Effective inhalation treatment for acute fungal infections

Delivery of aerosolized itraconazole directly to the lungs shows promise for reducing deaths from aspergillosis in immunocompromised patients

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Candida and *Aspergillus* are opportunistic species of fungi that pose significant threats to all immunocompromised patient groups and account for 9% of all infections in hospitalized patients worldwide [1]. Treatment following an acute fungal infection such as pulmonary aspergillosis is likely to include oral administration of an antifungal agent such as voriconazole, amphotericin-B and/or itraconazole. When administered orally, however, itraconazole shows a high degree of systemic variability, even when administered as a solution as in the case of Sporanox oral liquid. A standard course of oral itraconazole treatment for fungal pulmonary infections fails to provide significant benefits to immunocompromised patients; mortality rates are at 87% for bone marrow transplant patients and 86% for HIV/AIDS patients, highlighting the acute need for an improved therapy.

Itraconazole has a broad spectrum of activity in treatment of aspergillus, but its poor aqueous solubility (less than 1 mcg/ml at pH 7) limits its oral bioavailability for absorption in the small intestine. The drug has a low acid-ionization constant, with a pKa of 3.7 and, as a result, has greater solubility in more acidic environments. Some clinical studies have focused on administering an acidic beverage such as Coca Cola prior to administration of the oral drug in order to improve bioavailability. Variability in the acidity of patients' systems can influence the drug's absorption, so oral formulations of itraconazole must be administered in high doses in order to assure high levels systemically. However, even high doses of the oral formulation may fail to deliver enough drug to the lungs via the systemic route to ensure that the minimum lethal concentration for Aspergillus flavus of 0.5 mcg/ml itraconazole [2], which approximates to 0.5 mcg/g, is obtained.

The studies presented in this article, all of which use a mouse model, demonstrate the effectiveness of delivering itraconazole as an aerosol for the treatment of an acute fungal infection in the lung. The first part of the article describes the admistration of itraconazole nanoparticles using a nebulizer and demonstrates that effective lung concentrations may be achieved by this delivery method. The second part describes the determination of a therapeutic level that would be required for treating a lung infection. The final part demonstrates the effectiveness of inhaled itraconazole therapy compared to Sporanox liquid administered orally for treating pulmonary aspergillosis in mice.

Materials and methods

Preparation of amorphous itraconazole dispersions for nebulization. Amorphous itraconazole was prepared using the spray freezing into liquid technique developed at UT Austin. Itraconazole (0.1% w/v), polysorbate 80 (0.075% w/v), and poloxamer 407 (0.075% w/v) were dissolved in acetonitrile. The solution was then atomized directly into liquid nitrogen, rapidly freezing the atomized solution into amorphous microparticles. The frozen particles were then transferred to a tray lyophilizer. A stepwise lyophilization process formed a high surface area, low density white powder from the amorphous nanosized itraconazole. The resulting powder was dispensed with deionized water to form a colloidal dispersion with a 20 mg/ml itraconazole concentration prior to nebulization.

Cascade impactor testing. A 7 ml sample of the colloidal dispersion of the amorphous itraconazole was nebulized for a 10 minute period at a flow rate of 28.3 L/min to determine aerodynamic droplet size distributions from an Aeroneb Professional micropump nebulizer. An Anderson cascade impactor was used to evaluate total emitted dose per minute from the nebulizer, mass median aerodynamic diameter, geometric standard deviation, percentage fine particle fraction (defined as the percentage of droplets with an aerodynamic diameter less than 4.7 μm), and subsequent fine particle dose administered per minute.

Pharmacokinetic analysis of lung deposition. Fourteen male outbred ICR mice were dosed for 20 minutes in a specially designed whole body dosing chamber [3] in which the mice were allowed to move freely. The 20 mg/ml amorphous itraconazole colloidal dispersion was prepared and administered via nebulization into the chamber (equivalent to a 30 mg/kg dose exposure). Mice were euthanized by CO2 narcosis at 0.5, 1, 2, 4, 6, 10, and 24 hours post dose. Drug levels were determined using a validated HPLC analysis procedure for harvested lung samples for each time point [4]. A noncompartmental model was used to determine pharmacokinetic parameters.

Dose maintenance of inhaled itraconazole. Colloidal dispersions were prepared by dispersing 20 mg/ml itraconazole in saline. A group of 12 male outbred ICR mice was dosed again for 20 minute periods in the whole body exposure unit using the Aeroneb Professional micropump nebulizer. Another 12 mice were dosed twice daily with 0.4 ml Sporanox oral liquid (30 mg/kg itraconazole) directly to the stomach at 12 hour intervals. Both groups of mice were housed 4 per cage with access to food (standard rodent chow) and water and dosed twice daily at 12 hour intervals to obtain a steady state lung concentration of drug. After 3 days of dosing, 4 mice from each group were euthanized 12 hours after the last dose, when the drug would be its trough (lowest) level, and the lungs were harvested. On day 8, another third of the mice were euthanized. The dosing continued through day 12, when the remaining mice were euthanized.

Aspergillus challenge study. The aspergillus challenge study utilized four groups of 10 mice each. The control group (Group 1) was dosed with aerosolized sterile distilled water; another group (Group 2) received Sporanox oral liquid three times per day. The third group (Group 3) received twice daily doses of an aerosolized colloidal dispersion of amorphous itraconazole administered at 30 mg/kg dose exposure.

Each group was immunosuppressed using 100 mg/kg of subcutaneous cortisone acetate on the day prior to infection, and a single prophylactic dose of itraconazole was administered on the same day. Inoculation with *Aspergillus flavus* was performed via an inoculation flask, as indicated in a previous study [5], and cortisone acetate was administered on the day of infection, one day after that, and on day 6. The dosing regimens continued for a total of 12 days, and the study was terminated on the 20th day after infection. Animals that appeared moribund prior to the end of the study were euthanized, with the death recorded as occurring the next day. Survival was plotted by Kaplan-Meier analysis, and differences were analyzed using the log rank test.

Results and discussion

Cascade impactor testing. The amorphous itraconazole dispersion resulted in high total emitted dose values of approximately 1 mg/min. In addition, an expected high 85% fine particle fraction was determined for the nebulizer. A mass median aerodynamic diameter of 2.7 μm and geometric standard deviation of 1.7 was found. At that emission rate and fine particle fraction, the fine particle dose is calculated to be 964 mcg/min, resulting in a dose exposure time of 20 minutes, based on the mouse lung volume and breathing rate.

Pharmacokinetic evaluation of lung deposition. A high concentration of the drug was delivered and maintained in the lung for an extended period via nebulization. An initial maximum concentration of 13.4 mcg/g itraconazole was measured in the mouse lung tissue at 1 hour after dosing, a level significantly higher than the minimum 0.5 mcg/g concentration required to kill *Aspergillus flavus*. It was therefore considered to be clinically significant in the lung if a concentration greater than 0.5 mcg/g wet lung weight could be evaluated. The half life of the drug was calculated to be 5.5 hours, and the concentration of itraconazole was maintained above the minimum lethal concentration for *Aspergillus flavus* for at least 24 hours.

The exact mechanism of itraconazole depletion from the distal region of the lung is unclear; however, it is known that dissolution and macrophage uptake are active in this region. Itraconazole displays poor solubility at the elevated pH that is present at the alveolar surface, but the drug possesses a high surface area that may lead to enhanced dissolution rates even at the elevated pH.

Dose maintenance of inhaled itraconazole. The nebulized colloidal dispersion of itraconazole achieved 10-fold greater lung tissue concentrations

than did the orally administered Sporanox oral liquid. Multi-day dose exposure showed that the average 2.3 mcg/g lung levels of itraconazole were approximately 4 times the lethal concentration for *Aspergillus flavus* even at the steady state trough level. In contrast, the equivalent oral dosing of Sporanox oral liquid achieved only a 0.17 mcg/g concentration that is insufficient to kill the fungus, confirming that the currently available treatment regimens have a difficult time reaching therapeutic levels in the lung and explaining why oral formulations frequently fail to eradicate aspergillosis.

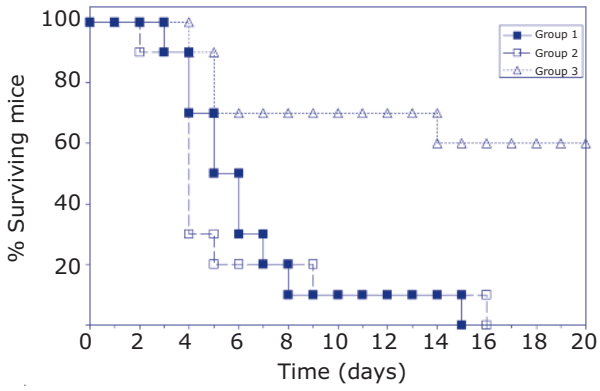
Nebulized itraconazole delivered directly to the lungs demonstrated a secondary benefit. Oral administration of Sporanox results in a high incidence of diarrhea, a side effect that was observed in the group of mice receiving the oral formulation. This side effect was not observed in the mice receiving the inhaled nanoparticles of itraconazole.

Survival study. The group of mice that received the nebulized itraconazole formulation displayed a significant improvement in survival compared to the control group and the that received the orally administered Sporanox liquid. Initial reductions in mice populations from each study group occurred between days 3 and 5. The control and oral Sporanox groups continued to decline following day 5, but the mice receiving the nebulized amorphous itraconazole stabilized.

In fact, all of the mice in the control and oral formulation groups died by the end of day 16, four days after cessation of dosing, while 70% of the mice in the inhalation treatment group survived until the end of the study on day 20 (Figure 1). Achieving similar results in immunocompromised humans would save many lives annually worldwide.

Figure 1

Survival curves for mice dosed with a nebulized colloidal dispersion of amorphous itraconazole compared against a control group and orally administered Sporanox oral liquid.



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