Background: The echinocandin antifungals anidulafungin (ANI) and micafungin (MICA) are recommended for treatment of candidemia. Their efficacy in peritonitis and thoracic empyema caused by Candida is less clear. Target-site concentrations, however, might be crucial for eradication of pathogens. Therefore, we refined an established method [1] for quantification of ANI and MICA in ascites and in pleural effusion in order to assess target-site pharmacokinetics. In addition, pharmacodynamics of ANI and MICA in ascites were assessed by in vitro simulation of fungal growth.

Methods: ANI and MICA were measured by high-pressure liquid chromatography (HPLC) and UV detection. Sample preparation was performed by protein precipitation with acetonitrile (ACN) and supernatant purification by solid phase extraction (SPE). Quantification was validated according to the bioanalytical method validation guidelines. Simulation of C. glabrata and C. albicans growth was performed in RPMI media and ascites spiked with ANI or MICA over a period of 144 h at 37°C. Numbers of fungal colony-forming units (CFU) were counted and assessed, considering the duplicit and the dilution.

Results: The lower limit of quantification (LLOQ) could be reduced from 0.1 to 0.05 µg/ml for ANI and MICA in ascites. Intra- and interday variability and reproducibility was within the required range (< 15%). Accuracy of linearity was within 85–115%. Extraction recovery could be doubled for ANI in ascites. Simulation of fungal growth in RPMI and ascites showed differences for C. glabrata and C. albicans proliferation. In untreated ascites, an increase in CFU by 10^3 within 144 h was observed for C. albicans, whereas no significant proliferation was seen for C. glabrata. An ANI concentration of 1 mg/l in ascites caused complete eradication of C. albicans. However, neither ANI nor MICA affected CFU numbers of C. glabrata in ascites.

Discussion: Implementation of SPE and a modified ACN precipitation technique lead to significant improvement in LLOQ and extraction rates for ANI and MIC. Due to higher accuracy and reproducibility, this method appears to be suitable for quantification of ANI and MICA in pharmacokinetic studies, particularly, when low echinocandin concentrations are anticipated. Results of fungal growth simulations suggest that antifungal efficacy in ascites may depend on the Candida species and on the applied echinocandin.

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Reference