Caspofungin: Cross-Reactivity in the *Aspergillus* Antigen Assay[▽]

The Platelia *Aspergillus* antigen enzyme-linked immunosorbent assay (ELISA) (Bio-Rad, Marnes-la-Coquette, France) has become an important tool for the early detection of invasive aspergillosis in immunocompromised patients (4). This ELISA uses a rat monoclonal antibody (EB-A2) which reacts with β -(1,5)-galactofuranose, a side chain of galactomannan (GM). Cross-reactivity of the Platelia ELISA with fungi other than *Aspergillus* was reported previously (2). Over 10 years ago, we discovered cross-reactivity of GM in antibiotics of fungal origin, such as ampicillin-sulbactam and piperacillin, by the Pastorex *Aspergillus* latex agglutination test, which used the same antibody (1). Industrially produced β -lactam antibiotics derived from *Penicillium* species also showed positive GM values *in vivo* (5).

The echinocandins inhibit the synthesis of glucan in the cell walls of several molds and are effective for treatment of candidemia. In addition, caspofungin is recommended for salvage therapy in invasive aspergillosis.

Echinocandins are sterile lyophilized agents for intravenous infusion that contain semisynthetic lipopeptide compounds synthesized from fermentation products of different fungi. Pneumocandin B, the semisynthetic derivative of caspofungin, is manufactured from the fungus *Zalerion arboricola*, anidulafungin from *Aspergillus nidulans*, and micafungin from *Coleophoma empetri*. These three filamentous fungi belong to the phylum Ascomycota. *Aspergillus* and *Penicillium*, which contain galactomannoproteins in the cell wall, also belong to this phylum.

The purpose of this study was to detect the presence of GM in preparations of industrially produced echinocandins by the *Aspergillus* antigen ELISA. In addition, the GM values of the supernatants of the ingredient fungi were tested by culturing them on Sabouraud agar at 37°C for 2 days. Broth cultures were obtained by inoculating 1 cm³ mature mycelia into 20 ml of the GM-negative isotonic solution (sterofundin). The isolates were incubated at room temperature for 5 days, and the

fungal supernatant was decanted; 300 μ l of the supernatant was used in the *Aspergillus* antigen assay.

All batches of Cancidas (caspofungin) infusion solutions tested were GM positive (cutoff index in serum is currently 0.5), whereas GM detection in Ecalta/Eraxis (anidulafungin) was variable in different batches and Mycamine (micafungin) gave negative test results (Table 1). The median GM indices ($n = 3$) were 4.4, 0.8, 0.65, <0.5, and <0.5 for 70 mg and 50 mg caspofungin, 100 mg anidulafungin, and 50 mg and 100 mg micafungin, respectively. In the supernatants of the fungi used for manufacturing the echinocandins, GM indices were also detected.

Due to the GM content of caspofungin, especially in the 70-mg dose which is used as the loading dose, the detection of circulating GM may be influenced during caspofungin administration. It is important to point out that the concentrations of GM in caspofungin infusion solutions do not reflect the concentrations of GM circulating in the bloodstream. Nevertheless, increases of GM levels have been reported *in vivo* and *in vitro* following echinocandin exposure (3). To our knowledge, this is the first report of GM detection in echinocandin preparations. These findings can have meaningful impact for interpretation of GM antigenemia in patients receiving echinocandins, especially caspofungin. Clinical studies are necessary to verify the relevance of positive serum GM levels and concomitant caspofungin administration.

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J. Steinmann*

J. Buer

P.-M. Rath

Institute of Medical Microbiology

University Hospital Essen

University of Duisburg-Essen

Hufelandstr. 55

45122 Essen, Germany

*Phone: 49 201 723 85771

Fax: 49 201 732 5602

E-mail: Joerg.Steinmann@uk-essen.de

TABLE 1. Galactomannan (GM) values of antifungal infusion solutions by blind testing^a

Drug name	Drug concn (mg)	Qualitative result ^b	GM index of antifungal infusion solution		GM index of fungal supernatant solution ^c
			Median	Range	
Caspofungin (Cancidas)	70	Positive	4.4	3.1–5.2	4.0 (<i>Zalerion arboricola</i>)
	50	Positive	0.8	0.5–1.0	
Anidulafungin (Ecalta)	100	Positive	0.65	0.1–1.8	6.1 (<i>Aspergillus nidulans</i>)
Micafungin (Mycamine)	50	Negative	<0.5	<0.5	3.7 (<i>Coleophoma empetri</i>)
	100	Negative	<0.5	<0.5	

^a Three batches were tested for each drug and each concentration.

^b The GM index was estimated by the ratio of the absorbance value to the cutoff value and considered positive when the value was ≥ 0.5 on repeat testing.

^c GM index of the supernatant solution from the ingredient fungus. The fungal species is shown in parentheses.

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