Research article

Inhaled Sodium Pyruvate Reduces Oxygen Radicals and Inflammatory Cytokines in COPD Patients

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Abstract

Background: As an anti-inflammatory and antioxidant, sodium pyruvate significantly reduces inflammatory cytokines and oxygen radicals such as IL-6, IL-8, MCP-1 and hydrogen peroxide. Thus, sodium pyruvate holds promise as a treatment for many lung diseases. Novel treatments for these conditions are needed as current medications, including steroids, often fail to treat severe symptoms. Methods: The data from two human clinical studies were analyzed for the effect of nebulized 0.5mM, 1.5mM or 5.0mM sodium pyruvate (N115), in patients with COPD. Hydrogen peroxide and inflammatory cytokine/chemokine levels were evaluated compared to a placebo control or a no-treatment baseline control. Results: Nebulization of sodium pyruvate in COPD patients significantly improved respiratory H_2O_2 (62% reduction compared to saline, p=0.0427) and inflammatory cytokines (80% reduction in IL-8, p=0.0001; 65% reduction in MCP-1, p=0.0001). Conclusions: Sodium pyruvate was safe and effective at reducing inflammatory markers including inflammatory cytokines and oxygen radicals. Although the trials reported here had small sample sizes and some were not blinded, these data provide a first look at the mechanisms by which sodium pyruvate improves inflammation in the human respiratory tract.

Keywords: Pyruvate, anti-inflammatory, COPD

Introduction

Oxidative stress is well known to contribute to chronic inflammation in lung diseases like COPD [1-4]. Reactive oxygen species (ROS), encompass a wide variety of molecules including superoxide anion, peroxynitrite, free hydroxyl radical, and hydrogen peroxide that are toxic to various tissues [5-9]. Elevated levels of ROS damage tissues through a variety of mechanisms including lipid peroxidation, production of cytokines and chemokines, and increased vascular permeability [1-4, 10-12]. Hydrogen peroxide, in particular, can increase inflammatory cytokine levels in the nasal cavity and lungs [12-14].

Importantly, antioxidant therapy has shown promise in animal models of inflammatory lung diseases [15-17]. In addition to its role in metabolism, pyruvate is an endogenous antioxidant that can neutralize ROS [18-20]. Exogenous administration of sodium pyruvate has protective antioxidant activity in various tissues [20-26] and decreases pro-inflammatory cytokine levels in vitro and in vivo [27, 28] .

COPD is a mechanistically complex disease, but can be classified as an inflammatory disease of the airways characterized by increased inflammatory cells (neutrophils, eosinophils, and lymphocytes) and increased inflammatory markers including hydrogen peroxide [29, 30]. Therapeutic approaches to managing this disease include various inhaled compounds like bronchodilators (e.g. beta agonists) and both inhaled and systemic steroid treatment [11, 13, 14, 29, 30]. However, the current therapies are not without adverse side effects and none of these treatments eliminate oxygen radicals [11, 13, 14, 29, 30].

Clinically, sodium pyruvate is safe [31, 32] and can be administered orally [33] and intranasally [34, 35]. Thus, sodium pyruvate is a potential alternative to current therapies for COPD to combat ROS and limit cytokine levels and inflammation. Importantly, sodium pyruvate has been used to reduce the severity and symptoms of all lung and sinus diseases tested thus far including COPD, pulmonary fibrosis, COVID-19 and Long COVID [34, 35].

The purpose of this study was to test the safety, antioxidant, and anti-inflammatory effects of sodium pyruvate in patients with COPD. We demonstrate that sodium pyruvate significantly decreases hydrogen peroxide and inflammatory cytokines in COPD patients.

Methods

Clinical trials

Informed consent was obtained prior to enrollment in all studies and all studies were reviewed under FDA IND 50089. There were 67 patients participating in two different clinical trials (Table 1). Specific details for each trial are given below. If patients were using other inhaled drugs, those were discontinued prior to participation in these trials. For all studies, a medical history was obtained, and a physical exam performed during the initial visit by a staff physician. Routine Blood analysis was performed, and vital signs (pulse rate, respiratory rate, and blood pressure) were checked. A urine pregnancy test was given to all women of childbearing age. Finally, an ECG and chest x-ray were performed.

For both studies, the exclusion criteria are as follows:

a. Pulmonary disease other than COPD

b. Clinically significant cardiac disease including uncontrolled congestive heart failure and unstable angina

c. Pregnancy

d. Females of childbearing potential age not on adequate contraception.

e. Lactating females

f. Subjects receiving systemic corticosteroid treatment within one month of screening visit

g. Subjects receiving inhaled corticosteroid treatment within 15 days of screening visit

h. Less than 18 years of age (except study 2 and 6, where the exclusion was less than 12 years of age)

i. Hospitalization within last 6 months due to acute exacerbation of airway disease

j. Subjects on escalating dose of immunotherapy

k. Subjects with a clinically significant abnormal chest x-ray within past 12 months

l. Medication changes within 1 month

m. Subjects who have participated in another investigational drug treatment study within the last month

n. Subjects with a current history of alcohol or recreational drug abuse

o. Subjects who have taken vitamins with antioxidant properties (E or C) or dietary supplements containing pyruvate within 24 hours prior to the screening visit

Inclusion criteria are as follows:

a. Study 1: Patients were recruited with mild COPD/bronchiolar asthma defined as 60-80% predicted FEV_1 or >12% reversibility to bronchodilator.

b. Study 2: Individuals with a clinical diagnosis of moderate to severe COPD, $\geq 50\%$ but <70% predicted FEV_1 and a stable pulmonary disease status were recruited for the study.

Study 1

Initially, fifteen healthy subjects were treated with a single nebulized dose of 0.9% saline as the placebo vehicle, and safety parameters and lung function were measured over a 240-minute experimental period to establish a baseline. Later, these same subjects were administered 5ml of either 0.5 mM, 1.5 mM or 5.0 mM sodium pyruvate (5 subjects per dose) in a 0.9% sodium chloride solution by nebulization. The safety and lung function measurements were again recorded. A DeVilbiss Pulmo-Aide Compressor and a Hudson RCI Up-draft Nebulizer were used for compound administration in this study.

Following the safety portion of the study, 45 individuals with mild COPD/bronchial asthma, were enrolled in the study protocol. These patients had a clinical diagnosis of the disease and during the screening visit demonstrated either an FEV_1 between 60-80% of predicted, or greater than or equal to 12% increase in

Table 1. Demographics for clinical patients from all eight clinical trials. All subjects were previous smokers and had asthma with a COPD component.

Study	Gender	Age Ave. (range)	Patient's Stated Ethnicity	Study Design
1	F=40 M=20	36.6 (18-66)	Caucasian=32 Hispanic=22 Black=5 Asian=1	Open label placebo-control
2	F=2 M=5	46.6 (18-66)	Hispanic=7	Double-blind placebo-control

FEV₁, post bronchodilator inhalation. Each patient was tested before and after treatment for hydrogen peroxide content in their breath condensate as an indicator of reactive oxygen species in the lung. Collection of exhaled breath for H_2O_2 analysis (Pre-Drug) was followed by administration of the 0.9% saline placebo by nebulization for 15 minutes. Then followed by collection of a second exhaled breath for H_2O_2 analysis one hour later (Post-Drug). On day 3, collection of exhaled breath for H_2O_2 analysis was followed by administration of single dose of 5mls of a 0.5 mM sodium pyruvate in 0.9% sodium chloride solution by nebulization for 15 minutes followed by collection of a second exhaled breath for H_2O_2 analysis one hour later. When the first fifteen patients had completed the study, a second group was enrolled. These patients followed the same protocol but were treated with nebulized 1.5 mM sodium pyruvate in 0.9% sodium chloride solution or saline control. Finally, 15 more patients were enrolled and treated with 5.0mM sodium pyruvate in 0.9% sodium chloride solution and compared to a saline control. All treatments and sample collections were performed in the clinic. For the 0.5mM pyruvate treatment, 14 of 15 patients completed the study. One could not product enough breath condensate for accurate analysis. For the 1.5mM pyruvate treatment, 13 of 15 completed; 2 could not produce enough breath condensate for analysis. Finally, for the 5.0 mM pyruvate treatment, 5 completed and 10 did not because of weather conditions. Due to insufficient completion, the 5.0mM data were not analyzed or included in the manuscript.

Measurement of H_2O_2

Expired breath condensate was collected by using a glass condensing device with an inner glass chamber that contained ice and was suspended in a larger chamber. Condensate was formed on the outside surface of the inside glass that was separated from ambient air. After rinsing their mouths, subjects breathed tidally through a mouthpiece connected to the inlet for 15 min while wearing a nose-clip. The mouthpiece was also used as a saliva trap. Approximately 1 mL of breath condensate was collected and stored at -70°C. H_2O_2 was measured using a colorimetric assay. Briefly, 100 μl of condensate was mixed with 100 μl of 420 μM 3'3'5'5'-tetramethylbenzidine in 0.42 M citrate buffer pH 3.8 and 10 μl of horseradish peroxidase (52.5 U/mL). The samples were incubated at room temperature for 20 min and the reaction stopped by the addition of 10 μl of 2 N sulfuric acid. The product was measured spectrophotometrically (Model AR 8003; Labtech Int. Ltd., Uckfield, UK) at 450 nm. A standard curve of H_2O_2 was performed for each assay with a detection limit of 0.1 μM.

Study 2

The subjects were told to administer 5ml of a 0.5mM sodium pyruvate solution or 0.9% saline control by nebulization (a) upon waking (between 7:00 and 9:00 am) but after flow meter reading; (b) during mid-day (2:00 to 4:00 pm); and (c) before bedtime (between 9:00 and 11:00 pm) but after flow meter reading. This was done daily for 21 days. Patients completed a daily log, which was used to verify patient compliance. All patients were compliant. Sputum for cytokine/chemokine analysis was collected at Day 3 and Day 14. Analyses were conducted for IL-6, IL-10, TNFα, IL-8, MCP-1 and Elastase. Nebulization was performed using a Pari Proneb Ultra Compressor and a Micro Air (NE-U22V) Nebulizer (Omron).

Cytokine testing

Sputum samples were collected in the clinical setting over a 12-minute period during which time the subject inhaled 3% physiological saline via a handheld nebulizer. Prior to the inhalation of 3% saline, the subject cleared themselves of as much saliva as possible by blowing their nose and spitting into a container. Sputum was then collected in 2–3-minute intervals (or upon need) after the inhalation of 3% saline. The sputum samples from each subject were pooled in a 50 mL conical polypropylene centrifuge tube, weighed, kept on ice, and processed within 2 hours of collection. The sputum sample was diluted 1:4 with phosphate buffered saline and then with an equal volume of 10% Sputolysin. The sputum solution was incubated in a stirring water bath for 15 minutes at 37°C. The cells were separated from the fluid via centrifugation at 250 x g for 10 minutes and the fluid stored in aliquots ($\leq$ -70OC) for measurement of various pro-inflammatory markers. Prior to freezing, one aliquot of fluid was treated with protease inhibitors PMSF and EDTA.

Sputum samples were analyzed at the University of Connecticut for the inflammatory markers, Interleukin-8 (IL-8), Monocyte Chemotactic Protein-1 (MCP-1), IL-6, IL-10, TNF-α, and elastase. The samples were thawed and pretreated with Protease Inhibitor Cocktail (Product #P8340; Sigma, St. Lois MO) at a ratio of 1μL per 500 μL sputum. Samples were then concentrated in Microcon YM-10 concentrators in an Eppendorf Centrifuge (1500RPM x 20min) and retentate utilized for cytokine and chemokine analyses. Samples were analyzed using a MILLIPLEX MAP Human Cytokine/Chemokine Panel (Cat #MPXHCYTO-60K; Millipore, Billerica, MA) following the manufacturer's instructions.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 6. Analyses for specific data sets are indicated in figure legends.

Results

Study 1: Examine N115's effect on expired hydrogen peroxide in patients with COPD

Elevated levels of hydrogen peroxide increase lung and sinus inflammation and the production of inflammatory cytokines [1-5, 12]. Thus, the primary objectives of this study were to examine safety and to determine the efficacy of various doses of nebulized sodium pyruvate on reactive oxygen species (hydrogen peroxide). This was a phase I/II study conducted at the University of Connecticut Health Center and Hospital for Special Care in Connecticut, and at Yale University School of Medicine, Yale New Haven Hospital, New Haven, Connecticut. As a preliminary test, we conducted an open label, placebo-controlled study as proof of concept. Patient demographics are presented in Table 1.

An initial safety study was performed. Fifteen healthy subjects were treated with a single 5ml nebulized dose of 0.9% saline as the placebo. Later, these same subjects were divided into 3 groups and administered a single dose of 5ml of nebulized 0.5 mM, 1.5 mM or 5.0 mM sodium pyruvate in 0.9% saline

solution. There were no significant differences observed in vital signs, blood chemistries, hydrogen peroxide, FEV_1, or PEF after treatment with any of the doses of sodium pyruvate when compared to the subject's baseline values after receiving 0.9% saline placebo. There were no serious adverse events reported for any of the subjects. There were three non-serious adverse events reported, two were not related to the study and one was "very likely related" to the study (dry mouth). No action was taken, and the subject recovered without sequalae.

Following the safety portion of the study, 45 individuals with mild COPD/bronchial asthma, were enrolled in this open label protocol. Each patient was tested before and after a single 5ml nebulized treatment with saline placebo and before and after a single 5ml nebulized sodium pyruvate treatment for hydrogen peroxide content in their breath condensate as an indicator of reactive oxygen species in the lung. Three different doses of sodium pyruvate were tested 0.5, 1.5 and 5.0mM in 15 patients each. Because only 5 of 15 patients finished the trial at the 5.0mM dose, do to winter weather, these data were excluded from the study and not included in the analysis. There was a statistically significant reduction in hydrogen peroxide with the sodium pyruvate treated patients when compared to the saline placebo with the 0.5mM (55% reduction p=0.0459) and with the 1.5mM nebulized sodium pyruvate formula (62% reduction p=0.0427) (Table 2). During the trial, there was also a significant percentage difference in FEV_1 values between the pyruvate group and the saline group after drug administration. FEV_1 values in those subjects administered 0.5mM sodium pyruvate averaged a 8% increase over saline treated patients (p<0.0001), and subjects administered 1.5mM sodium pyruvate averaged a 4% increase over saline treated patients (p<0.0001), (Table 2).

Study 2: Effects of sodium pyruvate on inflammatory cytokines in N115 treated COPD patients

As H_2O_2 is known to affect inflammation and inflammatory cytokine production [1-5, 12], we conducted a small pilot study of confirmed COPD patients to examine cytokine levels in the respiratory tract. This was a Phase II, double blind, placebo-controlled study conducted at the Instituto Nacional de Enfermedades Respiratorios, Mexico City, Mexico. Randomization was performed by computer generated random numbers that were applied to placebo and drug packaging. Both the patients and the clinicians were blinded to the patient's allocation. Patient demographics are presented in Table 1.

Patients were treated with placebo or sodium pyruvate daily for 21 days. Sputum for cytokine/chemokine analysis was collected on Day 3 and Day 14. Analyses were conducted for IL-6, IL-10, TNFα, IL-8, MCP-1 and Elastase. The concentrations of IL-6, IL-10, TNFα and elastase were too low to detect, so were not reported. At the 3-Day sample collection period, the subjects who received saline had a 32% and 31% reduction in IL-8 and MCP-1 respectively from baseline measurements on day 0. Patients treated with pyruvate therapy had a similar 33% and 32% reduction in IL-8 and MCP-1 respectively at Day 3 compared to their day 0 baseline (Table 3). After 14 days, treatment with saline showed no further change in MCP-1 and IL-8 increased by 144% compared to day 0 baseline (Table 3). Conversely, pyruvate showed an 80% reduction in IL-8 (p<0.0001) and a 65% reduction in MCP-1 (p<0.0007) compared to the day 0 baseline. During the trial, there was also a significant percentage difference in FEV_1 values between the pyruvate group and the saline group after drug administration. FEV_1 values in those subjects administered sodium pyruvate averaged a 12.7% increase by day 3 of the study compared to the saline group that averaged only a 2.1% increase (p=0.0023, Table 3). There was also an improvement in FEV1 on day 14 where saline treated patients averaged a 6.1% increase versus an 11.0% increase in sodium pyruvate treated patients, but this did not achieve statistical significance (p=0.06). These data suggest that improved pulmonary function precedes lower cytokines levels rather than resulting from them.

Discussion

The purpose of this research was to test the safety and therapeutic value of sodium pyruvate in patients with COPD. In our safety study with three doses of sodium pyruvate ranging from 0.5-5.0mM, there were no severe adverse events reported. From the standpoint of safety, sodium pyruvate is part of the body's natural endogenous metabolic and antioxidant systems. As a natural metabolite, it is not surprising that it has an excellent safety profile. It is secreted by cells, readily enters cells, and can react with ROS to detoxify them [18-20]. Additionally, sodium pyru-

Table 2. Change in breath condensate H_2O_2. H_2O_2 levels were measured in breath condensate before and after treatment with 5ml nebulized 0.5mM sodium pyruvate in 14 patients or 1.5mM sodium pyruvate in 13 patient's vs treatment with saline in the same patients. Patient lung function was also assessed by measuring forced expiratory volume in 1 second (FEV_1) and determining the percentage change compared to baseline after treatment with saline or sodium pyruvate. Statistical analysis was performed using a two-way ANOVA with Sidak's post-hoc test for H_2O_2 and an unpaired two-tailed student's t-test for FEV_1. p<0.05 was considered statistically significant.

Treatment	H_2O_2 (µM) (Baseline)	H_2O_2 (µM) (Post treat.)	P value	FEV_1 (% change)	P Value
Saline (0.9%)	1.12 ± 0.78	1.36± 0.93	0.466	3.0±1.0	
Pyruvate (0.5mM)	1.21± 0.75	0.55± 0.44	0.0459	11.0±3.0	<0.0001
Saline (0.9%)	1.08 ±1.4	0.80 ±0.89	0.5485	0.0±0.5	
Pyruvate (1.5mM)	2.43 ±2.44	0.93 ±1.29	0.0427	4.0±0.8	<0.0001

Table 3. Effects of sodium pyruvate on inflammatory cytokines in COPD patients. Absolute values and percentage change from day 0 baseline of inflammatory cytokines in sputum of patients treated with the 0.5mM nebulized sodium pyruvate (4 patients) or a 0.9% sodium chloride placebo control (3 patients) in COPD patients. Patient lung function was also assessed by measuring forced expiratory volume in 1 second (FEV_1). Statistical analysis was performed using an unpaired two-tailed student's t-test. $p < 0.05$ was considered statistically significant.

Treatment (Day3)	IL-8(pg/ml) (Baseline)	IL-8(pg/ml) (Post treat.)	(% change)	P Value
Saline (0.9%)	425±6	289±7	-32%±6	
Pyruvate (0.5mM)	217.8±8	152.5±5	-33%±7	0.8510
Treatment (Day3)	MCP1(pg/ml) (Baseline)	MCP1(pg/ml) (Post treat.)	(% change)	P Value
Saline (0.9%)	24.1±2.3	14.4±1.2	-31%±8	
Pyruvate (0.5mM)	14.7±1.4	9.4±0.9	-32%±7	0.8668
Treatment (Day3)	FEV$_1$ (ml/s) (Baseline)	FEV$_1$ (ml/s) (Post treat.)	(% change)	P Value
Saline (0.9%)	47.0±3.2	48.0±2.2	2.1%±0.7	
Pyruvate (0.5mM)	62.6±3.6	71.7±3.6	12.7%±3.1	0.0023
Treatment (Day14)	IL-8(pg/ml) (Baseline)	IL-8(pg/ml) (Post treat.)	(% change)	P Value
Saline (0.9%)	90.9±7	131.1±6	144%±18	
Pyruvate (0.5mM)	325±8	64±1	-80%±16	<0.0001
Treatment (Day14)	MCP1(pg/ml) (Baseline)	MCP1(pg/ml) (Post treat.)	(% change)	P Value
Saline (0.9%)	9.3±1.3	9.3±1.6	0%±5	
Pyruvate (0.5mM)	16.6±1.2	5.9±0.6	-65%±5	0.0007
Treatment (Day14)	FEV$_1$ (ml/s) (Baseline)	FEV$_1$ (ml/s) (Post treat.)	(% change)	P Value
Saline (0.9%)	49.8±3.6	53.0±4.6	6.1%±1.1	
Pyruvate (0.5mM)	62.8±3.9	70.6±3.1	11.0%±3.3	0.0600

vate prevents nitric oxide from forming toxic peroxynitrite in the presence of hydrogen peroxide, both of which are elevated in COPD [1-4]. Furthermore, we demonstrate that sodium pyruvate can not only significantly decrease oxidative stress (lowered H_2O_2 levels in Study 1), but also decrease inflammatory cytokine and chemokine levels (IL-8 and MCP-1 in Study 2).

The association between oxidative stress and chronic inflammation in lung diseases like COPD is well documented [1-4, 10]. As reactive oxygen species are toxic to various mammalian tissues [9], and elevated levels of ROS can induce production of chemo attractant molecules and increased vascular permeability [1-4, 10-12], we propose that the decreased hydrogen peroxide observed in COPD patients treated with N115 subsequently resulted in decreased IL-8 and MCP-1. Overall, this mechanism agrees with other studies where antioxidant treatment decreased inflammation and cytokine/chemokine responses both in vitro [20-24] and in vivo [25, 26]. More specifically, it was effective in several animal models of inflammatory lung diseases [10-12].

The data presented have some limitations. All studies were preliminary and subject enrollment was small. Study 1 was an open labeled study but patients were compared to a placebo control. Although bias is a confounding factor, the data are still informative, as they agree with the data in study 2, where clinical staff and patients were blinded (patients were assigned to groups by computer randomization), and a saline placebo was used as a control. In the future, more blinded, placebo-controlled trials

with larger patient enrolment are needed to address the therapeutic potential of sodium pyruvate in COPD and other respiratory diseases. We have shown that sodium pyruvate improves symptoms associated with long-COVID [34]. We have future studies planned to examine the ability of sodium pyruvate to treat pulmonary fibrosis resulting from COVID-19 infection and the contribution of pulmonary fibrosis in long-COVID.

In conclusion, the two current human clinical trials, as well as previously published trials, suggest that inhalation of sodium pyruvate (N115) is safe and effective regardless of the etiology of the respiratory disease (COPD, Pulmonary fibrosis, COVID-19, Long COVID) [34, 35], resulting in improve breathing and reduced inflammation, inflammatory cytokines, and oxygen radicals.

Author Contributions

Dr. Alain Martin: Conceptualization (Lead), Data Curation (Lead), Project Administration (Lead), Resources (Lead), Funding (Lead), Methodology (Supporting), Supervision (Lead), Validation (Equal), Writing-original draft (Lead), Writing-review & Editing (Equal), Formal analysis (Supporting). Dr. Christopher

Lupfer: Validation (Supporting), Writing-original draft (Supporting), Writing-review & Editing (Equal), Visualization (Lead), Formal analysis (Lead). Dr. Ronald Amen: Validation (Equal), Writing-original draft (Supporting), Supervision (Supporting), Formal analysis (Supporting).

Conflicts of Interest

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