Effect of freeze-thaw preparation of Ambisome on its activity against *Cryptococcus gattii*

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【Objectives】

The antifungal agent amphotericin B (AMPH-B) is available in two forms, Fungizone that is dissolved in sodium deoxycholate and the liposomal form Ambisome (L-AMB). Both drugs have the same mechanism of action, targeting fungal ergosterol. However, several studies have reported that the range of minimal inhibitory concentration (MIC) of Fungizone is 0.5 – 2 µg/mL but for Ambisome is wider with lower values, especially on *Cryptococcus gattii*. The aim of this study was to evaluate the effect of freeze-thaw preparation on the activity of Ambisome against *C. gattii*.

【Methods】

A total of 22 fungal strains, *C. neoformans* ($n = 10$), *C. gattii* ($n = 12$). *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as two quality control strains. (Table 1) MICs of Fungizone, Ambisome and Amphotericin B prepared freshly or frozen/thawed for one round, were assessed. Both drugs were dissolved according to the manufacturer's instructions. AMPH-B was dissolved in DMSO. Susceptibility tests were conducted following the M27-A3 protocol of CLSI; serial twofold dilutions were prepared with RPMI1640 medium.

【Results】

The MIC range was 0.12 – 1 µg/mL on freshly prepared Ambisome, while it was 0.06 – 0.5 µg/mL on Ambisome with freeze/thaw preparation. Moreover, the MIC of frozen plates of Fungizone and AMPH-B was 1 – 4 µg/mL. (Table 1.) A reduction of MIC of each strain by a freeze/thaw preparation was indicated below. (Fig 1) In addition, there was a tendency to show lower MIC values especially in *C. gattii* with a thick capsule. On the contrast, freeze/thaw preparation seems to have a less effect on strains of *C. neoformans* or *C. gattii* with a thin capsule. (Data not shown)

Table 1. Range of MIC

Species	Strains	AMPH-B		Fungizone		Ambisome	
		Freshly	freeze-thaw	Freshly	freeze-thaw	Freshly	freeze-thaw
<i>C. gatti sensu lato</i>	10	1~2	2	1~2	1~2	0.5~1	0.06~0.5
<i>C. neoformans sensu lato</i>	12	1~2	2~4	1~2	2~4	0.25~1	0.12~0.5
<i>C. krusei</i> ATCC 6258	1	4	4	2	4	2	2
<i>C. parapsilosis</i> ATCC 22019	1	1	2	1	2	1	0.5

C. neoformans with a thin capsule

C. gattii with a thick capsule

【Conclusion】

In conclusion, it suggests that both of liposome components and capsule thickness could be attributed to lower MIC values of Ambisome, as the thicker the capsule of fungi, the lower the MIC values. It is assumed that the main liposome components, such as other ingredients of the liposomal medicine, phosphatidylcholine, phosphatidylglycerol and cholesterol, could influence on the result. However, its insolubility toward the culture medium makes it difficult to explore each of them in detail. In our further study, we will consider the effect of structural change and the interaction among liposome components and capsule by the freezing preparation.

Fig 1. Effect of MIC by Freeze-thaw

Blue arrow AMPH-B
 Red arrow Fungizone
 Brown arrow Ambisome