A Comparison of Minimal Inhibitory Concentration (MIC) and Agar Diffusion Assays for Clinical use in Determining Minimal Effective Drug Concentrations

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Abstract

The incidence of life threatening fungal infections has increased since the development of improved medical interventions, such as organ transplantations and the wider use of anticoagulant chemotherapy. Identifying the etiologic fungal agent is important to more effectively treat the patient and to identify a potential lack of drug sensitivity (i.e., resistance). The use of a minimal inhibitory concentration (MIC) assay and an agar diffusion assay is useful to aid the clinician in developing a more effective therapeutic regimen to eliminate the fungal infection. In this study, we examined these assays using the yeast Candida albicans tested against the antifungal agents Amphotericin B (Ambisome) and Anidulafungin. Using these assays, we identified the minimal drug levels necessary to be achieved in the patient to effectively treat a potential systemic candidiasis infection.

Introduction

A number of fungal species have been identified as the etiological agents of human infections ranging from superficial, non-lethal colonization to systemic, the threatening infections since the first fungal infections were first reported in the 1800’s. Some of these species are Aspergillus rugus, Candida albicans, Pneumocystis carinii (Revankar & Dobel, 2014). In the past 50 years, the incidence of serious fungal infections has increased and corresponded to the increase use of chemical (drug) treatments that adversely affect the immune system, such as cancer chemotherapy and transplantation drugs.

Amphotericin B, isolated from the bacterium Streptomyces nodosum and marketed under the trade name Fungizone®, was identified in the mid-1950’s as having antifungal properties for effectively treating fungal infections. This chemical has a high binding affinity to sterol, one of the structural components of fungal cell membranes is the sterol ergosterol, not present in mammalian cells (Kagan et al. 2014). However, this amphotericin B was also has significant undesirable side effects including damage to the distal tubules of the kidney. To help reduce these adverse reactions in humans, amphotericin B was incorporated into the bilayer of liposomes (lipid nanoparticles) containing cholesterol (Kagan et al. 2014).

When amphotericin B is administered IV it is not easily able to enter the blood stream, making it less useful for combating certain yeast and fungal infections (Olson et al. 2011). When administered intravenously it tends to have many side effects including kidney damage that can be severe and/or irreversible (Olson et al. 2011). When amphotericin B was administered in a liposomal formulation the drug is encapsulated in a lipid membrane which disrupts when it reaches the cell wall of fungi, this allows for the drug to be administered in higher doses and with a lesser severity of the side effects (Olson et al. 2011).

AmBisome® is a liposomal formulation of amphotericin B approved by the FDA to treat systemic fungal infections. AmBisome has been shown in both pre-clinical and clinical use to retain the antifungal activity of amphotericin B with a reduction in the negative side effects even when given at higher doses than the conventional amphotericin B (Ambisome). AmBisome was also found to have a longer half-life compared to Fungizone which allows AmBisome to be dosed less frequently. Although there are other lipid formulations used to deliver amphotericin B, it is the structure of the liposome, and not just the lipids which confers the beneficial characteristics of AmBisome (Ambisome, 2008).

Due to increasing toxicity effects of amphotericin B treatments and the development of resistance to Azoles, there are many several different methods for testing the effectiveness of antifungal medications. Due to increasing toxicity effects of amphotericin B treatments and the development of resistance to Azoles, several different methods for testing the effectiveness of antifungal medications are used. MIC assays are beneficial characteristic of amphotericin B with a reduction in the negative side effects even when given at higher doses than the conventional amphotericin B (Ambisome). MIC assays are widely used to aid in the development of a more effective therapeutic regimens to eliminate fungal infections.

Materials and Methods

MIC Assay

- Subculture the yeast in Sabouraud’s daily for 3 consecutive days, wash with PBS and adjust the solution to 30% 50% 90% 5% 3%.
- The solution was sterilized by autoclaving for 15 minutes at 1 atm.
- A series of drug dilutions were prepared in RPMI medium; 30% 40% 50% 60% 70% 80% 90% 100%.
- The solution was carefully poured into an agar plate on a level surface and allowed to sit for approximately 30 minutes to allow the agar to solidify.
- A sterile template cutter was then sued to punch wells and the plugs were then vacuumed out.
- 100µL of the drug, 100µL of RPMI and 20µL of alamarBlue® were dispensed in the negative control.
- The plate was incubated for 20 hours at 35°C.
- The absorbance at 570nm minus the absorbance at 600nm.

Agar Diffusion

- 13.2 grams of AM91 medium was hydrated with 220mL of DI water and 75µL of saturated NaOH.
- The solution was sterilized by autoclaving for 15 minutes at 1 atm.
- The solution was then placed in a 48°C water bath for a minimum of 30 minutes.
- The medium was allowed to cool to approximately 25°C then 2.5% yeast cells per mL of solution.
- The solution was then added to sterile petri plates using a sterile pipette.
- The plates were read using Spectramax 340 plate reader and the numbers generated were the result of reading the absorbance at 570nm minus the absorbance at 600nm.

Discussion

Standard MIC assays are easily performed with minimal space requirements when compared to agar diffusion plates which require greater amounts of space. Agar diffusion and MIC assays can be performed with equal amounts of ease; however, Agar diffusion assays require more work and are slightly less precise as the zones of inhibition are open for interpretation, thus allowing more room for error. MIC assays can be visually interpreted by examining color changes and electronically with spectrophotometry for more precision; however, MIC assays are most effective at an identified drug concentration as the results plateau after certain drug concentrations.

Both MIC Assays and agar diffusion assays can be useful in clinical practice for developing effective therapeutic regimens for the treatment of fungal infections. The spectrophotometric results of varying drug concentrations from MIC assays can be plotted to determine the minimal effective drug concentration, and the zones of inhibition of agar diffusion assays can be plotted to develop a standard curve to determine drug concentrations for administration.

References