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Determination of micafungin and anidulafungin in human plasma: UV- or mass spectrometric quantification?

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ABSTRACT

Micafungin and anidulafungin are two newer antifungal drugs from the echinocandine class. They are used as monotherapy or in combination with azole-antifungal drugs. The optimized clinical treatment course for the echinocandin drugs with regard to the different infection types and patient subgroups (renal or hepatic impairment, overweight) is still under debate. Therefore, an easy and rugged assay for these two drugs is highly desirable. We here present a method for the quantification of micafungin or anidulafungin in human plasma, applying protein precipitation as sample preparation, reversed phase separation of the analytes and UV-detection and simultaneous tandem mass spectrometry. Anidulafungin served as I.S. for micafungin quantification and vice versa. The method was validated in the calibration ranges from 0.1 μg/ml to 20 μg/ml for both substances. Intra-day precision and accuracies recorded with the UV-detector were 1.80% and 2.65% for micafungin and 4.30% and 10.44% for anidulafungin at the 0.1 μg/ml level. The respective data at the 1 μg/ml level were 2.25% and −0.83% for micafungin and 4.35% and −1.85% for anidulafungin and at the 20 μg/ml level 0.97% and −2.98% for micafungin and 1.04% and 4.74% for anidulafungin, respectively. With the mass spectrometer, because of the unique properties of the analyte molecules, no acceptable validation results could be achieved. Therefore, the mass spectrometric chromatograms served only as identity confirmation of the observed UV-peaks.

1. Introduction

Micafungin and anidulafungin (chemical structures see Fig. 1) are, beside the more established caspofungin, two newer antifungal drugs from the echinocandin class. They display fungistatic activity against *Aspergillus* spp. and fungicidal activity against most *Candida* spp., including strains that are fluconazole-resistant [1]. They can be administered alone and in combination with an antifungal drug from the azole class [2,3]. After intravenous infusion, the drugs are normally well tolerated and effective. Generally, no dosage adjustments are required in patients with varying degrees of hepatic or renal impairment [4,5]. However, clinical data suggest that dose adjustments are needed for special subgroups of patients, such as infants [6], patients with severe liver dysfunction [7] or overweight or obese patients [8] to achieve effective systemic drug concentrations. Optimized drug concentrations have to avoid levels lower than the minimum inhibitory concentration (MIC) of the pathogen, as well as to high concentrations because of the so-called "Eagle effect". This is described as a paradoxical effect on the growth rates of pathogens at increasing drug concentration levels, which is reported for caspofungin and micafungin [9] and may also apply to anidulafungin. The optimized clinical treatment course for the echinocandin drugs with regard to the different infection types and patient subgroups is still under debate [10]. Thus, to facilitate dosage adjustments and to gain further insight in the clinical application of the echinocandin drugs, easy and precise methods for the determination of these drugs in human plasma are of great interest.

In the literature, some HPLC-fluorescence methods for the quantitative determination of micafungin in plasma were reported [7,11–16]. The simultaneous detection of two active metabolites of micafungin was also described [15,16]. However, because of their low concentrations in plasma, these metabolites were regarded as of no therapeutic relevance [15]. In general, there were only minor variations between these methods. Additionally, a tandem mass spectrometric method has been described in short [17], but does not give any validation data. In the case of anidulafungin, quantitative detection in plasma with HPLC UV-detection [4,18] and with mass spectrometric detection [2,19] was described. In all of the cited papers, with the exception of Zornes and Stratford [18], the focus was on clinical investigation of the echinocandin drugs and the description of the analytical method and its validation remained marginal. A dedicated publication described the simultaneous detection of micafungin and anidulafungin, together with caspo-

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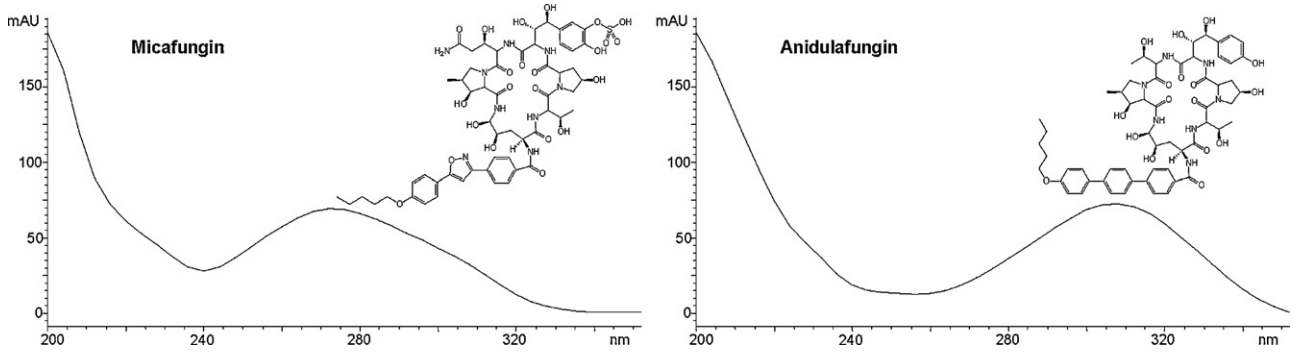

Fig. 1. Chemical structures and UV-spectra of micafungin and anidulafungin.

fungin and various azole antifungal drugs, utilizing HPLC–mass spectrometry [20]. However, this method suffered from the conflictive molecular nature of caspofungin (basic) and micafungin (acidic). Thus, the authors were able to optimize the chromatographic method only for caspofungin and the neutral anidulafungin, whereas micafungin resulted in distorted and broad peaks. This problem was avoided by Decosterd et al. [21] by excluding micafungin in their UPLC–mass spectrometric multiplex method for the quantification of anidulafungin and caspofungin together with various azol-antifungal drugs. In the analytical methodology of echinocandines, the choice of the I.S.'s proved to be difficult. Either the I.S.'s had no structural similarity to the analyte [2,21] or were custom synthesized analogs, which were not commercially available [4,7,12–15,17–20]. This makes it difficult to establish such a method outside a dedicated clinical study. For an everyday rugged routine analytical method, an easily available I.S. with similar physicochemical properties would be much more desirable.

Here, we described the development and validation of a method for the quantitative detection of micafungin or anidulafungin in human plasma, which is easy, precise and accurate. In the case of the quantification of micafungin, anidulafungin served as I.S., and vice versa. All other method parameters were identical for both substances. The method featured sample preparation by protein precipitation and chromatogragphic separation on a reversed phase column. We compared the performance characteristics of UV-detection and tandem mass spectrometric detection to investigate their respective pros and cons in everyday laboratory routine.

2. Materials and methods

2.1. Instrumentation

The HPLC system consisted of an Agilent 1100 system (Waldbronn, Germany) comprising a binary pump, an autosampler, a thermostatted column compartment and a diode array UV–vis detector. The analytical column was a Zorbax Eclipse XDB-C18 150 mm × 2.1 mm with 3.5 μm particle size (Agilent Technologies, Böblingen, Germany), protected by a SecurityGuard system (Phenomenex, Aschaffenburg, Germany) equipped with a 4 mm × 2 mm C18 filter insert. The mass spectrometric detection was performed on a Thermo Fisher Scientific TSQ Discovery Max triple quadrupole mass spectrometer (San Jose, CA, USA), equipped with an ESI ion source.

2.2. Chemicals

Reference substance of micafungin (Lot No. 122320KA, purity >98.5%, potency = 93.3%) was a kind gift of Astellas Pharma Inc. (Ibaraki, Japan) whereas reference substance of anidulafungin (Lot No. PF-3910960-0002, purity >98%, potency = 82.4%) was a kind gift of Pfizer Inc. (Groton, CT, USA). Ultra pure water was produced in our laboratory by a Barnstead EASYpure UV-system (Werner, Leverkusen, Germany). Drug free human plasma was obtained from the blood bank of the University Hospital Magdeburg (Germany). All other chemicals were of analytical grade or better.

2.3. Patient samples

Patient samples were obtained in the course of therapeutic drug monitoring during standard antifugal therapy with micafungin or anidulafungin, respectively. Steady state dosing was 100 mg/day for micafungin as well as for anidulafungin in all subjects studied. Blood samples were drawn into vacuum tubes without additives 30 min after the end of the infusion (C_{max}) and immediately prior to the next application (C_{min}). The blood samples were allowed to clot for 30 min and blood cells were separated by centrifugation at 1400 × *g* for 10 min. The serum samples were immediately frozen at −80 °C until analysis.

2.4. Stock solutions of micafungin and anidulafungin

Stock solutions were prepared as following: 5.34 mg of micafungin reference substance or 6.07 mg of anidulafungin reference substance were dissolved in 10 ml methanol/water 50/50 (v/v), respectively. The achieved concentrations in these stock solutions were 500 μg/ml in both cases. The stock solutions were stored at −80 °C until usage.

2.5. Calibration and quality control samples

To prepare calibration and quality control samples, 200 μl of the stock solution of micafungin or anidulafungin was diluted with 800 μl water to produce working solution A with the concentration of 100 μg/ml. A further dilution by factor 10 with water resulted in working solution B with a concentration of 10 μg/ml. By spiking 990, 980, 950 or 900 μl of drug free plasma with 10, 20, 50 or 100 μl of working solution B, respectively, and 980, 950, 900 or 800 μl drug free plasma with 20, 50 100 or 200 μl working solution A, plasma calibration samples with the concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μg/ml were produced. Quality control samples were prepared in a similar way in the concentrations of 0.1, 1 and 20 μg/ml. Calibration samples and quality control samples were prepared separately for the quantification of micafungin and anidulafungin, respectively.

2.6. Sample preparation

The sample preparation for both substances was identical. To 100 μl calibration sample, quality control sample or patient sample 20 μl of the I.S. solution (anidulafungin 10 μg/ml in the case