European Journal of Respiratory Medicine

2024; 5(1): 384- 388. doi: 10.31488/EJRM.140

Research Article

Inhaled Sodium Pyruvate Reduces Lung Inflammation and Fibrosis in a Bleomycin Rat Model

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Received: December 09, 2023; Accepted: December 28, 2023; Published: January 08, 2024

Abstract

Purpose: Interstitial lung disease encompasses a large group of chronic lung disorders associated with excessive tissue remodeling, scarring, fibrosis, and a decrease in lung function. Numerous studies have shown oxidative stress to be associated with interstitial lung diseases. Reactive oxygen species (ROS) can directly induce inflammation and tissue damage, especially in lung cells, which triggers myofibroblasts to produce interstitial fibrillar collagens and other fibrotic proteins resulting in fibrosis. The elevated production of these proteins by the activated myofibroblasts and their exaggerated accumulation in the interstitial space of lungs results in progressive fibrotic alterations. Sodium Pyruvate is a natural antioxidant that significantly reduces inflammatory cytokines IL-6, IL-8, MCP-1 and oxygen radicals including, hydrogen peroxide and peroxynitrite. Sodium pyruvate also increases the synthesis of nitric oxide. Current evidence from clinical trials indicate that NO exerts antifibrogenic effects during the early phase of fibrosis. As an anti-inflammatory and antioxidant, sodium pyruvate holds promise as a treatment for many lung diseases including pulmonary fibrosis. Materials and Methods: Rats received a single dose of bleomycin administered intratracheally at 0.5U/100g body weight in 0.3mls of a 0.15M sterile sodium chloride solution. Rats were then administered 0.3mls of sodium pyruvate, (5.0 mM, in 0.9% sodium chloride solution) or 0.9% sodium chloride (normal saline vehicle control) at various time points after bleomycin exposure. Results: Treatment with two doses of sodium pyruvate significantly reduced lung BAL protein and reduced inflammatory cell infiltrates in the lungs of bleomycin exposed rats compared to saline controls. Pyruvate treated animals also had a statistically significant improvement in lung compliance (87%) over saline (44%) (p=<0.0001). Furthermore, histopathological examination showed less fibrosis in the lungs of sodium pyruvate treated animals compared to saline controls. Conclusions: In the rat bleomycin model, sodium pyruvate decreased inflammation and lung fibrosis with only two treatments in two weeks. .

Keywords: Fibrosis, pyruvate, inflammation

Introduction

The association between chronic inflammation and oxidative stress is well documented [1-5]. Reactive oxygen species (ROS), such as superoxide anion, peroxynitrite, free hydroxyl radical, and hydrogen peroxide have been shown to be toxic to various mammalian cells and tissues [6, 7], including the lungs [8-10], and have been implicated in many human diseases, including pulmonary fibrosis [11]. Elevated levels of ROS induce a variety of pathological changes that are highly relevant in nasal and lung airway mucosa [12-15]. These include lipid peroxidation, increased airway reactivity, increased nasal mucosal sensitivity and secretions, production of chemoattractant molecules, increased vascular permeability and congestion [2-5, 12, 13]. Furthermore, hydrogen peroxide is known to increase inflammatory cytokines including IL-6 in both the nasal cavity and lungs [12, 16, 17].

ROS also activates latent TGF-β1 and the phenotypic conversion of quiescent fibroblasts and endothelial cells into profibrotic myofibroblasts [14, 15]. The newly generated myofibroblasts cells are capable of producing large amounts of interstitial fibrillar collagens and other fibrotic proteins. The elevated production of these proteins by the activated myofibroblasts and their exaggerated accumulation in the interstitial space of lungs results in severe progressive fibrotic lung diseases. Furthermore, these cells produce elevated ROS levels that are capable of inducing oxidative stress in various target cells as well as perpetuating the phenotypic changes in fibroblasts and endothelial cells, causing

their conversion into activated myofibroblasts. Additionally, ROS induced release and activation of TGF-β from the latent TGF-β protein complex in the extracellular matrix that can establish a paracrine pathway by causing further stimulation of NOX4 production. The activation of the ROS/NOX4 pathway creates a positive feedback loop resulting in chronic tissue fibrosis [18].

In the development of Neosporin, the addition of sodium pyruvate caused a marked inhibition of intracellular ROS and reduction in the expression of genes encoding fibrosis-associated proteins and abrogated several molecular pathways involved in the fibrotic process, including a significant reduction in the synthesis of TGF-β that reduced scar formation in wounds [19]. Previous studies demonstrate that pro-inflammatory cytokine levels were decreased by treatment with sodium pyruvate, which correlated with decreased ROS [20, 21]. Furthermore, the inhalation of sodium pyruvate in mice produced statistically significant reductions in IL-6 and IL-1β over the inhalation of saline controls [22].

In the early phase of the wound-healing process, NO is produced by macrophages and KCs, contributing to the restoration of lung functions. iNOS produces NO, which modulates collagen formation, cell proliferation, and wound contraction. NO down regulates type I collagen in dermal and cardiac fibroblasts, smooth muscle cells [14], and chondrocytes [15], and it induces antifibrotic effects in animal models of lung damage [23]. Importantly, the inhalation of sodium pyruvate increases the synthesis of nasal and lung nitric oxide [1], which may contribute to its antifibrotic wound healing effects.

The purpose of this study was to test the therapeutic value of sodium pyruvate in a fibrotic animal model. We demonstrate that sodium pyruvate decreases inflammation and improves lung function in the bleomycin fibrotic lung disease model in rats.

Materials and Methods

The Bleomycin rat model is the standard model to determine the effect of drugs in interstitial lung disease (pulmonary fibrosis). Rats were purchased from Charles River and all experiment protocols were performed as approved by the University of Connecticut and the FDA under IND 50,089. Seventy-two rats were divided into three study groups with 24 animals per group (untreated, saline treated, and pyruvate treated). The saline and pyruvate groups received a single dose of bleomycin administered intratracheally at 0.5U/100g body weight in 0.3mls of a 0.15M sterile sodium chloride solution. Untreated control rats were not treated with saline or pyruvate or injured with bleomycin. A single dose of 0.3mls of sodium pyruvate, (5.0 mM, in 0.9% sodium chloride solution) or 0.9% sodium chloride (normal saline vehicle control) was then administered by intratracheal injection 6 hours later. Six rats per study group (untreated, saline treated, and pyruvate treated) were sacrificed at each time period (24 hours, 72 hours, one week) for sample collection. Additionally, 6 rats per study group were sacrificed at two weeks post bleomycin. However, these animals were administered sodium pyruvate or saline control on the third and seventh-day post bleomycin insult as opposed to a single treatment at 6h post bleomycin. All animals were euthanized by CO2 asphyxiation.

Results

Effect of Intratracheal Administration of Sodium Pyruvate on Lung Injury Caused by Bleomycin

Table 1. Bleomycin Rat Study. Six rats were placed into each group (Control, Saline and Pyruvate) and time point (Day 1, 3, 7, 14). The control group was not administered bleomycin or any treatment. The saline and pyruvate groups were administered bleomycin and then treated with saline or pyruvate after 6 hours. Samples were collected at 1, 3 or 7 days after bleomycin injury. Six additional rats per group were treated with bleomycin and then treated with saline or pyruvate on day 3 and 7, and then, samples collected on day 14. Statistical analysis was performed using a two-way ANOVA with Sidak's post-hoc test. $p<0.05$ was considered statistically significant.

Table 1	Day 1	Day 3	Day 7	Day 14
	BAL protein (mg/ml)			
Control	0.01 ±0.02			
Saline	0.08 ±0.03	0.40 ± 0.07	0.38 ± 0.11	0.18 ± 0.04
Pyruvate	0.11 ± .02	0.56 ± 0.15	0.34 ± 0.01	0.16 ± 0.02
P value	0.9280	0.0020	0.8213	0.9830
	BAL Inflammatory Cells (x106/ml)			
Control	0.05 ± 0.1			
Saline	1.4 ± 0.4	5.0 ± 0.2	2.4 ± 0.6	3.6 ± 0.3
Pyruvate	1.2 ± 0.5	3.2 ± 0.9	1.9 ± 0.7	1.7 ± 0.3
P value	0.9474	<0.0001	0.3820	<0.0001
	Lung compliance (ml/cm H20)			
Control	0.75 ± 0.1			
Saline	0.42 ±0.04	0.50 ±0.05	0.45 ±0.07	0.42 ±0.06
Pyruvate	0.55 ±0.06	0.55 ±0.09	0.43 ±0.12	0.65 ±0.05
P value	0.0127	0.6568	0.9816	<0.0001

All bleomycin treated animals had lung injury as seen by the significant increases in bronchoalveolar lavage (BAL) protein levels and in total inflammatory cells, including lymphocytes, and a decrease in lung compliance (Tables 1-2). Both saline and pyruvate treatments had no effect on the progression of bleomycin induced lung injury in the acute stages (twenty-four hours to one week). At week two, there was a statistically significant reduction in inflammatory cells (-57%, p=<0.0001) in the bleomycin-pyruvate treated animals when compared to the bleomycin-saline treated animals. Pyruvate treated animals also had a statistically significant improvement in lung compliance (87%) over saline (44%) (p=<0.0001) (Table 1). At week two, there was a statistically significant reduction in lymphocytes (p<0.0001) and reduction in total cells found in the BAL, indicating a reduction in airway inflammation in the pyruvate treated animals (a return to normal), when compared to saline treated animals (Table 2). Finally, prior to sample collection, the researchers noted that the pyruvate treated rats on day 14 did not exhibit the labored breathing noted in the saline treated rats (data not shown). As reported by the investigators, analysis of rat lung tissue showed that sodium pyruvate decreased collagen deposition that inhibited fibrosis, specifically measured by changes in sub-epithelial matrix deposition, using histochemical and immunohistochemical staining.

Table 2. Percentage of leukocytes in BAL on day 14 after bleomycin injury in rats. Six rats were placed into each group (Control, Saline and Pyruvate). The control group was not administered bleomycin or any treatment. The other groups were treated with bleomycin and then treated with saline or pyruvate on day 3 and 7, and then, samples collected on day 14. Statistical analysis was performed using a two-way ANOVA with Sidak's post-hoc test. p<0.05 was considered statistically significant.

Table 2	Neut.	Lymph.	Alveo. Mac.
	Day 14 BAL cell percentages		
Control	3.0%±0.07	22%±1.09	74%±4.01
Saline	1.0%±0.05	51%±3.14	47%±5.10
Pyruvate	2.0%±0.04	30%±2.34	67%±4.3
P value	0.9298	<0.0001	<0.0001

Discussion

The approval of (Nintedanib and pirfenidone) offers a glimmer of hope for successful medical therapy for pulmonary fibrosis. Unfortunately, most of these therapies are associated with substantial toxicity, and although they slow disease progression, they are not curative. Importantly, the mechanisms by which these therapies act to affect lung fibrosis are incompletely understood, greatly hampering the development of drugs with reduced toxicity or identifying combination therapies that might act additively or synergistically to slow or prevent disease progression.

Here, we demonstrate that sodium pyruvate was effective in reducing inflammation and lung damage in this chronic fibrotic stage of lung injury in bleomycin treated rats. At week two, there was a statistically significant reduction in lymphocytes (p<0.0001) and reduction in total cells found in the bronchoalveolar lavage, indicating a reduction in airway inflammation in the pyruvate treated animals (a return to normal), when compared to saline treated animals. Finally, prior to sample collection, the researchers noted that the pyruvate treated rats on day 14 did not exhibit the labored breathing noted in the saline treated rats (data not shown). Analysis of rat lung tissue showed that sodium pyruvate decreased collagen deposition that inhibited fibrosis, specifically measured by changes in sub-epithelial matrix deposition, using histochemical and immunohistochemical staining. It was concluded that sodium pyruvate was effective in reducing inflammation and lung damage in this chronic fibrotic stage of the lung injury. (It should be noted that this type, of injury, with the subsequent fibrotic infiltration, is typical of the fibrosing group of interstitial diseases in humans.)

Clinically, the safety of sodium pyruvate has been proven, and it has been given to patients for a variety of other disorders ranging from Friedreich's ataxia [24] to open heart operations [25]. It has been administered via several routes including intravenous [25], topical administration for hyperkeratotic disorders [26], and in dietary supplementation [27]. In a study with 75 COPD patents, the inhalation of sodium pyruvate produced a statistically significant reduction in exhaled hydrogen peroxide, IL-8 and MCP1 when compared the placebo control in the same patients [21]. Thus, the ability of N115 to reduce H2O2 and inflammatory cytokine levels correlates with improved lung functions. In patients with Allergic Rhinitis, the Sodium pyruvate nasal spray reduced the severity of nasal inflammation and congestion caused by oxygen radicals [28]. The inhalation of sodium pyruvate also reduced nasal inflammation caused by oxygen radicals to reduce hypoxemia and dyspnea associated with chronic respiratory diseases [29]. In another study, nine patients with pulmonary fibrosis with COPD and 6 patients with idiopathic pulmonary fibrosis without COPD that remained on their normal medications, were administered the 20 mM (2.2 mg/mL) sodium pyruvate nasal spray 3 times per day for 22 days. The data from this study showed that coughing episodes per day was significantly reduced in all 15 patients. Especially important was the reduction of nightly coughing episodes. Nightly coughing episodes was reduced on day 8 by 30% (p = 0.007) and continued to decrease on day 14 by 55% (p = 0.0001) and on the 22 day of the trial, coughing decreased by 59% (p = 0.0001). This correlated with a significant (p = 0.010) improvement in nasal irritation/erythema with most patients being free of irritation by day 22 (p < 0.001); and a significant (p = 0.010) increase in the group average expelled NO by day 8 (1). Reducing nightly coughing episodes allowed these patients to finally get a full night's sleep. The inhalation of the 20mM sodium pyruvate nasal spray increases SaO2 by reducing hypoxemia and dyspnea in patients with various lung diseases including COPD, allergic rhinitis, sinusitis, long COVID and in patients with pulmonary fibrosis and IPF, clearly demonstrating the ability of sodium pyruvate to reduce lung and nasal inflammation and congestion [1, 28, 29].

In conclusion, in animal and human clinical trials, the inhalation of Sodium pyruvate (N115) has shown safety and efficacy regardless of the etiology of the diseases (COPD, Pulmonary fibrosis, Idiopathic pulmonary fibrosis and Cystic fibrosis) [1, 21, 28, 29] to reduce nasal and lung inflammation, inflammatory cytokines, and oxygen radicals that can reduced the activation of myofibroblasts, thus potentially reduce progression of lung

fibrosis.

Disclosure Statement

Alain Martin is the CEO of Emphycorp/Cellular Sciences Inc. Christopher R. Lupfer is a paid consultant for Emphycorp/Cellular Sciences Inc.

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To cite this article: Martin A, Lupfer CR. Inhaled Sodium Pyruvate Reduces Lung Inflammation and Fibrosis in a Bleomycin Rat Model. European Journal of Respiratory Medicine. 2024; 6(1): 384 - 388. doi: 10.31488/EJRM.140.