

## IN VITRO ACTIVITIES OF AMPHOTERICIN-B IN COMBINATION WITH RIFAMPIN AGAINST *ASPERGILLUS* SPECIES

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### ABSTRACT

**Objective:** The main goal of study was finding the synergism effect of amphotericin B (AMB) and rifampin (RIF) on 3 species of *Aspergillus*.

**Methodology:** Activities of amphotericin B in combination with rifampin were tested in buffered yeast-nitrogen base using checkerboard method. Plates were inoculated with 20µl spores suspensions of each organism and incubated at 30°C for 24h. For this method, the MICs were defined as the lowest antimicrobial concentration inhibiting visible fungal growth on the plates. Minimal fungicidal concentration was defined as the first tube showing no growth on the plate.

**Results:** The MIC of amphotericin B for 100% of isolates of *A. fumigatus* and *A. flavus* were inhibited by 4mg/lit amphotericin B. 100% of isolates of *A. niger* were inhibited by 8mg/lit amphotericin B. When amphotericin B was combined with rifampin, amphotericin B MICs decreased to 2, 1 and 4mg/lit in *A. fumigatus*, *A. flavus* rephrase and *A. niger* respectively.

**Conclusion:** The results indicate that combination of amphotericin B and rifampin was synergistic on *A. fumigatus*, *A. flavus* and *A. niger*.

**KEY WORDS:** Aspergillus, Amphotericin B, Rifampin, Synergism.

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### INTRODUCTION

Invasive fungal infections (IFI) are most important causes of death in severely immunocompromised patient.<sup>1,2</sup> A move in the epidemiology of IFIs has been observed over the past two decades, associated with an expansion of the antifungal armamentarium and a widening spectrum of immunosuppressive agents. Since the early 1990s, invasive aspergillosis has become the predominant IFI.<sup>1,2</sup>

Amphotericin B (AMB) is the drug of choice for treatment, but its use is restricted by its narrow therapeutic index.<sup>3</sup> A few studies have been performed to test antifungal combinations

against fungi. Combination of amphotericin B with rifampin has been confirmed to be synergistic in vitro against yeasts,<sup>4,5</sup> dimorphic fungi,<sup>6,7</sup> and *Rhizopus*.<sup>8</sup> Interaction between amphotericin B and flucytosine has been revealed to be additive or synergistic against yeasts in vitro and in vivo in animal models as well as in patients.<sup>9</sup> Concerning *Aspergillus*, indifferent-to-synergistic and in some instances antagonistic interactions have been reported for this combination.<sup>10</sup>

In vitro studies have indicated a possible role for combining rifampin with amphotericin againsts *Aspergillus* spp., though reports have provided conflicting results.<sup>11,12</sup> Therapy with rifampin or flucytosine combined with amphotericin B was shown to increase survival over amphotericin B alone in a mouse model of disseminated *A. fumigatus* infection.<sup>13</sup>

In this in vitro study, we combined amphotericin B (AMB) with relevant concentration of rifampin (RIF) to screen for active combinations against *A. fumigatus*, *A. flavus*, and *A. niger*.

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## MATERIALS AND METHODS

**Organisms:** Strains of *A. fumigatus*, *A. flavus*, & *A. niger* collected by the medical mycology laboratory at the Ahwaz Jondishapor of Medical Sciences. Cultures of *A. fumigatus*, *A. flavus*, and *A. niger* were grown on Sabouraud's dextrose agar at 37°C for three days. Spores were obtained by swabbing the surface of the colonies with glass beads suspended in distilled water including tween 80. Final suspensions were adjusted to 90% transmittance at 530nm, corresponding to 10<sup>6</sup>CFU/m.

**Drug solutions:** Stock solutions of amphotericin B (Squibb) and Rifampin (Hakim) were dissolved in dimethyl sulfoxide. These solutions were incubated at room temperature for 30 minutes for self-sterilisation. The drug solutions were then stored at -70°C for further experiences.

**Method:** The susceptibility testing of amphotericin B and rifampin were performed in yeast nitrogen base glucose (YNBG) agar buffered with phosphate buffer (pH 7). In the first step both amphotericin B and rifampin were tested separately. Final drug concentrations for amphotericin B and rifampin were 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25mg/l. Combination rows each contained amphotericin B in dilutions from 8 to 0.5mg/ml with rifampin from 64 to 0.5mg/ml. Drug activity was assessed by a checkerboard method. Growth control tube contained media with and without each of drugs and dimethyl sulfoxide in plates. Plates were inoculated with 20µl spores suspensions of each organism and incubated at 30°C for 24h. For this method, the MICs were defined as the lowest antimicrobial concentration inhibiting visible fungal growth on the plates. Minimal fungicidal concentration (MFC) was defined as the first tube showing no growth on the plate.

**Statistics:** *P* values were calculated by using the paired *t* test.

## RESULTS

The checkerboard approach was used in this study to investigate the interaction between AMB and rifampin against isolates of *A.*

*fumigatus*, *A. flavus* and *A. niger*. The susceptibility results obtained for the isolates are reported in Table-I.

All isolates were uniformly resistant to rifampin. In this study, 14 isolates of *A. fumigatus*, 30 isolates of *A. flavus* and 34 isolates of *A. niger* were tested. All isolates of *A. fumigatus* and *A. flavus* were inhibited by 4mg/lit amphotericin B. While all isolates of *A. niger* were inhibited by 8mg/lit amphotericin B (Table-I). Amphotericin B MICs ranged from 2.5 to 3.5mg/lit for *A. fumigatus*. The range of MICs for *A. flavus* and *A. niger* were 2.5-4 and 5.5-7 respectively.

The addition of rifampin to AMB gave variable results. When AMB was combined with rifampin, there were significant reductions in concentration of AMB. Synergy was demonstrated against *A. fumigatus*, *A. flavus* and *A. niger*. In all isolates, the concentration of AMB in combination with rifampin was reduced.

Table-I shows a list the MIC at which 100% of the strains were inhibited by AMB and amphotericin B + rifampin. When inhibitory effects of AMB were tested with rifampin, amphotericin B MICs decreased to 2,1 and 4mg/lit in *A. fumigatus*, *A. flavus* and *A. niger* respectively (Table-I). While AMB MIC for *A. flavus* was 2mg/lit with 16mg/lit of rifampin, it was decreased to 1mg/lit using 32mg/lit rifampin. Addition of 16mg/lit rifampin prevented growth of *A. niger* and *A. fumigatus* and felled the concentration of AMB to 4 and 2mg/lit respectively. The Table-I shows the activities of AMB with different concentration of rifampin.

Rifampin alone was inactive against all isolates tested. However, when sub inhibitory

Table -I: Summary of drug concentrations for 3 species of *Aspergillus*.

Species	MICs of amphotericin B (mg/l)		MICs of AMB with RIF (mg/l)	
	100% of isolates	Range	AMB	RIF
<i>A. fumigatus</i>	4	2.5-3.5	2	16
<i>A. niger</i>	8	5.5-7	4	16
<i>A. flavus</i>	4	2.5-4	1	32

AMB, amphotericin B; RIF, rifampin.

concentrations of AMB were used, rifampin MICs decreased significantly in isolates. No antagonism was noted with these drug combinations against any of the isolates tested.

## DISCUSSION

The antifungal activity of AMB rifampin combination has been previously proven in vitro against a variety of fungal species including *Candida* spp,<sup>4</sup> *Histoplasma capsulatum*,<sup>6</sup> *Coccidioides immitis*,<sup>7</sup> *Cryptococcus neoformans*,<sup>5</sup> and zygomycetes.<sup>14</sup> In each study, rifampin alone had no effect on the *Aspergillus* strains, while the addition of AMB resulted in obvious synergy at clinically achievable drug concentrations.

Kitahara et al<sup>15</sup> previously verified a decrease of MICs of AMB against *A. fumigatus* with the addition of rifampin or flucytisine in concentrations higher than clinically achievable. Recently, amphotericin B has been tested in combination with many other drugs to determine whether it possibly has improved activity when used in combinations.<sup>16,17</sup> In vitro and in vivo studies have shown wide variations in effect when the polyene is combined with fluconazole or itraconazole.<sup>16,18</sup>

The lack of efficient antifungal therapy encouraged us to test the *Aspergillus* isolates against combinations of drugs that have been shown activity against other fungi. With establishing dose-response based on fungal spore suspensions, we were able to confirm our finding by the checkerboard method. Our data suggests that MIC strains were relatively higher to amphotericin B and the concentration of the amphotericin B was decreased significantly when added rifampin was added in the media. The lowest MIC was 1mg/l for *A. flavus* and the highest was 4mg/l for *A. niger*. Based on our data the addition of rifampin to amphotericin B might be expected to have enhanced activity against selected isolates. This study indicates that susceptibility tests in vitro may be valuable predictors of the most appropriate drug or drugs to use clinically against a given infecting isolate.

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