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Biafungin (CD101), a Novel Echinocandin, Displays Excellent Stability in Plasma, Aqueous Solution, and at Elevated Temperature

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ABSTRACT

Background: The echinocandins are an important class of antifungal agents, but all are administered once daily by intravenous infusion. They are known for susceptibility to thermal, hydrolytic, and (for micafungin) photodegradation, which impose limitations for manufacturing, storage, and usage. We now present data for biafungin, an echinocandin that displays prolonged stability in plasma, aqueous solution, and at elevated temperature.

Methods: Stabilities of biafungin and anidulafungin were evaluated in rat, dog, monkey, and human plasma (pH-stabilized with 0.1 M sodium phosphate, pH 7.4) and phosphate buffered saline (PBS, 0.1 M, pH 7.4). Each analyte (10 µg/mL) was incubated at 37 ° C over 44 h. Aliquots (0.5 mL) at various time points were added to acetonitrile (0.5 mL) to precipitate proteins. Quantitation of the respective compounds was performed by LC/UV analyses compared to an internal standard.
Long-term stability of biafungin was evaluated by storage as a lyophilized powder at 40 ° C. It was also evaluated without stabilizers in solutions in clear glass vials at 40 ° C (acetate and lactate buffers) or at RT (sterile water, 5% dextrose, and 0.9% saline). Quantitation at various time points was performed by LC/UV analyses.

Results: After incubation for 44 h in rat, dog, monkey, and human plasma, the percent of biafungin remaining (91, 79, 94, and 93), respectively, was consistently greater than the percent of anidulafungin remaining (7, 15, 14, and 7), respectively. In PBS, the percent of biafungin remaining was also greater than that of anidulafungin (96 vs 42), respectively. Biafungin exhibited less than 3% degradation after long-term storage at 40 ° C as a lyophilized powder (9 mos) and at RT in 5% dextrose (15 mos), 0.9% saline (12 mos) and in sterile water (18 mos). Degradation levels were acceptable at 40 ° C in acetate and lactate buffers (6 to 9 mos at pH 4.5-5.5).

Conclusions: Biafungin was more stable than anidulafungin in plasma and buffer solutions. The limited chemical degradation/hydrolysis observed in the different plasma/buffer matrices is consistent with the extended pharmacokinetic half-lives observed across various species. The long-term stability as a lyophilized powder and in aqueous solution could provide convenient storage, handling, and usage for hospital pharmacies.

BACKGROUND

Since their introduction in 2001, the echinocandins have become an increasingly important class of antifungal agents. They have very few drug interactions, a low incidence of resistance, and no dose adjustments based on renal function. Despite these advantages, the pharmacokinetic and stability properties of the approved echinocandins impose restrictions on their use. Short half-lives and poor oral absorption mean they must each be administered once daily by intravenous (IV) infusion. Poor stability leads to dosing preparations that must be used within 24 h or be discarded. It also precludes the introduction of other dosage forms, such as subcutaneous preparations and topical creams. Consequently, the echinocandins presently cannot be used for indications in which daily infusion of the drug is impractical. In short, the susceptibility to hydrolytic, thermal, and (for micafungin) photodegradation of the approved echinocandins impose limitations for manufacturing, storage, usage, and acceptable dosage forms for this attractive drug class.

Biafungin (formerly SP 3025) is a highly stable echinocandin in development for intermittent IV administration. The compound was found to be comparable to the approved echinocandins in terms of MIC/MEC against panels of recent *Candida* and *Aspergillus* clinical isolates.[1] In addition, it was also found to have a long half-life ($T_{1/2}$) and slow clearance compared to other echinocandins in multiple species.[2,3] We now report the uncommon stability of biafungin as a lyophilized powder, in aqueous solution, at elevated temperature, and in plasma. Adding to the obvious benefits for manufacturing and storage in pharmacies, an echinocandin that is stable both *in vivo* and *in vitro* could be administered by routes not available for the currently approved echinocandins, such as subcutaneous injections and topical applications. These alternate routes could find utility for prophylaxis or treatment of indications for which an echinocandin would be beneficial, but intravenous administration is not practical.

METHODS

Stability of Biafungin in Plasma: Blank plasma (rat, dog, monkey, and human) was mixed with a 10% volume of sodium phosphate (1.0 M, pH = 7.4). In the control, phosphate buffered saline (PBS, pH = 7.4) was used in the place of plasma. Each matrix was mixed separately with 1.0% stock solutions of biafungin and anidulafungin, bringing the starting concentration of analyte in each sample to ~ 10 µg/mL. The sample volume for each solution was 5.0 mL. Each stability sample was divided into aliquots of 500 µL each, which were incubated at 37 ° C. At each stability time point (1, 2, 8, 21, and 44 h), an aliquot was quenched with acetonitrile (500 µL, with internal standard) and centrifuged. The supernatant (100 µL) was removed and analyzed by reversed phase LC/UV.

Stability of Biafungin as a Lyophilized Powder and in Solutions: Biafungin acetate (lyophilized powder) was stored at 40 ° C in capped, glass vials. Stability was assayed for nine months.

Biafungin acetate in a prototype IV formulation (3.33 mg/mL, lactate buffer) was diluted to 1.1 or 0.77 mg/mL with 5% dextrose or 0.9% saline infusion solutions, respectively. The dextrose formulation solution (infusion bag) and the saline formulation solution (clear glass vial) were stored at room temperature (RT) with exposure to light. Stability was assayed up to fifteen months.

Biafungin stock solutions (acetate buffer, lactate buffer, and sterile water; 3.33 mg/mL) without fructose or stabilizers were stored at either 40 ° C or RT in capped, clear glass vials. Stability was assayed up to eighteen months.

Analysis of Biafungin as a Lyophilized Powder and in Solutions: Stability of biafungin was assayed using two different LC/UV methods. One was to assess purity and to identify degradation products. Another was used to look for epimerization of the hemiaminal ether stereocenter. Appearance was also monitored. Degradation products that arose above 0.5% were isolated and characterized by LC/MS methods.

RESULTS

Stability of Biafungin in Plasma: After incubation at 37 ° C in rat, dog, monkey, and human plasma, the biafungin remaining after 44 h was 91, 79, 94, and 93%, respectively. Stability in PBS buffer was similar (96% remaining after 44 h). The time curves for biafungin in the various matrices are shown in Figure 1.

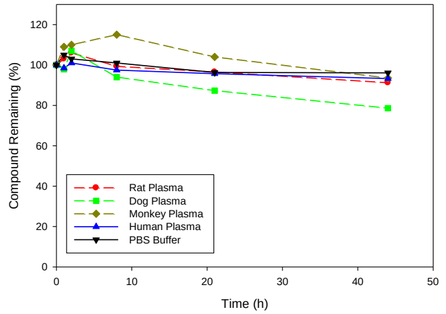

Figure 1. Degradation of biafungin in plasma from different species and in PBS buffer at 37 ° C. The amount of intact biafungin remaining is expressed as a percentage of the 0 h time point. No stabilizers were used in the reactions.

RESULTS cont.

Degradation of anidulafungin was much faster compared to biafungin in each of the four plasma matrices. After incubation at 37 ° C in rat, dog, monkey, and human plasma, the anidulafungin remaining after 44 h was 7, 15, 14, and 7%, respectively. Degradation of anidulafungin in PBS buffer (42% remaining after 44 h) was also faster than that of biafungin in the same buffer, although the difference was not as stark as in the plasma experiments. Degradation of anidulafungin was much faster in each of the plasma matrices than it was in PBS buffer. The time curves for anidulafungin in the various matrices are shown in Figure 2.

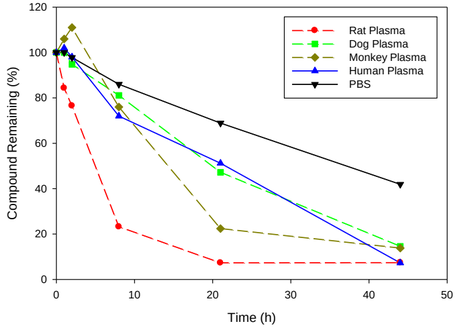

Figure 2. Degradation of anidulafungin in plasma from different species and in PBS buffer at 37 ° C. The amount of intact anidulafungin remaining is expressed as a percentage of the 0 h time point. No stabilizers were used in the reactions.

Stability of Biafungin as a Lyophilized Powder: The results from stability studies under accelerated conditions of two lots of biafungin lyophilisates are presented in Table 2. After storage at 40 ° C for nine months in the absence of stabilizers or other excipients, biafungin acetate evidenced less than 3% degradation. No epimerization of the hemiaminal ether was observed.

Biafungin Lot	Test	Time (Months)				
		0	1	3	6	9
1	Chromatographic Purity (% AUC)	**99.0**	**98.6**	**98.4**	**98.3**	**96.5**
1	Impurity & Degradation Products (% AUC)	1.0	1.4	1.6	1.7	3.5
2	Chromatographic Purity (% AUC)	**97.5**	**97.1**	**96.8**	**96.3**	**96.4**
2	Impurity & Degradation Products (% AUC)	2.5	2.2	3.2	3.3	3.5

Table 2. Stability data of biafungin acetate as a lyophilized powder at 40 ° C for 9 months. Values reported are the % AUC for biafungin and for the total of all impurities and degradation products. Less than 2.5% increase in degradation products was evident, and no epimerization of the hemiaminal ether was observed.

RESULTS cont.

Stability of Biafungin in IV Infusion Solutions: The results from stability studies of biafungin acetate in two different IV infusion solutions are presented in Table 3. After storage at RT and exposure to light in the absence of stabilizers or other excipients, biafungin exhibited negligible degradation both in 5% dextrose solution after 15 months and in 0.9% saline solution after 12 months. No epimerization of the hemiaminal ether was observed.

Biafungin Infusion Solution	Conc. (mg/mL)	Test	Time (Months)						
			0	1	3	6	9	12	15
5% Dextrose	1.1	Assay (mg/mL)	1.11	1.12	1.10	-	1.13	1.12	1.11
		Purity (% AUC)	97.3	97.1	97.2	-	98.1	96.9	97.7
0.9% Saline	0.77	Assay (mg/mL)	0.80	0.81	0.79	0.82	0.83	0.79	-
		Purity (% AUC)	93.7	93.5	93.7	94.4	94.2	94.0	-

Table 3. Stability data of biafungin in IV infusion solutions at RT and unprotected from light for up to 15 months. The assay values are in reference to an internal standard and are an indication of the amount of biafungin in solution. Values between 90 and 110% of the target concentration were considered to be within the specification. Purities are reported as the % AUC for biafungin out of the total chromatogram. Degradation was negligible in each solution, and no epimerization of the hemiaminal ether was observed.

Stability of Biafungin in Aqueous Solutions: The results from stability studies of biafungin acetate in different aqueous solutions at varying pH in the absence of stabilizers are presented in Table 4. Under accelerated conditions (40 ° C), biafungin displayed less than 7% degradation in acetate buffer (pH 4.5) and less than 5% degradation in lactate buffer (pH 4.5 and 5.5) over periods of 6 and 9 months, respectively. In sterile water at RT, degradation of biafungin was negligible over 18 months. No epimerization of the hemiaminal ether was observed in any of the samples.

Biafungin Solution	Conc. (mg/mL)	Temp (° C)	Test	Time (Months)						
				0	1	3	6	9	12	18
Acetate Buffer (20 mM, pH 4.5)	3.33	40	Assay (mg/mL)	3.28	3.24	3.14	3.25	-	-	-
			Purity (% AUC)	97.7	96.0	94.5	91.2	-	-	-
Lactate Buffer (20 mM, pH 4.5)	3.33	40	Assay (mg/mL)	3.18	3.21	3.11	3.20	3.16	-	-
			Purity (% AUC)	93.9	92.6	92.3	91.1	89.3	-	-
Lactate Buffer (20 mM, pH 5.5)	3.33	40	Assay (mg/mL)	3.32	3.42	3.21	3.41	3.18	-	-
			Purity (% AUC)	95.6	93.5	92.2	92.3	90.9	-	-
Sterile Water	3.33	RT	Assay (mg/mL)	3.20	3.20	3.14	3.20	3.49	3.32	3.33
			Purity (% AUC)	97.3	96.6	96.7	96.1	96.7	97.4	97.4

Table 4. Stability data of biafungin in aqueous solutions at varying pH. The assay values are in reference to an internal standard and are an indication of the amount of biafungin in solution. Values between 90 and 110% of the target concentration were considered to be within the specification. Purities are reported as the % AUC for biafungin out of the total chromatogram. Degradation was slow under accelerated conditions in the acidic solutions, but it was negligible in sterile water at RT. No epimerization of the hemiaminal ether was observed.

CONCLUSIONS

- Biafungin was more stable than anidulafungin in plasma (rat, dog, monkey, and human) and in PBS buffer.
- The stability of biafungin in plasma suggests that (unlike the other echinocandins) chemical degradation may not be the primary mechanism for clearance.
- Biafungin acetate lyophilisate, free of fructose or stabilizers, exhibited less than 3% degradation over 9 months at 40 ° C.
- Biafungin acetate, free of stabilizers or other excipients, exhibited negligible degradation at room temperature in the presence of light in sterile water (18 months), 5% dextrose (15 months), and 0.9% saline (12 months) solutions.
- Biafungin acetate, free of stabilizers or other excipients, exhibited acceptable stability (6 – 9 months) in acetate and lactate buffers (pH 4.5 – 5.5) at 40 ° C accelerated conditions.
- Biafungin dosing solutions may not need to be discarded within 24 h, but perhaps could be stored at room temperature under light for much longer periods.
- Precautions such as refrigeration, controlled room temperature, protection from light, and the use of stabilizers and solubilizers may not be required for dosage preparations and storage stability.
- Further work needs to be done to determine if the stability of biafungin could enable viable subcutaneous or topical dosage forms.

REFERENCES

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ACKNOWLEDGEMENTS

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