Synergistic Antifungal Activity of Amphotericin B, Essential Oils, and Low-Frequency Ultrasound on Rhizopus oryzae Biofilm

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Introduction

Fungal infections have gained clinical importance in the last decade. These serious and sometimes fatal infections are often associated with biofilm formation, which can increase resistance to antifungal agents when compared to free living colonies. This increased resistance makes it vital to test antifungal susceptibility using biofilms and not planktonic cells. Amphotericin B has been used as the first line of treatment for mucormycosis since the 1950's. However, it can have many adverse side effects including chills, fever, headaches, and muscle pain as well as the fatal syndromes of hepato and nephrotoxicity. These side effects, in conjunction with mortality rates of 97% (untreated) and 39% (treated with amphotericin B) demonstrate the need for alternative treatment options. Previous work has been performed using essential oils in combination with Amphotericin B (Amp B). Another possibility is the use of Low Frequency Ultrasound (LFUS) which in combination with antibiotics has proven to be promising for biofilm removal and treatment of chronic rhinosinusitis. LFUS disrupt biofilms, increasing their sensitivity to antibiotics, and could conceivably be used in tandem with any one or more anti-microbial agents. This study aims to investigate fungal biofilm disruption using LFUS alone, and in combination with amphotericin B and various essential oils, to determine if LFUS and essential oils enhance the effectiveness of current treatments.

Hypothesis

Decreased concentrations of Amp B will be required for treatment of mucormycosis when used in synergism with low frequency ultrasound and thyme oil.

Methodology

Results and Discussion

Individual Treatments of LFUS with Amp B or Thyme Oil

![Graph showing individual treatment inhibition rates for Amp B and LFUS alone, and with Thyme oil combined.]

Thyme & Amp B MIC dilution Test

- Highest to lowest concentration of Amp B
- Highest to lowest concentration of Thyme oil

Figure 1: Effect of LFUS and AmpB on R. oryzae biofilm. The first three rows were treated with Amp B and LFUS. Better inhibition was obtained when Amp B and LFUS were used in combination compared to LFUS only.

Figure 2: Comparison of the biofilm inhibition when treated with Amp B alone and in combination with LFUS. The MIC₆₀ for Amp B alone was at 0.25 μg/mL. When treated in combination with LFUS, the MIC₆₀ was at 0.0125 μg/mL.

Figure 3: The first three rows were treated with LFUS and Thyme oil. The concentration of Thyme oil decreases from left to right. The last three rows were treated with only LFUS and suggest the need for Thyme oil and LFUS.

Figure 4: Comparison of the biofilm inhibition when treated with Thyme oil alone and in combination with LFUS. The MIC₆₀ for Thyme oil alone was at 0.0125 μg/mL. When treated in combination with LFUS, the MIC₆₀ was at 0.00625 μg/mL.

Figure 5: Combination therapy of Amp B, Thyme oil, and LFUS. Thyme oil concentration decreases from left to right and Amp B concentration decreases from top to bottom showing the greatest amount of inhibition at higher concentrations.

Synergism of Thyme Oil, Amp B, and LFUS

![Graph showing synergistic inhibition rates for Amp B alone, Thyme oil alone, and in combination with LFUS.]

Figure 6: Treatment with 0.0625 μg/mL of Amp B and LFUS with 0.1 μg/mL of Thyme oil resulted in 91% Inhibition Rate. In comparison, without LFUS, the inhibition rate was at 72%, suggesting a 19% increase in synergism.

Impact of LFUS

- Inhibition rate increased by 19% when LFUS was added to 0.1 μg/mL of Thyme oil and 0.0625 μg/mL of Amp B. This suggests the capability of LFUS has on disrupting the biofilms and allowing better penetration of the antimicrobial agents.

Future Work

Further studies could be performed with those organisms.

Thyme oil is only one of many essential oils with antimicrobial properties. Oils such as Clove, Oregano, Lemongrass, and Cinnamon could be studied.

An intriguing method found in the disruption of biofilm is the use of Extracorporeal Shockwave Therapy. Shockwave therapy could be used in synergism with essential oils and Amp B to inhibit mucormycosis infections.

The breakdown of biofilm would be different when integrated with human tissues or cells. Because of the possible difficulty to apply essential oils on infected tissue, LFUS in combination with Amp B could be used to breakdown the biofilm and ultimately cause fungal cell death while embedded in tissues.

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