

Hyphal Damage Activity of Human Monocytes Primed with Amphotericin B Formulations against *Aspergillus Fumigatus*

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INTRODUCTION

Invasive fungal infections are an important and increasing problem in recent years associated with significant morbidity and mortality. Deoxycholate amphotericin B (DAMB) and its lipid formulations, including liposomal amphotericin B (LAMB), lipid complex (ABLC) and colloidal dispersion (ABCD), are common antifungal agents used in immunocompromised patients with invasive aspergillosis (1). We studied the effects of different amphotericin B formulations, at low and high concentrations on the antihyphal activity of human monocytes (MNCs) against *Aspergillus fumigatus*.

MATERIALS AND METHODS

MNCs were isolated from healthy blood donors and purified by Ficoll centrifugation and plastic adherence and then rested for 24 hours. One million MNCs were incubated with 1 and 5 mg/L DAMB as well as with 5 and 25 mg/L lipid formulations at 37°C for 24 hours. MNCs were then

assayed for hyphal damage activity against *A. fumigatus* using the XTT (2,3-bis(2-methoxy-4-nitro-5-[(sulfonylamino) carbonyl]-2H-tetrazolium-hydroxide) colorimetric assay (2). Ten thousand conidia were grown in YNB at 37 °C for 17 hours and incubated with MNCs at an effector:target (E:T) ratios of 10:1 and 20:1 for 2 hours. After hypotonic lysis of MNCs, hyphal damage was evaluated spectrophotometrically by measuring the colour change of XTT produced by surviving hyphae at 550 nm with a reference wavelength 690 nm. Experiments were performed fivefold and data were statistically analyzed using ANOVA parametric repeated measures with Dunnett test for multiple comparisons.

RESULTS

Results of the study are summarized in the table below (*p<0.05, **p<0.01).

		% Hyphal Damage									
E:T ratio	MNCs only	DAMB		LAMB		ABLC		ABCD			
		1	5	5	25	5	25	5	25		
10:1	17.08±	21.38±	48.10±	26.93±	42.54±	79.91±	91.18±	37.21±	49.40±		
	6.02	5.37	8.12**	4.30	3.79*	6.38**	3.02**	8.51	8.68**		
20:1	16.12±	31.37±	63.78±	42.57±	58.52±	89.60±	95.21±	57.85±	66.49±		
	3.88	8.01**	12.82**	6.18	6.02**	3.82**	1.23**	6.67**	7.47**		

Pre-incubation of MNCs with all amphotericin B formulations, used at high concentrations (5 mg/L of DAMB and 25 mg/L of lipid formulations), resulted in a significant increase of MNC-induced hyphal damage at 10:1 E:T ratio as compared to MNCs alone. At E:T ratio 20:1, MNCs pretreated with either high or low concentrations of all amphotericin B formulations had similar effects. ABLC induced an increase in *A. fumigatus* hyphal damage at E/T ratio 10:1 even at the lower concentration constituting the formulation with the most potent enhancing activity among all amphotericin B formulations.

CONCLUSIONS

The hyphal damage induced by amphotericin B lipid formulation-pretreated MNCs is higher than that induced by DAMB-pretreated MNCs. Moreover, the effects of amphotericin B formulations on MNC-induced hyphal damage are concentration-dependent. ABLC-induced MNCs showed

the highest antihyphal activity. The mechanisms of superior induction of antihyphal activity of MNCs by ABLC may be based on either production of immunoenhancing cytokines or higher intracellular concentrations of the drug and subsequent release of it to the hyphal microenvironment. While further work is required to study the antifungal defense mechanisms of MNCs, these data show that amphotericin B lipid formulations are more effective in inducing innate host defense to damage *A. fumigatus* hyphae than DAMB.

REFERENCES

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