Unraveling Drug Penetration of Echinocandin Antifungals at the Site of Infection in an Intra-Abdominal Abscess Model

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ABSTRACT

Background: Intra-abdominal candidiasis (IAC) is a persistent invasive fungal infection associated with high mortality. Prompt antifungal therapy and source control are crucial for successful treatment. Echinocandin antifungal drugs are first-line treatment options for IAC, but their efficacy in the site of infection is unknown due to the lack of pharmacokinetic information in IAC.

Methods: Female 6-8 week old CD1 mice weighing 18-22 g were infected intraperitoneally (IP) with 5x10^7 CFU of C. albicans SC5314 mixed with sterile stool matrix. Single or 3 doses of CD101 or micafungin were administrated at day 2 post-infection. Mice were sacrificed at 24 and 48 h post-dose. Liver and kidney lesions were collected for MALDI imaging. Laser capture microdissection (LCM) followed by liquid chromatography coupled tandem mass spectrometry (LC-MS/MS) was applied to 6 and 24 h samples for drug quantitation measurement.

Conclusions: Drug accumulation within lesions was observed with both drugs at their humanized therapeutic dose. However, drug accumulation was only detected from lesion enters at steady state (after 3 doses). CD101 diffused into lesions which may account for a considerable amount of treatment failures. Yet, data on the infection site pharmacokinetics (PK) of echinocandins are extremely scarce and nothing is known about penetration into tissue lesions. These findings indicate that current echinocandin drugs may be limited by penetration at the site of infection, which has implications for clinical outcomes and emergence of resistance in patients with IAC.

INTRODUCTION

Intra-abdominal candidiasis (IAC) is one of the most common yet poorly understood types of invasive candidiasis associated with high mortality. Non-invasive Candida species are responsible for life-threatening infections within abdominal organs from humans with IAC. Echinocandins are recommended as first-line therapy for many types of invasive candidiasis, but treatment failures occur in up to 40% of cases. Limited data have suggested that echinocandin delivery to infection sites is often insufficient to achieve concentrations that eliminate Candida or suppress resistance. Characterization of infection site pharmacokinetics (PK) of echinocandins may account for a considerable amount of treatment failures. Yet, data on the infection site pharmacokinetics (PK) of echinocandins are extremely scarce and nothing is known about penetration into tissue lesions. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) has emerged as a powerful tool to acquire spatially allocated molecular information about drug distributions in tissues. Here, we report our initial attempt to apply this technology, as well as standard analytical techniques, to investigate echinocandin drug penetration at the site of infection in a clinically relevant IAC mouse model involving C. albicans.

MATERIALS AND METHODS

Ethics statement: All animal experiments were approved by Rutgers Institutional Animal Care and Use Committee.

Mice model of intra-abdominal candidiasis and tissue sample collection: A mouse model of IAC was established by Zhong et al. (15). Female 6-8 week old CD1 mice were infected intraperitoneally (IP) with 5x10^7 CFU of C. albicans SC5314 mixed with sterile stool matrix as previously described. Single doses of CD101 (20 mg/kg) or micafungin (5 mg/kg) were administered at day 2 post-infection. In a separate experiment, mice were treated with 2 or 3 doses of micafungin (3 mg/kg) or single dose of CD101 (20 mg/kg). Drug accumulation was analyzed at 48 and 72 h post the first dose.

Drug accumulation within lesions was observed with both drugs at their humanized therapeutic dose. However, information on the infection site pharmacokinetics (PK) of echinocandins is extremely scarce and nothing is known about penetration into tissue lesions. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) has emerged as a powerful tool to acquire spatially allocated molecular information about drug distributions in tissues. Here, we report our initial attempt to apply this technology, as well as standard analytical techniques, to investigate echinocandin drug penetration at the site of infection in a clinically relevant IAC mouse model involving C. albicans.

RESULTS

Figure 1. Overview of MALDI-mass spectrometry imaging (left) and LCM (right).

Figure 2. Drug distribution in infected liver tissues after single dose micafungin and CD101. (a) Upper row: low magnification images of micafungin in representative liver tissues collected at 1, 3, 6, 24, and 48 h post a single dose of micafungin at 5 mg/kg. Signal intensity color bar is fixed for micafungin, with gradually increased intensity from blue (no signal) to red (maximum signal). H&E and GMS staining of adjacent sections are shown below each set of six images. Outlines highlight the lesion area on each tissue section. Scale bars, 3 mm.

Figure 3. Quantification of drug exposure in liver lesions and surrounding tissues. Drug concentration was measured in lesions and surrounding uninvolved tissues by laser capture microdissection of liver sections collected at 6 h and 24 h post single dose of micafungin at 5 mg/kg or CD101 at 20 mg/kg. Error bars, mean ± s.d. of 5-10 liver pieces or distinct lesions.

Figure 4. Liver burden comparison at 24 h (pretreated, 6 and 24 h post single dose treatment of CD101 at 20 or 5 mg/kg, micafungin at 5 mg/kg, and vehicle control. Each symbol represents liver burden of a single mouse. Error bars are mean burdens determined for 5 mice in each treatment group. Symbols on the x-axis represent mice with no liver burden (sterilization). Percentage of mice with liver sterilization were 0% for mice treated with pretreatment or 20 mg/kg CD101, and 24% for micafungin at 5 mg/kg.

Figure 5. Drug penetration after (a) multi-dosing micafungin and (b) single dosing CD101. Micafungin is steadily being accumulated in abscesses upon 2 and 3 doses. Micafungin signal was only detected from lesion enters at steady state (after 3 doses). CD101 diffusion into lesions throughout 48 h post single dosing, and accumulated in necrotic area of each lesion at 72 h. H&E and GMS staining of adjacent sections are shown below each set of ten images. Outlines highlight the lesion area on each tissue section. Scale bars, 3 mm.

Figure 6. Drug accumulation comparison between multiple doses of micafungin (5 mg/kg) and single dose of CD101 (20 mg/kg). Absolute drug level in liver lesions and surrounding uninvolved tissues from liver samples collected at 48 and 72 h post the 1st dose of micafungin and those treated with a single dose of CD101 and collected at the matched time points. Error bars, mean ± s.d. of 5-10 liver pieces or distinct lesions. ***P < 0.001, drug levels of CD101 were significantly higher than micafungin at all sites (lesion or uninvolved tissue) at specified time point.

CONCLUSIONS

- These findings indicate that current echinocandin drugs may be limited by penetration at the site of infection, which have implications for clinical outcomes and emergence of resistance in patients with IAC.

- Drug candidate CD101 appears to overcome this penetration limitation.

- More broadly, these types of studies are relevant to a wide range of infectious and non-infectious diseases that require effective drug levels for clinical response.

REFERENCES


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