

4. Exercise and Pulmonary Rehabilitation

Recently, pulmonary rehabilitation for patients with pulmonary fibrosis has received considerable attention [74]. Exercise programs have been found to reduce dyspnea and improve exercise capacity and quality of life in IPF patients. Like patients with chronic obstructive pulmonary disease (COPD), IPF patients present reduced tolerance to exercise and reduced life quality [75]. Jastrzêbski *et al.*, 2006 evaluated the level of dyspnea and quality of life in patients with interstitial lung disease after 6 weeks of rehabilitation [76]. These authors observed that rehabilitation caused a reduction in the dyspnea sensation and improved the quality of life, without, however, altering lung function [76]. The

rehabilitation program included training of the respiratory muscles and lower limbs in the cycle ergometer. Similar results were observed by Holland *et al.*, 2008 who submitted IPF patients to the supervised pulmonary rehabilitation program performed on treadmill and cycle ergometer associated with endurance training of upper limbs and strength training of lower limbs [77]. Rehabilitation improved exercise capacity and symptoms. According to the data, physical training for this group of patients is safe and improves functional capacity, dyspnea and quality of life. In another study, patients undergoing twelve weeks of controlled exercise showed significant improvement in ventilatory functions [78]. The authors speculate that the increased ventilatory need during exercises promoted chest expansion and stretching of the chest muscles.

Studies in exercise physiology have used laboratory animals to simulate physical stress conditions observed in humans to better monitor the systemic, cellular and molecular changes of physical activity [79]. Chronic aerobic exercise is responsible for changes in the cardiorespiratory system, both at rest and during exercise, especially when aerobic training is performed in low to moderate intensity [74,80]. In rodent swimming protocols, incremental exercise is obtained by adding loads that are progressively heavier relative to the rodent body weight and that are placed in the animal tail [41]. The use of swimming as a model of exercise for mice avoids the animal selection since they need to swim to avoid water submersion. The measurement of the time that the animal can swim with different loads, allows the researcher to calculate its functional capacity, which includes its functional performance [81]. In a study conducted by us, mice submitted to a swimming protocol obtained improved functional capacity and reduced bleomycin-induced lung fibrosis [41]. In this study, a condition of interstitial fibrosing pneumonitis similar to what is seen in humans was observed in the pulmonary parenchyma of sedentary animals receiving bleomycin. Similar results were obtained in the qualitative and quantitative analysis of type I collagen, the main constituent of the pulmonary connective tissue. In the sedentary animals treated with bleomycin, thick areas of positive marking for collagen I were observed, especially in the alveolar septa and peribronchiolar and perivascular interstices, associated or not with clusters of alveolar epithelial cells, fibroblasts and macrophages. As verified by Gomori trichrome staining, animals treated with bleomycin and submitted to physical exercise showed reduction of fibrosis in all interstitial spaces, when compared to sedentary animals [41].

Tolerance to functional exercise and improvement in quality of life are factors that make the patient with IPF a good candidate to participate in this type of pulmonary rehabilitation program [75].

5. Exercise associated with pharmacological treatment

There are still many doubts regarding the pathogenesis of interstitial lung diseases and the efficacy of different pharmacological agents in interfering with the development of these lesions. In this sense, it is important to perform more human and experimental studies on the practice of physical activity, associated or not with pharmacological treatment, as a method of reconditioning this group of lung diseases. Therefore, we evaluated the effects of pharmacological treatment with DIZE associated with physical exercise on lung changes experimentally induced by bleomycin [82]. Ninety-six male Balb/c mice were divided into Control (C) and bleomycin groups (BLM). Animals from the BLM groups received 8U/kg of bleomycin intratracheally and the C groups received saline. The groups were subdivided into sedentary control, sedentary control with DIZE, exercise control, exercise control with DIZE; sedentary bleomycin, bleomycin with DIZE, bleomycin exercise and bleomycin exercise with DIZE. The animals were trained 5 days/week, 1h/day, with 60% of the maximum load obtained in the evaluation of functional capacity, during 4 weeks. Groups with DIZE were treated (1mg/kg, via gavage) daily until the end of the experiment. The lungs were collected 48 hours after the last functional capacity assessment, fixed in buffered formalin and analyzed by Gomori trichrome, immunohistochemistry of type I collagen, TGF-β1, beta-prolyl-4-hydroxylase, MMP-1 and MMP- 2. This study showed that the association of physical exercise with the activation of the ACE 2 caused a reduction of bleomycin-induced pulmonary fibrosis and an increase in the endurance capacity of the animals submitted to joint treatment (Figure 2).
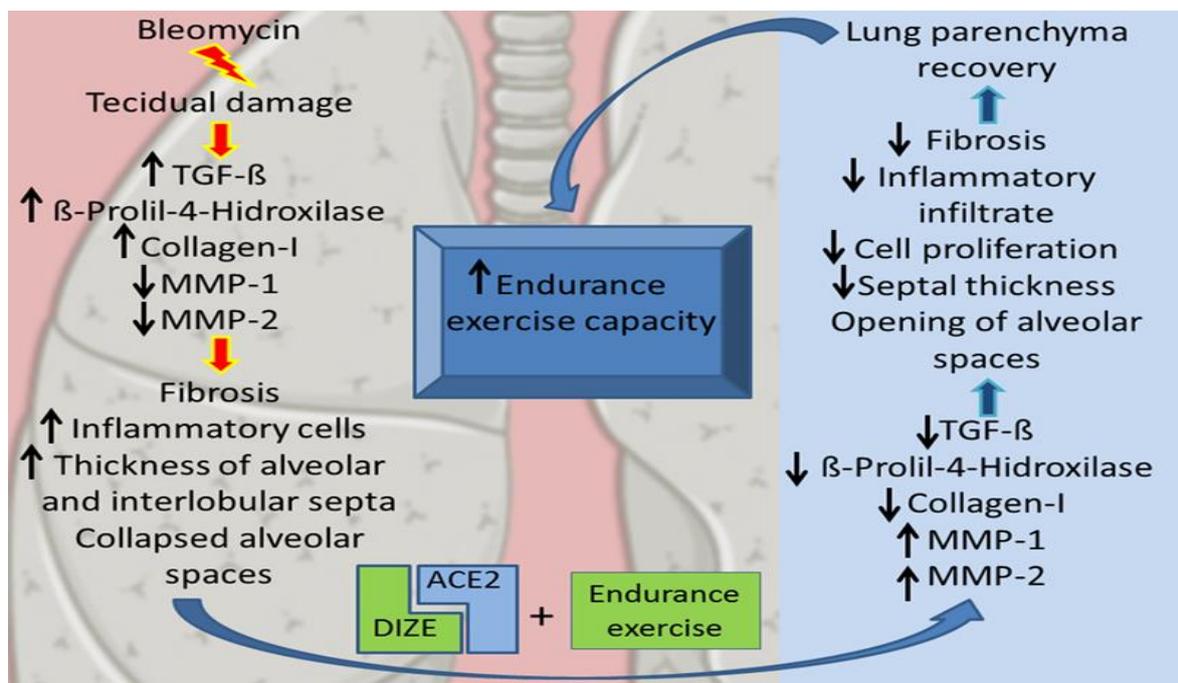
Figure 2 Effects of DIZE treatment associated with endurance exercise on bleomycin-induced interstitial fibrosis.

Fibrosis is a remarkable lesion that characterizes chronic fibrosing pneumonitis induced by bleomycin. Type I collagen deposition is associated with an increase in ß-Prolyl-4-Hydroxylase (fibroblast marker) and TGF-ß, which is the main growth factor associated with migration, proliferation and activation of fibroblasts. The reduction of MMP-1 and MMP-2 decreases the extracellular matrix remodeling, favoring fibrosis. Along with the inflammatory infiltrate, fibrosis causes thickening of the interalveolar and interlobular septa and collapse of the alveolar spaces. Pharmacological treatment with DIZE, an ACE 2 activator, associated with endurance exercise reduced TGF-ß and ß-Prolil-4-Hydroxylase and increased MMP-1 and MMP-2. Such changes led to an increase in the extracellular matrix remodeling and a reduction of biological mechanisms that could favor the deposition of more type I collagen. The reduction of inflammatory infiltrate and fibrosis led to a decrease in the thickness of the alveolar and interlobular septa, as well as the opening of the alveolar spaces. With the reduction of the lung lesions intensity, there was an increase in the capacity to perform the endurance exercise. TGF-ß = Transforming Growth Factor-Beta; MMP-1 = metalloproteinase 1; MMP-2 = metalloproteinase 2; DIZE = diminazene aceturate; ACE 2 = Angiotensin Converting Enzyme 2.

The combination of treatments was able to further reduce pulmonary fibrosis by decreasing fibrous connective tissue, type I collagen, expression of TGF-β1 and beta-prolyl-4-hydroxylase. In addition, it increased the expression of MMP-1 and MMP-2 that are involved in collagen degradation. In this way, the reduction of extracellular matrix synthesis mechanisms and the increase of their degradation were involved in the pulmonary fibrosis reduction proposed in the study. Although the mechanisms are not well understood, the results of this work showed that the association of these two therapeutic methods might be useful in the treatment of pulmonary diseases associated with fibrosis [82].

6. Conclusion

Recent studies on IPF pharmacological treatment show great advances of the use of nintedanib and perfenidone in the effectiveness of reducing functional decline. However these drugs do not seem capable of significantly improve patients' survival. Even with a better understanding of pathophysiology and therapeutic advancement, the disease remains incurable and patients experience different symptoms such as chronic cough, shortness of breath, impaired quality of life, along with the presence of various comorbidities. In conclusion, IPF is complex, progressive, and lethal and the therapeutic options are limited, justifying the search for new alternative treatments that can increase survival and quality of life. Multidisciplinary methods experimentation, such as the pharmacological treatment associated with physical exercise, can be one of the ways to stop the progression of the disease, reduce mortality and improve quality of life. With reference to COVID-19, since SARS-Cov-2 uses the spike protein to bind to the ACE 2 receptor, further analyses are needed to evaluate the use of molecules, which are capable of activating ACE 2 [83–85]. Recent studies have shown that the administration of recombinant ACE 2 apparently reduced the severe pulmonary effects caused by

infection with the H5N1 virus and the respiratory syncytial virus, suggesting the possibility of using recombinant ACE 2 as a tool to treat severe forms of Covid- 19 [86,87].

Compliance with ethical standards

Disclosure of conflict of interest

Authors have no conflicts of interest.

References

[1]   Meyer KC. Diagnosis and management of interstitial lung disease. Transl Respir Med 2014; 2: 1-13.

[2]   Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. Lancet 2017; 389: 1941–52.

[3]   Dai H, Zhang X, Xia J, Zhang T, Shang Y, Huang R, et al. High-resolution Chest CT Features and Clinical Characteristics of Patients Infected with COVID-19 in Jiangsu, China. Int J Infect Dis. 2019; 51: 186–02.

[4]   WHO. (World Health Organization): Coronavirus disease (COVID-2019) situation report. 2020; 51.

[5]   WHO. World Health Organization. Coronavirus disease (COVID-2019). Situation Report 99.

[6]   Thompson BT, Chambers RC, Liu KD. Acute Respiratory Distress Syndrome. N Engl J Med 2017; 377: 562–72.

[7]   Ratjen F, Bell SC, Rowe SM, Goss CH, Quittner AL, Bush A. Cystic fibrosis. Nat Rev Dis Prim. 2015; 1: 1-19.

[8]   Wolters PJ, Collard HR, Jones KD. Pathogenesis of Idiopathic Pulmonary Fibrosis. Annu Rev Pathol Mech Dis. 2014; 9: 157–79.

[9]   Olson AL, Gifford AH, Inase N, Fernández Pérez ER, Suda T. The epidemiology of idiopathic pulmonary fibrosis and interstitial lung diseases at risk of a progressive-fibrosing phenotype. Eur Respir Rev 2018; 27(150), 180077.

[10]   Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An Official ATS/ERS/JRS/ALAT Statement: Idiopathic pulmonary fibrosis: Evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011; 183: 788–24.

[11]   Hutchinson J, Fogarty A, Hubbard R, McKeever T. Global incidence and mortality of idiopathic pulmonary fibrosis: A systematic review. Eur Respir J. 2015; 46: 795–06.

[12]   Natsuizaka M, Chiba H, Kuronuma K, Otsuka M, Kudo K, Mori M, et al. Epidemiologic survey of Japanese patients with idiopathic pulmonary fibrosis and investigation of ethnic differences. Am J Respir Crit Care Med. 2014; 190: 773–9.

[13]   Baddini-Martinez J, Pereira CA. Quantos pacientes com fibrose pulmonar idiopática existem no Brasil? J Bras Pneumol. 2015; 41: 560–1.

[14]   Algranti E, Saito CA, Silva DRM e, Carneiro APS, Bussacos MA. Mortality from idiopathic pulmonary fibrosis: a temporal trend analysis in Brazil, 1979-2014. J Bras Pneumol. 2017; 43: 445–50.

[15]   Zoz DF, Lawson WE, Blackwell TS. Idiopathic pulmonary fibrosis: A disorder of epithelial cell dysfunction. Am J Med Sci. 2011; 341: 435–8.

[16]   Sugahara K, Tokumine J, Teruya K, Oshiro T. Alveolar epithelial cells: Differentiation and lung injury. Respirology. 2006; 11: 28–31.

[17]   McElroy MC, Kasper M. The use of alveolar epithelial type I cell-selective markers to investigate lung injury and repair. Eur Respir J. 2004; 24: 664–73.

[18]   Han SH, Mallampalli RK. The role of surfactant in lung disease and host defense against pulmonary infections. Ann Am Thorac Soc. 2015; 12: 765–74.

[19] Greene KE, King JE, Kuroki Y, Bucher-Bartelson B, Hunninghake GW, Newman LS, et al. Serum surfactant proteins-A and -D as biomarkers in idiopathic pulmonary fibrosis. Eur Respir J 2002;19:439–46.

[20] Chapman HA. Disorders of lung matrix remodelling. J Clin Invest. 2004; 113: 148–57.

[21] Gharaee-Kermani M, Gyetko MR, Hu B, Phan SH. New insights into the pathogenesis and treatment of idiopathic pulmonary fibrosis: A potential role for stem cells in the lung parenchyma and implications for therapy. Pharm Res. 2007; 24: 819–41.

[22] Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, Du Bois RM, et al. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-β1: Potential role in idiopathic pulmonary fibrosis. Am J Pathol. 2005; 166: 1321–32.

[23] Phan SH. Biology of Fibroblasts and Myofibroblasts. Proc Am Thorac Soc. 2008; 5: 334–7.

[24] Plantier L, Cazes A, Dinh-Xuan AT, Bancal C, Marchand-Adam S, Crestani B. Physiology of the lung in idiopathic pulmonary fibrosis. Eur Respir Rev. 2018; 27: 1–14.

[25] Laurent GJ, McAnulty RJ, Hill M, Chambers R. Escape from the matrix: Multiple mechanisms for fibroblast activation in pulmonary fibrosis. Proc Am Thorac Soc. 2008; 5: 311–5.

[26] Burgstaller G, Oehrle B, Gerckens M, White ES, Schiller HB, Eickelberg O. The instructive extracellular matrix of the lung: basic composition and alterations in chronic lung disease. Eur Respir J. 2017; 50: 1601805.

[27] Glasser SW, Hagood JS, Wong S, Taype CA, Madala SK, Hardie WD. Mechanisms of Lung Fibrosis Resolution. Am J Pathol. 2016; 186: 1066–77.

[28] Corbel M, Boichot E, Lagente V. Role of gelatinases MMP-2 and MMP-9 in tissue remodeling following acute lung injury. Brazilian J Med Biol Res. 2000; 33: 749–54.

[29] Pardo A, Selman M. Matrix metalloproteases in aberrant fibrotic tissue remodeling. Proc Am Thorac Soc. 2006; 3: 383–8.

[30] Greenlee KJ, Werb Z, Kheradmand F. Matrix metalloproteinases in lung: Multiple, multifarious, and multifaceted. Physiol Rev. 2007; 87: 69–98.

[31] Kim JY, Choeng HC, Ahn C, Cho SH. Early and late changes of MMP-2 and MMP-9 in bleomycin-induced pulmonary fibrosis. Yonsei Med J. 2009; 50: 68–77.

[32] Mura M, Ferretti A, Ferro O, Zompatori M, Cavalli A, Schiavina M, et al. Functional predictors of exertional dyspnea, 6-min walking distance and HRCT fibrosis score in idiopathic pulmonary fibrosis. Respiration. 2006; 73: 495–02.

[33] Richeldi L, Du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014; 370: 2071–82.

[34] Noble PW, Albera C, Bradford WZ, Costabel U, Bois RMD, Fagan EA, et al. Pirfenidone for idiopathic pulmonary fibrosis: Analysis of pooled data from three multinational phase 3 trials. Eur Respir J. 2016; 47: 243–53.

[35] Kim ES, Keating GM. Pirfenidone: A review of its use in idiopathic pulmonary fibrosis. Drugs. 2015; 75: 219–30.

[36] Ahluwalia N, Shea BS, Tager AM. New Therapeutic Targets in Idiopathic Pulmonary Fibrosis. Aiming to Rein in Runaway Wound-Healing Responses. Am J Respir Crit Care Med. 2014; 190: 867–78.

[37] Wollin L, Maillet I, Quesniaux V, Holweg A, Ryffel B. Antifibrotic and anti-inflammatory activity of the Tyrosine Kinase inhibitor Nintedanib in Experimental Models Of Lung Fibrosiss. J Pharmacol Exp Ther. 2014; 349: 209–20.

[38] Wollin L, Wex E, Pautsch A, Schnapp G, Hostettler KE, Stowasser S, et al. Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis. Eur Respir J. 2015; 45: 1434–45.

[39] Hilberg F, Tontsch-Grunt U, Baum A, Le AT, Doebele RC, Lieb S, et al. Triple angiokinase inhibitor nintedanib directly inhibits tumor cell growth and induces tumor shrinkage via blocking oncogenic receptor tyrosine kinases. J Pharmacol Exp Ther. 2018; 364: 494–03.

[40] Graney BA, Lee JS. Impact of novel antifibrotic therapy on patient outcomes in idiopathic pulmonary fibrosis: patient selection and perspectives. Patient Relat Outcome Meas. 2018;9: 321–8.

[41] Prata LO, Oliveira FMS, Ribeiro TMS, Almeida PWM, Cardoso JA, Rodrigues-Machado M da G, et al. Exercise attenuates pulmonary injury in mice with bleomycin-induced pulmonary fibrosis. Exp Biol Med. 2012; 237: 873–83.

[42] Watson RA, De La Peña H, Tsakok MT, Joseph J, Stoneham S, Shamash J, et al. Development of a best-practice clinical guideline for the use of bleomycin in the treatment of germ cell tumours in the UK. Br J Cancer. 2018; 119: 1044–51.

[43] Moore BB, Hogaboam CM. Murine models of pulmonary fibrosis. Am J Physiol - Lung Cell Mol Physiol. 2008; 294: 152–60.

[44] Hay J, Shahzeidi S, Laurent G. Mechanisms of bleomycin-induced lung damage. Arch Toxicol. 1991; 65: 81–94..

[45] Martin WG, Ristow KM, Habermann TM, Colgan JP, Witzig TE, Ansell SM. Bleomycin pulmonary toxicity has a negative impact on the outcome of patients with Hodgkin's lymphoma. J Clin Oncol. 2005; 23: 7614–20.

[46] Azambuja E, Fleck JF, Batista RG, Menna Barreto SS. Bleomycin lung toxicity: who are the patients with increased risk? Pulm Pharmacol Ther. 2005; 18: 363–6.

[47] Ferreira AJ, Shenoy V, Yamazato Y, Sriramula S, Francis J, Yuan L, et al. Evidence for angiotensin-converting enzyme 2 as a therapeutic target for the prevention of pulmonary hypertension. Am J Respir Crit Care Med. 2009; 179: 1048–54.

[48] Ferreira AJ, Santos RA, Almeida AP. Angiotensin-(1-7): cardioprotective effect in myocardial ischemia/reperfusion. Hypertension. 2001; 38: 665–8.

[49] Tallant EA, Ferrario CM, Gallagher PE. Angiotensin-(1-7) inhibits growth of cardiac myocytes through activation of the mas receptor. Am J Physiol - Hear Circ Physiol. 2005; 289: 1560–6.

[50] Ferrario CM. Angiotensin-converting enzyme 2 and angiotensin-(1-7): An evolving story in cardiovascular regulation. Hypertension. 2006; 47: 515–21.

[51] Oudit GY, Kassiri Z, Patel MP, Chappell M, Butany J, Backx PH, et al. Angiotensin II-mediated oxidative stress and inflammation mediate the age-dependent cardiomyopathy in ACE2 null mice. Cardiovasc Res. 2007; 75: 29–39.

[52] Fraga-Silva RA, Pinheiro SVB, Gonçalves ACC, Alenina N, Bader M, Souza Dos Santos RA. The antithrombotic effect of angiotensin-(1-7) involves Mas-mediated NO release from platelets. Mol Med. 2008; 14: 28–35.

[53] Mercure C, Yogi A, Callera GE, Aranha AB, Bader M, Ferreira AJ, et al. Angiotensin(1-7) blunts hypertensive cardiac remodeling by a direct effect on the heart. Circ Res. 2008; 103: 1319–26.

[54] Shenoy V, Ferreira AJ, Qi Y, Fraga-Silva RA, Díez-Freire C, Dooies A, et al. The angiotensin-converting enzyme 2/angiogenesis-(1-7)/Mas axis confers cardiopulmonary protection against lung fibrosis and pulmonary hypertension. Am J Respir Crit Care Med. 2010; 182: 1065–72.

[55] Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme: Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem. 2000; 275: 33238–43.

[56] Santos RAS, Simoes e Silva AC, Maric C, Silva DMR, Machado RP, De Buhr I, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci U S A. 2003; 100: 8258–63.

[57] Rigatto K, Casali KR, Shenoy V, Katovich MJ, Raizada MK. Diminazene aceturate improves autonomic modulation in pulmonary hypertension. Eur J Pharmacol. 2013; 713: 89–93.

[58] Kuriakose S, Uzonna JE. Diminazene aceturate (Berenil), a new use for an old compound? Int Immunopharmacol. 2014; 21: 342–5.

[59] Richard Brodersen HL, Kelkheim e Heinrich Ott. Readily'soluble and stable salts of DI. (4-AMIDINO-PHENYL) - TRIAZENE - (N-13) and a process of preparing them. United States Pat Off. Patented: June 10, 1958.

[60] Heinrich Jensch Hochst FHA. BAsic diazoamanobenzene compounds. United States Pat Off. Patented. 23 Mar 1954.

[61] Vial HJ, Gorenflot A. Chemotherapy against babesiosis. Vet Parasitol. 2006; 138: 147–60.

[62] Gohil S, Herrmann S, Günther S, Cooke BM. Bovine babesiosis in the 21st century: Advances in biology and functional genomics. Int J Parasitol. 2013; 43: 125–32.

[63] Mamman M, Aliu YO, Peregrine AS. Comparative pharmacokinetics of diminazene in noninfected Boran (Bos indicus) cattle and Boran cattle infected with Trypanosoma congolense. Antimicrob Agents Chemother. 1993; 37: 1050–5.

[64] Onyeyili PA, Anika SM. The influence of Trypanosoma congolense infection on the disposition kinetics of diminazene aceturate in the dog. Vet Res Commun. 1989; 13: 231–6.

[65] González VM, Pérez JM, Alonso C. The berenil ligand directs the DNA binding of the cytotoxic drug Pt- berenil. J Inorg Biochem. 1997; 68: 283–7.

[66] T Haaf', W Feichtinger, M Guttenbach, L Sanchez, CR Müller, MS. Berenil-induced undercondensation in human heterochromatin. Cytogenet Cell Genet. 1989; 50: 27-33.

[67] Poot M, Kausch K, Köhler J, Haaf T, Hoehn H. The Minor-Groove Binding DNA-Ligands Netropsin, Distamycin A and Berenil Cause Polyploidisation via Impairment of the G2 Phase of the Cell Cycle. Cell Struct Funct. 1990; 15: 151–7.

[68] Uzonna JE, Kaushik RS, Gordon JR, Tabel H. Cytokines and antibody responses during Trypanosoma congolense infections in two inbred mouse strains that differ in resistance. Parasite Immunol. 1999; 21: 57–71.

[69] Kuriakose S, Muleme HM, Onyilagha C, Singh R, Jia P, Uzonna JE. Diminazene Aceturate (Berenil) Modulates the Host Cellular and Inflammatory Responses to Trypanosoma congolense Infection. PLoS One. 2012; 7: 1–8.

[70] Kulemina L V., Ostrov DA. Prediction of off-target effects on angiotensin-converting enzyme 2. J Biomol Screen. 2011; 16: 878–85.

[71] Shenoy V, Gjymishka A, Jarajapu YP, Qi Y, Afzal A, Rigatto K, et al. Diminazene attenuates pulmonary hypertension and improves angiogenic progenitor cell functions in experimental models. Am J Respir Crit Care Med. 2013; 187: 648–57.

[72] Mecca AP, Regenhardt RW, O'Connor TE, Joseph JP, Raizada MK, Katovich MJ, et al. Cerebroprotection by angiotensin-(1-7) in endothelin-1-induced ischaemic stroke. Exp Physiol. 2011; 96: 1084–96.

[73] Foureaux G, Nogueira JC, Nogueira BS, Fulgêncio GO, Menezes GB, Fernandes SOA, et al. Antiglaucomatous effects of the activation of intrinsic angiotensin-converting enzyme 2. Investig Ophthalmol Vis Sci. 2013; 54: 4296–306.

[74] Vainshelboim B. Exercise training in idiopathic pulmonary fibrosis: Is it of benefit? Breathe. 2016; 12: 130–8.

[75] Spruit MA, Janssen DJA, Franssen FME, Wouters EFM. Rehabilitation and palliative care in lung fibrosis. Respirology. 2009; 14: 781–7.

[76] Jastrzębski D, Gumola A, Gawlik R, Kozielski J. Dyspnea and quality of life in patients with pulmonary fibrosis after six weeks of respiratory rehabilitation. J Physiol Pharmacol. 2006; 57: 139–48.

[77] Holland AE, Hill CJ, Conron M, Munro P, McDonald CF. Short term improvement in exercise capacity and symptoms following exercise training in interstitial lung disease. Thorax. 2008; 63: 549–54.

[78] Vainshelboim B, Oliveira J, Yehoshua L, Weiss I, Fox BD, Fruchter O, et al. Exercise training-based pulmonary rehabilitation program is clinically beneficial for idiopathic pulmonary fibrosis. Respiration. 2014; 88: 378–88.

[79] Vu Thi Thu, Hyoung Kyu Kim and JH. Models, Acute and Chronic Exercise in Animal. Adv Exp Med Biol. 2017; 1000: 55–71.

[80] Nystoriak MA, Bhatnagar A. Cardiovascular Effects and Benefits of Exercise. Front Cardiovasc Med. 2018; 5: 1–11.

[81] Gobatto CA, De Mello MAR, Sibuya CY, De Azevedo JRM, Dos Santos LA, Kokubun E. Maximal lactate steady state in rats submitted to swimming exercise. Comp Biochem Physiol - A Mol Integr Physiol. 2001; 130: 21–7.

[82] Prata LO, Rodrigues CR, Martins JM, Vasconcelos PC, Oliveira FMS, Ferreira AJ, et al. Original Research: ACE2 activator associated with physical exercise potentiates the reduction of pulmonary fibrosis. Exp Biol Med. 2017; 242: 8–21.

[83] Morse JS, Lalonde T, Xu S, Liu WR. Learning from the Past: Possible Urgent Prevention and Treatment Options for Severe Acute Respiratory Infections Caused by 2019-nCoV. ChemBioChem. 2020; 21: 730–8.

[84] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020: 1–10.

[85]   Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci. 2020; 63: 457–60.

[86]   Zou Z, Yan Y, Shu Y, Gao R, Sun Y, Li X, et al. Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. Nat Commun. 2014; 5: 1–7.

[87]   Gu H, Xie Z, Li T, Zhang S, Lai C, Zhu P, et al. Angiotensin-converting enzyme 2 inhibits lung injury induced by respiratory syncytial virus. Sci Rep. 2016; 6: 1–10.