

4th Year Selectives Proposal

Student's Name: Richard Thrasher
Student's Phone: 224-1007 (home) 7088661 (pager)
Dates: 12/1/98-12/30/98 & 3/1/98-3/30/98
Selective Category: Research
Location: John Dempsey Hospital & Hospital for Special Care (New Britain)
Name and Address of Advisor: Roger Thrall, Ph.D.
UConn Health Center Rm L-2078
Phone: ~~263-2841~~ 679-3585
Fax: 679-4827

I. Phase I Clinical Trial of Inhaled Sodium Pyruvate on Bronchial Asthma

II & III. Hypothesis & Description of Importance of Study

Bronchial asthma is a disease which involves the conducting airways of the respiratory track and affects 5-10% of individuals in the US. The morbidity and mortality associated with this disease are increasing in both the US and worldwide'. The mechanisms involved in the pathogenesis of bronchial asthma are complex but it can be classified as an inflammatory disease of the airways characterized by an increase in inflammatory cells both in the mucosa and in bronchoalveolar lavage fluid*. Some of the many therapeutic approaches to managing this disease include various inhaled compounds, such as beta agonists and both inhaled and systemic steroid treatment. The purpose of this study is to test the therapeutic value of an endogenous compound, sodium pyruvate, with anti-inflammatory properties (anti-oxidant) in patients with bronchial asthma.

Reactive oxygen species (ROS), such as superoxide anion, free hydroxyl radical, and hydrogen peroxide, have been shown to be toxic to various mammalian tissue³, including lung⁴⁻⁶, and have been implicated in many human diseases⁷. Anti-oxidant therapy has been shown to be effective in several animal models of inflammatory lung disease⁸⁻¹⁰. Sodium pyruvate is a part of the body's natural endogenous anti-oxidant defense system. It is secreted by cells, readily enters cells, and can react with peroxide to detoxify¹¹⁻¹³. Since hydrogen peroxide is also a precursor to other ROS, inhibition of it has broad anti-oxidant effects. Sodium pyruvate has been shown to have protective anti-oxidant activity both in vitro^{11,14-17} and in vivo¹⁸⁻¹⁹.

Clinically, sodium pyruvate has been given to patients for a variety of disorders ranging from Friedreich's ataxia²⁰ to open heart operations²¹. It has been administered via several routes including intravenous²⁰⁻²³, topical administration for hyperkeratotic disorder?, and in dietary supplementation²⁵⁻²⁹. Although sodium pyruvate has been given to patients via several routes, it has not yet been administered via the airways as is proposed in this trial. Animal model studies in rats and rabbits were conducted to assess potential adverse effects. Rats were used to assess the impact of sodium pyruvate administered directly to the lungs by injection. Rabbits were used to assess the impact of

sodium pyruvate administered as an inhaled mist using a nebulizer. There were no adverse effects noted in the parameter which were monitored for acute lung injury (histology, bronchoalveolar lavage, fluid content, lung compliance, and arterial blood gas analysis). In addition, it was shown that the intratracheal administration of sodium pyruvate was beneficial in diminishing the development of acute lung injury in rats induced with bleomycin. The generation of toxic radicals is believed to be a major mechanism of bleomycin lung toxicity.

There are two groups of asthma medications: 1) bronchodilator that help stop asthma attacks after they have started and 2) anti-inflammatories help to prevent asthma attacks from beginning. Asthma attacks occur in two stages. The first stage is characterized by a rapid airway constriction and obstruction by mucosal agents over a period of minutes. The second stage of asthma attacks occurs 6-10 hours after the initial attack, resulting in air passages narrowing and the inflammatory production of mucous. The second phase of asthma is the most damaging, as the inflammation occurs on a cellular level and becomes a self-perpetuating cycle of attacks followed by an increased sensitivity to external stimuli, which lead to another attack. It is at this stage of asthma that sodium pyruvate is expected to be beneficial because of its action at the cellular level, by reducing the hydrogen peroxide levels and resuscitating the injured cells.

Again, the importance of this study is manifest by the demonstrated safety of sodium pyruvate in both animal and human models, and by its reported efficacy both in vitro and in vivo on reactive airway disease.

IV. Methods

- A. Subject Selection-test subjects will be recruited from outpatient clinics in the area (UConn, Grove Hill, New Britain General, and Hospital for Special Care). A minimum of 45 test subjects diagnosed with bronchial asthma will be recruited for this study. There will also be 15 healthy test subjects used as a control population.
- B. Inclusion Criteria-Normal controls will be healthy individuals with no known or detectable pulmonary or cardiovascular disease. Test group includes those with bronchial asthma. Test subjects with a clinical diagnosis of bronchial asthma with $<80\%$ predicted FEV₁, and a spirometry within the last year showing 12% or $>$ improvement in FEV₁, in response to inhaled bronchodilators. A full spirometry will be done at the screening visit to ensure that the subjects satisfy these criteria. All study subjects will have a chest X-ray taken within a year of the study, and all those with abnormal CXR's will be excluded.
- C. Exclusion Criteria -
 1. Subjects with Severe Asthma (FEV₁, $< 60\%$ predicted)
 2. Cardiopulmonary disease other than bronchial asthma
 3. Pregnancy
 4. Females of child bearing potential not on adequate contraception or lactating
 5. Subjects with systemic steroid treatment within one month
 6. <18 yo

7. hospitalization within last 6 months due to acute exacerbation of airway disease
 8. subjects on escalating dose of immunotherapy
 9. recent dose changes in asthma medication
 10. recent addition of new drugs
 11. subjects who have participated in another investigational drug treatment within the previous month
 12. subjects who are currently using tobacco products
 13. subjects who are using recreational drugs
- D. Washout – subjects who have not taken the following Albuterol in the last 8 hours, Serevent in the last 12 hours, or Atrovent in last 72 hours prior to visit.
- E. Special Exclusions for Day of Visit
1. Subjects who have not adhered to above washout times
 2. Individuals that have taken vitamins with anti-oxidant properties (E or C) or dietary supplements containing pyruvate at least 24 hours prior to start of study
 3. Subjects who have had changes in medication since last visit
 4. Subjects who need to use inhaled medication on day of visit
- F. Assignment Number and Randomization – as enrolled, each subject will be sequentially issued a unique subject number. The subjects at UConn will start with 001. Those from Hospital for Special Care will begin with 100. Once a number has been assigned it cannot be re-assigned. Subjects may withdraw at any time without prejudice. Details of the reason why a subject terminates participation will be recorded in their chart. Replacement subjects will be added with new numbers as needed.
- G. Discontinuation Criteria for Individual Subjects
1. subject demonstrates a drop of 10% or > in FEV₁, or a >25% decrease in PEF in response to inhaled saline or drug.
 2. Subjects with clinically significant asthma exacerbation manifested by chest tightness and wheezing
 3. In the event that two of the five healthy subjects or six of fifteen asthma subjects exhibit severe reaction to a given dose of the drug, then the study will not continue to proceed until a full review is instituted.
- H. Subject Procedures
1. Routine Testing – a medical history will be taken and a routine physical exam will be given during the screening visit (including an EKG). Blood will be drawn for CBC, Chem-7, and LFT's at the beginning of the trial and at its completion, Prior to and after the administration of placebo and drug, both vital signs and 12 lead EKG's will be obtained. Vital signs will also be repeated before and after each spirometry test. All study subjects must have a CXR taken within a year of the study and have the results available, otherwise a new one must be obtained and evaluated prior to admission in the study.
 2. Baseline Measurements – Spirometry will be performed to obtain at least three flow/volume loops at each designated time as determined by the FDA, Hydrogen Peroxide Measurement – subjects will breathe through a

sterile mouthpiece with a 2-way valve and the expired air conducted through a tube (2 feet long and wide enough not to increase resistance) with a cooling collection system to form breath condensate. A sufficient volume of 1 ml is usually collected within 15 minutes. The hydrogen peroxide levels will be measured in this condensate via photospectrometry. Levels of hydrogen peroxide in expired breath condensate have been shown to be elevated in patients with COPD³⁰⁻³¹ and in patients with ARDS^{4,32-33}. This procedure will be performed under the direct supervision of a respiratory therapist.

3. Drug Administration – a standard hand held nebulizer will be used to administer the sodium pyruvate or physiological saline as a placebo. Test drug will be provided by Cellular Sciences, Inc. Nebulization and inhalation will occur under direct supervision of a pulmonary physician. A 5 ml volume of saline or drug will be administered via nebulization and inhalation.

I. Protocol

1. Duration – in this study there will be at least 3 visits planned for each subject. Visit 1 is a screening visit that will last about 1 hour. Visits 2 & 3 will last for approximately 4 hours each during which time the subject will remain in the hospital. A follow-up contact of the patient will be made 8 hours after administration of the drug.
2. Study Drug – confidential (available upon request, but has already been approved by UConn IRB and FDA)
3. General Divided into 3 Stages
 - a. First stage involves only the healthy subjects, whereas stages 2 and 3 will involve those with bronchial asthma. Physiological saline will be inhaled as a placebo and subjects meeting the criteria for the administration of the drug will be scheduled for a return visit. Three concentrations of the drug will be administered. The healthy subjects and the asthmatic subjects will be divided into three groups. Each group will receive only 1 concentration of the drug. The first group will receive the lowest concentration. After this group successfully received this concentration, the second group will receive a concentration 3x that amount. With demonstrated safety at this stage, then the third group will receive a concentration at 10x the first dose. Each subject will inhale 5 ml of the nebulized drug at the desired concentration. In stage 2, a baseline FEV₁ will be obtained for each subject with bronchial asthma in response to inhaled physiological saline. Spirometry testing will be performed prior to and at designated times after administration of the saline. If the subject then meets the inclusion criteria, the subject will be entered into the next stage which involves administration of the drug.

J. Laboratory Measurements of Hydrogen Peroxide in Expired Breath

1. briefly 1 ml of breath condensate will be added to 1 ml of tetramethyl benzidine in the presence of horseradish peroxidase for 30 minutes at

room temperature, The solution is then acidified with sulfuric acid and read at a wavelength of 450 nm in a spectrophotometer. The absorbance has been shown to be proportional to the concentration of hydrogen peroxide. A standard curve, using known concentrations of hydrogen peroxide will be used for quantification. Specificity of the assay will be determined by inhibition with the addition of catalase.

2. Statistical analysis will be performed using Student's paired and unpaired T-test.

V. Guidance

- A. provided by Dr. Thrall during laboratory evaluation and by selected clinicians at each location who will be responsible for monitoring patient health during time of drug administration.
- B. the evaluation of breath condensate will take place in Dr. Thrall's lab as outlined above.

VI. Analysis of Data – see above

- A. Because of the confidential nature of the project since it is a phase I clinical trial funded entirely by venture capitalists with high amounts of money at stake, a paper and presentation will be produced in publishable form but not so submitted, The evaluation of performance will be done by Dr. Thrall who will evaluate the student's performance and comprehension of collected data.

VII. REFERENCES

1. Current estimates from the National Health Interview Survey United States 1992: Data from the National Health interview Survey. Series 10, No. 189 (January 1994).
2. McFadden ER, Gilbert IA. Asthma *New Engl J Med* 327:1928-1939, 1992.
3. Jamieson D. Oxygen toxicity and reactive oxygen metabolites in mammals. *Free Rad Biol & Med* 7:87-108, 1989.
4. Baldwin SR, Grum C, Boxer LA, Simon RH, Ketani LH, Devall LJ. Oxidant activity in expired breath of patients with adult respiratory distress syndrome. *Lancet* 8471:11-13, 1986.
5. Ward PA, Till GO, Hetherill JR, Annesley TM, Kunkel RG. Systemic complement activation, lung injury, and products of lipid peroxidation. *J Clin Invest* 76:517-527, 1985.
6. Perkowski SZ, Havill AM, Flynn JT, Gee MH. Role of intrapulmonary release of eicosanoids and superoxide anion as mediators of pulmonary dysfunction and endothelial injury in sheep with intermittent complement activation. *Circ Res* 53:574-583, 1983.
7. Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, McCord JM, Harman D. Oxygen radicals and human disease. *Ann' Int Med* 107:526-545, 1987.
8. Bernard GR, Lucht WD, Neidermeyer ME, Snapper JR, Ogletree ML, Brigham KL. Effect of N-acetylcysteine on the pulmonary response to endotoxin in the awake sheep and upon in vitro granulocyte function. *J Clin Invest* 73:1722-1784, 1984.
9. Twonsley MI, Taylor GI, Korthuis RJ, Taylor AE. Promethazine or DPPD pretreatment attenuates oleic acid-induced injury in isolated canine lungs. *J Appl Physiol* 59(1):39-46, 1985.
10. Brigham KL. Role of free radicals in lung injury. *Chest* 89(6):859-863, 1986.
11. O'Donnell-Torney J, Nathan CF, Lanks K, DeBoer CJ, de la Harpe J. Secretion of pyruvate in antioxidant defense of mammalian cells. *J Exp Med* 165:500-514, 1987.
12. Burton CA. Oxidation of alpha-diketones and alpha-keto-acids by hydrogen peroxide. *Nature* 163:444, 1949.

13. Melzer E, Schmidt HL. Carbon isotope effects on the **deca4rboxylation** of carboxylic acids: comparison of the lactate oxidase reaction and the degradation of PYruvate by H_2O_2 . *Biochem J* 252:913-915, 1988.
14. Andrae U, Singh J, Ziegler-Skyiakakis K. Pyruvate and related alpha-ketoacids protect mammalian cells in culture against hydrogen peroxide-induced cytotoxicity. *Toxicol Lett* 28:93-98, 1985.
15. Varma SC, Morris SM. Peroxide damage to the eye tens in vitro prevention by PYruvate. *Free Rad Res Comm* 4:283-290, 1988.
16. Nath KA, Enright H, Nutter L, Fischereder M, Jing-Nan Z, Hebbel RP. Effect of PYruvate on oxidant injury to isolated and cellular DNA. *Kidney International* 45:166-176, 1994.
17. Brunkhorst R, Mahiout A. Pyruvate neutralizes peritoneal dialysate cytotoxicity: Maintained integrity and proliferation of cultured human mesothelial cells. *Kidney International* 48:177-181, 1995.
18. Shah SH. Role of reactive oxygen metabolites in experimental **glomerular** disease. *Kidney international* 35: 1093-1 106, 1989.
19. Paller MS, Hoidal JR, Fem's TP. Oxygen free radicals in ischemic acute renal failure in the rat *J Clin Invest* 74:1156-1 164, 1984.
20. Dijkstra U, Gabreels F, Joosten E, Wevers R. Lamers K, **Doesburg W**, Reiner W. Friedreich's ataxia: intravenous pyruvate load to demonstrate a defect in pyruvate metabolism, *Neurology* 34:1493-1497, 1984.
21. Giannelli S, McKenna JP, Bordiuk JM, Miller LD Jerome CR. Prevention of Increased hemoglobin-oxygen **affinity** in open-heart operations **with inosine-phosphate-pyruvate** solution, *Ann Thoracic Surg* 21:386-396, 1976.
22. Tsukiyama T, Hara T, Lio M, Kido G, **Tsubokawa T**. Preferential accumulation of ^{11}C -1 PYruvate. *Eur J Nucl Med* 12:244-248, 1986.
23. Companacci L, Guarnieri GF, Faccini L, Bellini G. **Pyruvate** tolerance test in chronic uremic patients. *Nephron* 10:232-237, 1973.
24. Levy SB, Goldsmith LA. Sodium pyruvate treatment for hyperkeratotic disorders. *South Med J* 72(5):307-310, 1979.
25. Stanko RT, Robertson RJ, Galbreath RW, Reilly JJ **Greenawalt KD Goss FL**. Enhanced leg **exercise** endurance with a high-carbohydrate diet and dihydroxyacetone and pyruvate. *J Appi Physiol* 69:1651-1656, 1990.

26. Stanko RT, Robertson RJ, Spina RJ, Reilly JJ Jr., Greenawalt KE Goss FL. Enhancement of arm exercise endurance capacity with dihydroxyacetone and pyruvate. *J Appl Physiol* 68: 119-124, 1989.
27. Stanko RT, Reynolds HR, Lonchar KD, Arch JE. Plasma lipid concentrations in hyperlipidemic patients consuming a high-fat diet supplemented with pyruvate for 6 wk. *Am J Clin Nutr* 56:950-954, 1992
28. Stanko RT, Tietz DL, Arch JE. Body composition, energy utilization, and nitrogen metabolism with a severely restricted diet supplemented with dihydroxyacetone and pyruvate. *Am J Clin Nutr* 55:771-776, 1992.
29. Stanko RT, Reynolds HR, Hoyson R, Janosky JE, Wolf R. Pyruvate supplementation of a low-cholesterol, low-fat diet: effects on lipid concentrations and body composition in hyperlipidemic patients. *Am J Clin Nutr* 59:423-427, 1994.
30. Dekhuijsen PNR, Aben KKH, Dekker I, Aarts LPHJ, Wielders PLML Van Herwaarden LCA, Bast A. Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 154:813-816, 1996.
31. Dohlman AW, Black HR, Royall JA. Expired breath hydrogen peroxide is a marker of acute airway inflammation in pediatric patients with asthma. *Am Rev Respir Dis*, 148:995-960, 1993.
32. Kietzmann, Kahl R, Muller M, Burchardi H, Kettler D. Hydrogen peroxide in expired breath condensate of patients with acute respiratory failure and with ARDS. *Intensive Care Med*, 19:78-81, 1993.
33. Sznajder JJ, Fraiman A, Hall JB, Sanders W, Schmidt G Crawford G Nahum A, Factor P, Wood LDH. Increased hydrogen peroxide in the expired breath of patients with acute hypoxemic respiratory failure. *Chest*, 96:606-612, 1989.