Cardiac Effects of Echinocandins after Central Venous Administration in Adult Rats

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Echinocandins have become the agents of choice for early and specific antifungal treatment in critically ill patients. In vitro studies and clinical case reports revealed a possible impact of echinocandin treatment on cardiac function. The aim of our study was to evaluate echinocandin-induced cardiac failure. Using an in vivo rat model, we assessed hemodynamic parameters and time to hemodynamic failure after central venous application (vena jugularis interna) of anidulafungin (low-dose group, 2.5 mg/kg body weight [BW]; high-dose group, 25 mg/kg BW; caspofungin (low-dose group, 0.875 mg/kg BW; high-dose group, 8.75 mg/kg BW), micafungin (low-dose group, 3 mg/kg BW; high-dose group, 30 mg/kg BW), and placebo (0.9% sodium chloride). Left ventricular heart tissue was collected to determine mitochondrial enzyme activity via spectrophotometric measurements. mRNA expression of transcriptional regulators and primary mitochondrial transcripts, mitochondrial DNA (mtDNA) content, and citrate synthase activity were also explored. Animals receiving high-dose anidulafungin or caspofungin showed an immediate decrease in hemodynamic function. All of the subjects in these groups died during the observation period. Every animal in the untreated control group survived (P < 0.001). Hemodynamic failure was not noticed in the anidulafungin and caspofungin low-dose groups. Micafungin had no impact on cardiac function. In analyzing mitochondrial enzyme activity and mitochondrial transcripts, we found no association between echinocandin administration and the risk for hemodynamic failure. Further experimental studies are needed to elucidate the underlying mechanisms involved in cardiotoxic echinocandin effects. In addition, randomized controlled clinical trials are needed to explore the clinical impact of echinocandin treatment in critically ill patients.

Fungal infections represent a relevant risk for critically ill patients (1, 2), resulting in prolonged intensive care unit (ICU) stay and increased mortality (3–5). Antifungal therapy is crucial in septic patients to prevent irreversible injuries due to microbial load, systemic inflammation, and organ failure (6). Echinocandins are an established class of antifungal agents with activity against Candida and Aspergillus species. They are semisynthetic cyclic hexapeptides derived from various natural fungal products presenting a lipophilic side chain that interacts with the phospholipid bilayer of the fungal cell membrane, where they serve as noncompetitive inhibitors of β-1,3-1,6-glucan synthase (7, 8). The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines and the guidelines of the Infectious Diseases Society of America recommend echinocandins for the primary treatment of candidemia (9, 10). Development of septic cardiomyopathy reflects a crucial pathogenic part of hemodynamic instability in septic shock that aggravates tissue hypoperfusion and organ failure (11). Cardiac effects following echinocandin administration were seen in our ICU patients (12), in isolated rat hearts (Langendorff model) (13), and in isolated cardiomyocytes of rats (14). Following these approaches, we performed hemodynamic measurements after central venous administration of anidulafungin, caspofungin, and micafungin in clinically relevant concentrations in adult male rats to assess echinocandin-induced cardiotoxicity. Previous studies claimed that mitochondrial toxicity is the underlying mechanism behind echinocandin-induced cardiac failure (13, 15). To test this hypothesis in our model, we also determined left ventricular mitochondrial enzyme activity.

MATERIALS AND METHODS

Animal model. A total of 42 male Lewis rats (weighing 275 to 300 g), delivered by Charles River (Sulzfeld, Germany), were used in a randomized controlled model. All procedures involving animals were conducted in compliance with the standards for animal experiments and were approved by the local committee for animal care (GI 20/26 Nr.3/2012; Regierungspresidium, Giessen, Germany). Studies were performed in rats anesthetized with isoflurane (Baxter, Unterschleissheim, Germany). After endotracheal intubation with a 16-gauge catheter, animals were ventilated with a rodent respirator (Harvard Inspira, MA, USA) using volume-controlled ventilation in a weight-adjusted manner. Heart rate and body temperature were recorded. Ringer solution (10 ml/kg/h; Braun, Melsungen, Germany) and fentanyl (10 μg/kg/h; Ratiopharm, Ulm, Germany) were continuously administered intravenously through the lateral tail vein with a syringe pump (Braun, Melsungen, Germany). Arterial blood pressure was monitored using a tail-cuff system (Sphygmocare, IITC Life Science, Williston, VT, USA). Cytocardiographic parameters such as left ventricular pressure (LVP) and heart rate (HR) were recorded from aortic catheters and an ECG lead II, respectively. Hemodynamic data were recorded using a Safelab monitor (Safelab System, Böblingen, Germany). Infusions were performed through a 21-gauge catheter inserted into the left antecubital vein. Anidulafungin, caspofungin, and micafungin were dissolved in 10 ml of 0.9% sodium chloride (Braun Melsungen, Germany) and were continuously administered intravenously through the lateral tail vein with a syringe pump (Braun, Melsungen, Germany). Arterial
blood pressure was also measured continuously using a microtip catheter (SPR-1000; Millar Instruments, Houston, TX, USA) inserted in the animal’s tail artery. Experimental groups received clinically relevant human doses of echinocandins, and 10-fold higher drug doses were administered over a period of 60 min via a central venous catheter placed in the right jugular vein using a syringe pump (Harvard Apparatus, MA, USA). The animals were randomly divided into 7 groups: anidulafungin low-dose group (2.5 mg/kg body weight [BW] Ecalta; Pfizer, NY); anidulafungin high-dose group (25 mg/kg BW), caspofungin low-dose group (0.875 mg/kg BW Cancidas; Merck and Co., NJ), caspofungin high-dose group (8.75 mg/kg BW), micafungin low-dose group (3 mg/kg BW Mycamine; Astellas Pharma, Inc., IL), micafungin high-dose group (30 mg/kg BW), and untreated controls receiving 0.9% sodium chloride (placebo). Animals were observed for 6 h after echinocandin or placebo administration or until hemodynamic failure. Body temperature was kept at about 37°C throughout the experiment using a feedback-controlled heating pad and an infrared heater. After the rats were euthanized, their hearts were excised, dissected into left and right ventricle, and flash frozen in liquid nitrogen. Storage was carried out at −80°C.

**Hemodynamic measurements.** Cardiac function of the left ventricle was measured using a pressure-volume conductance catheter (SPR-838, Millar, Houston, TX, USA) (15). After blunt preparation and puncture of the carotid artery, the catheter was inserted, fixed with a suture, and carefully moved into the left ventricle. Pressure-volume signals were recorded using the PowerLab 8/30 signal converter (ADInstruments, Spechbach, Germany) and LabChart7 (ADInstruments). Parallel conductance catheter calibration was performed in each animal, where 30 μl of hypertonic saline (10%) was injected through a polyethylene catheter into the right jugular vein. Once, cuvette calibration to determine blood conductivity was performed with fresh heparinized warm blood. Recorded data were analyzed using PVAN 1.1 (Millar). Hemodynamic parameters, including cardiac output (CO), left ventricular ejection fraction (EF), arterial blood pressure, left ventricular end-diastolic volume (EDV), and stroke volume (SV) were calculated using PVAN software. Results are expressed as mean percentage of baseline ± SEM; n = 6/group.

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Primer sequences were designed using the corresponding reference sequences deposited in GenBank.

**FIG 1** Hemodynamic measurements in control and anidulafungin (ANID) high- and low-dose-treated rats. Animals treated with high-dose anidulafungin showed a large decrease in arterial blood pressure (A), cardiac output (B), and left ventricular ejection fraction (C). (D) Left ventricular end-diastolic volume decreased during the observation. Open squares, sham group; filled circles, low-dose (2.5 mg/kg BW) anidulafungin group; filled squares, high-dose (25 mg/kg BW) anidulafungin group. Data are mean percentages of baseline levels ± SEM; n = 6/group.
pressure (ABP), stroke volume (SV), left ventricular end-diastolic volume (EDV), and heart rate (HR) were recorded.

Determination of mitochondrial enzyme activities. Left ventricular (LV) tissues were homogenized in a solution containing 50 mM Tris buffer (pH 7.5), 100 mM potassium chloride, 5 mM MgCl₂, and 1 mM EDTA using a glass/glass homogenizer (2 ml, 0.025 mm clearance; Kontes Glass Co., Vineland, NJ). Enzymatic activities were spectrophotometrically measured at 30°C (Cary 50 photometer; Varian, Darmstadt, Germany). Each assay was performed at least in duplicate and normalized to citrate synthase (CS) activity and noncollagen protein (NCP). The analyses of the mitochondrial respiratory chain complexes NAD (NADH):coenzyme Q1 oxidoreductase (complex I), NADH-cytochrome c oxidoreductase (complex I + III), succinate-cytochrome c oxidoreductase (complex II + III), ubiquinone-cytochrome c oxidoreductase (complex III), cytochrome c oxidase (complex IV), and citrate synthase as mitochondrial marker enzyme were performed in accordance with a protocol described previously (17).

Real-time PCR analysis. Total RNA was isolated from frozen LV tissue using Tris Fast (Peqlab) according to the manufacturer’s instructions. The quality and integrity of the RNA samples were confirmed by agarose gel electrophoresis. RNA concentration was determined by measuring UV absorption (Nanodrop 1000 spectrophotometer; Thermo Fisher Scientific, Wilmington, DE, USA). Reverse transcription of RNA samples (500 ng total RNA) was carried out for 30 min at 42°C using the SuperScript III First-Strand cDNA synthesis kit (Invitrogen). Real-time PCR (primer sequences) (Table 1) and data analysis were performed using the StepOnePlus quantitative PCR system (Applied Biosystems, Life Technologies GmbH, Darmstadt, Germany). Expressed 18S rRNA was used as a housekeeping gene. All PCR results were calculated using the delta threshold cycle (C₀T) method and given as relative units to 18S rRNA concentrations. The relative copy number of mitochondrial DNA (mtDNA) per diploid nuclear genome was measured as described previously (18), using a fragment of mtDNA (cytochrome c oxidase [COX-1]) and a fragment of hypoxanthine phosphoribosyltransferase 1 (HPRT-1; housekeeping gene).

Statistical analysis. Hemodynamic results are expressed as the mean percentage of baseline levels plus or minus the standard error of the mean (SEM). Enzyme activity data are given as means ± SEMs for animals. All statistical analyses were performed using GraphPad Prism version 5.0 for Mac (GraphPad Software, La Jolla, CA, USA). Kaplan-Meier graphs were drawn for visualization of survival time, and the groups were compared using the log-rank test. For group comparison, we used the global survival function (Fig. 2). Rats treated with low-dose micafungin showed significantly increased mortality. Data are mean percentages of baseline levels ± SEM. *** P < 0.001.

RESULTS

Hemodynamic measurements in untreated rats over 6 h under anesthesia. All untreated controls (n = 6) survived the observation period. We found no change in CO (53 ± 21 ml/min at baseline versus 69 ± 20 ml/min at 6 h) (Fig. 1B), EF (69% ± 9% at baseline versus 55% ± 9% at 6 h) (Fig. 1C), and ABP (63 ± 6 mm Hg at baseline versus 51 ± 11 mm Hg at 6 h) (Fig. 1A). EDV increased slightly over the observation period (200 ± 55 µl at baseline versus 341 ± 47 µl at 6 h) (Fig. 1D).

Time to hemodynamic failure in low-dose- and high-dose-treated rats. All animals in the control group (n = 6) survived during the observation period (t = 360 min). The times to hemodynamic failure in the high-dose anidulafungin group (175 min versus 360 min; P < 0.001) and the high-dose caspofungin group (94 min versus 360 min; P < 0.001) were significantly reduced compared to those of the untreated controls (Fig. 2). Among the anidulafungin and caspofungin high-dose groups (n = 6 each), hemodynamic failure was observed in all animals. Animals in all the low-dose groups did not show significant differences compared to the placebo group in time to hemodynamic failure. In the high-dose micafungin group, 5 out of 6 rats survived during the observation period, while 1 animal showed hemodynamic failure 150 min after the start of infusion.

Hemodynamic measurements in treated animals. CO, EF, ABP, and EDV did not differ between the low-/high-dose micafungin groups and the nontreated controls during the experiments (Fig. 3A to D). Rats treated with low-dose anidulafungin or caspofungin also showed no significant differences in hemodynamic parameters (Fig. 1 and 4).

Animals treated with high-dose anidulafungin showed significant decreases in ABP (Fig. 1A) and EDV (Fig. 1D) that resulted in a consecutive decrease in CO (Fig. 1B) during our experiments. All animals treated with high-dose anidulafungin died during the observation period, and similar results were obtained after treatment with high-dose caspofungin. In the latter group, rats also showed a massive decrease in ABP (Fig. 4A) and CO (Fig. 4B). Each animal died during the first 120 min after the beginning of
drug administration. In contrast to animals receiving high-dose anidulafungin, those receiving high-dose caspofungin presented no significant decrease in EF (Fig. 4C).

Mitochondrial enzyme activities in echinocandin-treated animals. Spectrophotometric measurements of LV mitochondrial enzyme activity (complexes I to III, cytochrome c oxidase, succinate dehydrogenase) were performed. Results were normalized to citrate synthase activity. High-/low-dose anidulafungin groups, high-/low-dose caspofungin groups, and the low-dose micafungin group did not differ from the control group (Fig. 5). The high-dose micafungin group showed a significant reduction compared to the control group in complex III activity ($P < 0.05$) (Fig. 5B). No differences in COX-1 were observed between the control and treated groups. Regarding succinate dehydrogenase (SDH), we found a significant decrease in animals treated with low-dose micafungin ($P < 0.05$) (Fig. 5F).

Influence of echinocandin administration on LV mitochondrial gene expression. The mRNA expressions of ND-1 (complex I), cytochrome b (CYTB, complex III), COX-1 (complex IV), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), nuclear respiratory factor 1 (NRF-1), and mitochondrial transcription factor A (Tfam) were measured to explore possible influences of echinocandin treatment on mitochondrial gene expression. Treated animals did not show significant changes compared to the control group in mRNA expression. Specifically, there was no induction of ND-1, CYTB, COX-1, PGC-1α, NRF-1, or Tfam (see Table S1 in the supplemental material). Accordingly, no significant differences in mtDNA content were observed between echinocandin-treated animals and the controls (data not shown).

DISCUSSION
Our present study reveals a dose-dependent cardiac depression with anidulafungin and caspofungin treatment in the in vivo rat model. The current data also showed significantly higher rates of hemodynamic failure in animals treated with high-dose anidulafungin or caspofungin during the observation period. To our knowledge, this is the first study using a left ventricular catheterization system in rats for longitudinal hemodynamic measurements following echinocandin treatment. Our current results are in line with our previous findings in isolated cardiomyocytes treated with different concentrations of echinocandins (14). In these experiments, we discovered a dose-dependent decrease in contractility after echinocandin administration. In agreement with the data on cardiac side effects seen in our ICU patients after echinocandin administration (12) and case reports of hemodynamic instability (19) and pulmonary edema (20) during anidulafungin administration, we found decreased cardiac function after treatment with anidulafungin and caspofungin. Ex vivo cardiotoxicity studies in rats performed with a Langendorff heart model revealed similar findings (13). We searched the FDA Adverse Events Reporting System (FAERS) database and found four...
cases of arrhythmias and cardiac failure which may also reflect cardiac events after echinocandin administration (21). However, in a search of phase 2 and 3 drug studies, we did not find reports of adverse cardiac events after echinocandin application. Otherwise, cardiovascular decompensation in critically ill patients is common, so echinocandin-related side effects may have been considered a result of the primary disease. Stover et al. (13, 15) suggest that there are different possible mechanisms of drug-induced cardiotoxicity, including direct toxic effects, alterations in mitochondrial oxidative function, modulation of cardiac gene expression, alterations in myocyte protein synthesis/function, apoptosis, oxidative stress/free-radical generation, neurohormonal activation, and arrhythmia (22, 23).

In our model of continuous in vivo hemodynamic measurement in rats, we examined hemodynamic parameters following echinocandin administration. In animals treated with high-dose anidulafungin or caspofungin, we found an immediate decrease in cardiac output starting 30 min after the beginning of drug administration. Correspondingly, SV, LV EDV, and ABP decreased in these two high-dose groups. These findings may reflect an increased ventricular contraction that leads to a reduction in end-diastolic volume and consecutive failure of the Frank-Starling mechanism, which leads to a depression in cardiac output. In contrast to the measured decrease in EF in the high-dose caspofungin group, we found an increase in EF in the high-dose anidulafungin group compared to baseline. This fact might reflect different mechanisms that lead to impaired left ventricular function.

We performed spectrophotometric measurements of LV mitochondrial enzyme activity in cardiac tissue samples in order to investigate the underlying mechanisms of echinocandin-induced cardiac failure. We did not see an association between LV mitochondrial enzyme activity and hemodynamic dysfunction. Further analyses of mRNA did not demonstrate echinocandin-induced changes in mitochondrial gene expression in the study animals.

Animals treated with low-dose echinocandins, at a dosage commonly used in patients, showed no significant decrease in cardiac function. Because of these findings, we suggest a dose-dependent component in echinocandin-induced cardiac toxicity, but the underlying mechanisms of the described effects need further evaluation. Nevertheless, according to the findings of Stover et al. (13), micafungin did not cause relevant changes in cardiac function.

In our experiments, we found no evidence for the hypothesis that arrhythmia, as a side effect of echinocandin treatment, was the reason for the demonstrated changes in cardiac function. Arrhythmias were not described after antifungal drug administration in the Langendorff studies or in our ICU patients (12, 13). The effects of echinocandin treatment observed in our in vivo and in vitro studies, in the Langendorff heart studies, and in the published case reports were rapid. Therefore, modulations of cellular

![Hemodynamic measurements in controls and rats treated with high- and low-dose caspofungin. Animals treated with high-dose caspofungin showed a strong decrease in arterial blood pressure (A) and cardiac output (B). Left ventricular end-diastolic volume decreased during the observation (D). Rats treated with low-dose caspofungin showed no difference in left ventricular ejection fraction (C) or EDV. Open squares, sham group; filled circles, low-dose (0.875 mg/kg BW) caspofungin group; filled squares, high-dose (8.75 mg/kg BW) caspofungin group. Data are mean percentages of baseline ± SEM; n = 6/group.](http://aac.asm.org/)

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gene expression, alterations in cardiac protein synthesis, and neurohormonal activation are unlikely causes of the observed cardiac effects. Other possible mechanisms of toxicity are oxidative stress and the generation of free radicals, which were not analyzed in the present study. In summary, our findings revealed a dose-dependent cardiac depression in an *in vivo* rat model. The drug concentrations used in our studies were equal to or 10-fold higher than clinical dosages used in humans. Previous studies explored concentrations of caspofungin in ICU patients that were even higher than those used in healthy volunteers (24). Also, caspofungin peak plasma concentrations in healthy patients can reach 20 μg/ml (25), and preclinical distribution studies in animals revealed that heart tissue reaches about 30% of plasma concentrations (26–28). Anidulafungin plasma peak concentrations were measured up to 14 μg/ml. Micafungin peak plasma concentrations have reached 8.8 μg/ml (29). We conclude that rapid infusion of loading or maintenance doses via central venous catheters in critically ill patients with impaired cardiac function might lead to high echinocandin peak concentrations and cardiac depression in humans. Patients with sepsis-induced cardiomyopathy especially may be at risk for further aggravation of cardiac dysfunction following echinocandin administration due to the existing inflammation-induced endothelial damage. Nevertheless, our study only provides data for doses clinically equivalent to those used in humans and

![Spectrophotometric measurement of LV mitochondrial enzyme activity (complexes I to III, cytochrome c oxidase, succinate dehydrogenase) in left ventricular tissue samples of echinocandin-treated rats. Ctrl, sham animals; L, low dose; H, high dose. Data are means ± SEM; n = 6/group. *, P < 0.05 compared to control.](attachment:image.png)
for doses that are 10-fold higher. Further studies are necessary to delineate the potential dose dependence of the effect. Moreover, we did not reveal the mechanism that is responsible for our findings. Taking into consideration the results in isolated cardiomyocytes treated with echinocandins from our earlier in vitro study (14), we hypothesize that the observed effect might be driven by echinocandin-induced alterations in calcium homeostasis or direct toxic effects, but these mechanisms need further exploration. Randomized controlled trials are needed to explore drug-induced cardiac toxicity in critically ill patients and advise safe and effective antifungal treatment. Mechanisms leading to echinocandin-induced heart failure also need further evaluation.

In conclusion, echinocandin administration in an in vivo rat model of hemodynamic measurement reduces cardiac function and time to hemodynamic failure. Changes in mitochondrial function or mitochondrial biogenesis are unlikely causes of this cardiac dysfunction. This study may influence antifungal treatment. Mechanisms leading to echinocandin-induced heart failure also need further evaluation.

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