Cardiac Effects of Echinocandins in Endotoxemic Rats

Christian Koch, a Matthias Wolff, a Michael Henrich, a,c Markus A. Weigand, b Christoph Lichtenstern, b Florian Uhle b

Department of Anesthesiology and Intensive Care Medicine, University Hospital of Giessen and Marburg, Giessen, Germany a; Department of Anesthesiology, Heidelberg University Hospital, Heidelberg, Germany a; Department of Anesthesiology and Intensive Care Medicine, St. Vincentius Clinics, Karlsruhe, Germany c

Echinocandins are known as effective and safe agents for the prophylaxis and treatment of different cohorts of patients with fungal infections. Recent studies revealed that certain pharmacokinetics of echinocandin antifungals might impact clinical efficacy and safety in special patient populations. The aim of our study was to evaluate echinocandin-induced aggravation of cardiac impairment in septic shock. Using an in vivo endotoxemic shock model in rats, we assessed hemodynamic parameters and time to hemodynamic failure (THF) after additional central-venous application of anidulafungin (2.5 mg/kg of body weight [BW]), caspofungin (0.875 mg/kg BW), micafungin (3 mg/kg BW), and control (0.9% sodium chloride). In addition, echinocandin-induced cytotoxicity was evaluated in isolated rat cardiac myocytes. THF of the animals in the caspofungin group (n = 7) was significantly reduced compared to that in the control (n = 6) (136 min versus 180 min; P = 0.0209). The anidulafungin group (n = 7) also showed a trend of reduced THF (136 min versus 180 min; log-rank test P = 0.0578). Animals in the micafungin group (n = 7) did not show significant differences in THF compared to those in the control. Control group animals and also micafungin group animals did not show altered cardiac output (CO) during our experiments. In contrast, administration of anidulafungin or caspofungin induced a decrease in CO. We also revealed a dose-dependent increase of cytotoxicity in anidulafungin- and caspofungin-treated cardiac myocytes. Treatment with micafungin did not cause significantly increased cytotoxicity. Further studies are needed to explore the underlying mechanism.

Sepsis is one of the most serious and urgent infectious conditions in clinical practice. Although bacterial infections are the main cause of sepsis, fungal infections also represent a relevant risk factor for critically ill intensive care unit (ICU) patients (1, 2). Echinocandins (anidulafungin [ANID], caspofungin [CASP], and micafungin [MICA]) are an established class of antifungal agents recommended for the empirical and specific treatment of invasive Candida infections and aspergillosis (3, 4).

Randomized controlled trials and meta-analysis described echinocandins as effective and safe agents for the prophylaxis and treatment of different cohorts of patients with (suspected) fungal infections (5). Nevertheless, evidence is mounting that the pharmacokinetics of echinocandins in special patient populations prone to systemic inflammatory responses (e.g., critical ill patients, burn patients, patients with severe organ dysfunction) may impact clinical efficacy and safety (6).

Recent case reports describe adverse cardiac effects following echinocandin administration, especially in certain cohorts of critical ill patients (7–9). Also, in experimental studies using isolated rat hearts (Langendorff model) (10) or isolated cardiomyocytes of the rat (11), echinocandins were found to impair function properties.

Hemodynamic measurements after central venous administration of high doses of anidulafungin or caspofungin in adult rats provided evidence of significantly reduced cardiac output, which was associated with a significantly reduced survival rate compared to that in control animals (12). These results, which suggest a dose-dependent mechanism of echinocandin-induced cardiac depression, were also confirmed by a study of rats treated with different doses of echinocandins (13). Previous investigations claimed mitochondrial toxicity to represent the underlying mechanism behind echinocandin-induced cardiac failure (10, 13, 14, 28). However, spectrophotometric measurements in echinocandin-treated rats did not reveal any altered mitochondrial enzyme activity (12).

Septic shock is characterized by impaired hemodynamic function, microcirculatory alterations, and mitochondrial damage, which all reduce cellular energy production. The mechanisms of sepsis-induced cardiac dysfunction include the attenuation of the adrenergic response on the cardiomyocyte level, alterations of intracellular calcium trafficking, and blunted calcium sensitivity of contractile proteins (15). Hypothesizing an echinocandin-induced aggravation of cardiac impairment in patients already suffering from septic shock, we performed in vivo hemodynamic measurements in endotoxemic rats.

MATERIALS AND METHODS

Animals. Male Lewis rats (275 to 300 g) were obtained from Charles River (Sulzfeld, Germany). All procedures involving animals were conducted in compliance with standards for animal experiments and were approved by the local committee for animal care (GI 20/26 Nr.3/2012, JLU-Nr. 540_M; Regierungspräsidium Giessen, Germany). Studies were performed in anesthetized rats using isoflurane (Baxter, Unterschleissheim, Germany).

Experimental groups. Animals were randomly assigned into 4 study groups: the anidulafungin (ANID) group, which received 2.5 mg/kg of body weight (BW) (Ecalta; Pfizer, NY, USA) plus lipopolysaccharides (LPS) (1 mg/kg BW) (n = 7) (16); the caspofungin (CASP) group, which received 0.875 mg/kg BW (Cancidas; Merck, NJ, USA) plus LPS (1 mg/kg BW) (n = 7) (16).
In vivo hemodynamic rat model. Animals were handled and hemodynamic data were obtained as described previously (12, 27). Briefly, after endotracheal intubation with a 16G catheter, animals were ventilated with a rodent respirator (Inspira; Harvard Apparatus, MA, USA) using volume-controlled ventilation in a weight-adjusted manner. Ringer solution (10 ml/kg/h; Braun, Melsungen, Germany) and fentanyl (10 μg/kg/h; Ratiopharm, Ulm, Germany) were continuously administered intravenously over the lateral tail vein with a syringe pump (Braun, Melsungen, Germany). Experimental agents (anidulafungin, caspofungin, or micafungin) were administered via a central venous catheter placed in the right jugular vein using a syringe pump (Harvard Apparatus, MA, USA) over a period of 1 h. Simultaneously, animals received a lipopolysaccharide (LPS) bolus (LPS-EB Ultrapure from Escherichia coli O111:B4 strain TLR4 ligand; InvivoGen, San Diego, CA, USA) (1 mg/kg in 1 ml saline intravenously over tail vein) for >20 min via a syringe pump (Harvard Apparatus). LPS are the major constituents of the outer membrane of Gram-negative bacteria and are recognized by the Toll-like receptor 4 (TLR4), which is expressed from many cell types (e.g., monocytes), leading to a strong activation of the immune cells and to subsequent cytokine secretion. As a result of their amphiphile structure, LPS are capable of forming micelles in aqueous solution. Therefore, it was sonicated for 30 min before injection. Body temperature was kept at about 37°C during the experiment using a feedback-controlled heat pad and an infrared heater. Arterial blood pressure was also measured continuously using a Mikro-Tip catheter (SPR-1000; Millar Instruments, Houston, TX, USA) inserted in the animal’s tail artery. Cardiac function of the left ventricle was measured using a pressure-volume conductance catheter (SPR-838; Millar, Houston, TX, USA) (20). Recorded data were analyzed using PVAN 1.1 (Millar Chemical Company, Ann Arbor, MI, USA) according to the manufacturer’s instructions. The background control values were subtracted, and the mean percentage of treatment-induced cytoktoticity for each sample was calculated using the following equation: 100 × [(experimental value − spontaneous release)/(maximum release − spontaneous release)]. Spontaneous release was defined as the LDH activity in the supernatant of untreated samples, while maximum release was measured from untreated cell samples lysed by Triton X-100, reflecting the total capacity of intracellular LDH within the sample. Plasma LDH levels were also analyzed using the described cytoktoticity assay. At the end of the observation period or at the appearance of hemodynamic breakdown, animal blood samples were taken and plasma was separated. Samples were diluted 1:10 and optical density (OD) was measured. The absorbance at 490 nm was read using an automated plate reader (Epoch; BioTek Instruments GmbH, Heilbronn, Germany).

Statistical analysis. Hemodynamic results are expressed as the mean percentage of the baseline level plus or minus the standard error of the mean (SEM). Cytotoxicity data are given as a normalized percentage of cytoktocity of each LDH test sample plus or minus the SEM. Plasma LDH levels were expressed as mean OD plus or minus the SEM. 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 the visualization of survival time, and the groups were compared using the log-rank test. For group comparison, we used a global Kruskal-Wallis test followed by a Dunn’s multiple comparison test. A P value of 0.05 or smaller was regarded as significant.

RESULTS
Hemodynamic measurements in control animals. Among the control animals receiving 0.9% sodium chloride (NaCl) plus LPS (1 mg/kg BW) (n = 6), all animals survived the whole observation period. We observed only slight changes in CO (t₀ versus t₁, 6.34% ± 11.07%) (Fig. 1A), EF (t₀ versus t₁, −8% ± 4.62%) (Fig. 1B), and ABP (t₀ versus t₁, −10.94% ± 4.34%) (Fig. 1C). EDV (t₀ 19.16% ± 6.14%) (Fig. 1D) increased over the observation period.

Time to hemodynamic failure. All animals (n = 6) of the control group (LPS plus 0.9% saline) survived the complete observation period (t = 180 min). The time to hemodynamic failure of the endotoxemic animals in the caspofungin group (n = 7) was significantly reduced compared to that in the control animals (Fig. 2B; log-rank test, P = 0.0209). The anidulafungin group (n = 7) also showed a trend to reduced time to hemodynamic failure (Fig. 2A; P = 0.0578). Animals of the micafungin group (n = 7) did not show any significant difference in time to hemodynamic failure compared to that in the animals of the control group (Fig. 2C).

Hemodynamic measurements. Hemodynamic data were recorded during the first hour of each experiment. All animals survived during that period. In control group animals and in micafungin group animals, we found an increased CO during the experiments, while the CO decreased in anidulafungin and caspofungin group animals compared to baseline CO (Fig. 1A). Similar results were found regarding the animals’ SV (data not shown).

Further, our experiments revealed no alterations to the EF of the control group or of the micafungin group animals, while anidulafungin and caspofungin group animals showed a reduced EF (Fig. 1B). Common over all experimental groups, we observed a time-dependent decrease of ABP during the observation period (Fig. 1C). Also, all groups showed an at least slight tendency toward an increased EDV (Fig. 1D). Finally, we did not find any changes in a 96-well plate and were incubated for 2 h. They were then treated in triplicate with ANID, CASP, or MICA concentrations of 1, 2.5, 5, 10, 15, 20, 50, or 100 μg/ml and were incubated for another 20 h. The LDH released from cells was measured by a cytoktoticity detection kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer’s instructions. The background control values were subtracted, and the mean percentage of treatment-induced cytoktoticity for each sample was calculated using the following equation: 100 × [(experimental value − spontaneous release)/(maximum release − spontaneous release)]. Spontaneous release was defined as the LDH activity in the supernatant of untreated samples, while maximum release was measured from untreated cell samples lysed by Triton X-100, reflecting the total capacity of intracellular LDH within the sample. Plasma LDH levels were also analyzed using the described cytoktoticity assay. At the end of the observation period or at the appearance of hemodynamic breakdown, animal blood samples were taken and plasma was separated. Samples were diluted 1:10 and optical density (OD) was measured. The absorbance at 490 nm was read using an automated plate reader (Epoch; BioTek Instruments GmbH, Heilbronn, Germany).

Statistical analysis. Hemodynamic results are expressed as the mean percentage of the baseline level plus or minus the standard error of the mean (SEM). Cytotoxicity data are given as a normalized percentage of cytoktocity of each LDH test sample plus or minus the SEM. Plasma LDH levels were expressed as mean OD plus or minus the SEM. 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 the visualization of survival time, and the groups were compared using the log-rank test. For group comparison, we used a global Kruskal-Wallis test followed by a Dunn’s multiple comparison test. A P value of 0.05 or smaller was regarded as significant.

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Time to hemodynamic failure. All animals (n = 6) of the control group (LPS plus 0.9% saline) survived the complete observation period (t = 180 min). The time to hemodynamic failure of the endotoxemic animals in the caspofungin group (n = 7) was significantly reduced compared to that in the control animals (Fig. 2B; log-rank test, P = 0.0209). The anidulafungin group (n = 7) also showed a trend to reduced time to hemodynamic failure (Fig. 2A; P = 0.0578). Animals of the micafungin group (n = 7) did not show any significant difference in time to hemodynamic failure compared to that in the animals of the control group (Fig. 2C).

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HR among the different groups during the observation period (data not shown).

**Plasma lactate dehydrogenase.** At the end of the observation period or at the appearance of hemodynamic breakdown, blood samples of each study animal were taken and serum LDH levels were measured and expressed as mean OD. The highest values were found among the anidulafungin group animals. The mean OD values of the caspofungin group and of the micafungin group did not differ from that of the control group (Fig. 3A).

**Cell viability assay.** In order to link the observed effects to an actual influence of echinocandins on the cardiomyocytes, we assessed the cytotoxicity of the compounds by the release of LDH on freshly isolated rat cardiac myocytes. This revealed a dose-dependent increase of cytotoxicity in anidulafungin- and caspofungin-treated cardiac myocytes. Treatment with micafungin did not cause a significant increase in cytotoxicity (Fig. 3B).

**DISCUSSION**

Echinocandins are known as effective and safe agents for the prophylaxis and treatment of different cohorts of patients with fungal infections (5). Recent studies revealed that the pharmacokinetics of echinocandins in special patient populations might impact clinical efficacy and safety (6). Septic cardiomyopathy is a well-known organ dysfunction in sepsis, which is associated with reduced left ventricular contractility (19). Our study demonstrates cardiac depression and a reduced time to hemodynamic failure after administration of clinically used doses of anidulafungin (2.5 mg/kg BW) and caspofungin (0.875 mg/kg BW) in endotoxemic rats. In contrast, administration of micafungin (3 mg/kg BW) did not alter cardiac function or survival time.

Recent clinical case reports demonstrated unsuspected side effects after echinocandin treatment (7–9). In our previous studies, we discovered a dose-dependent decrease of contractility after echinocandin administration in isolated cardiomyocytes (11). Furthermore, in our model of continuous in vivo hemodynamic measurement in rats, we examined hemodynamic parameters following echinocandin administration. In animals treated with high doses of anidulafungin (25 mg/kg BW) or caspofungin (8.75 mg/kg BW), we found an immediate decrease of CO and a reduced survival time compared to those of the control and those of animals treated with low doses (anidulafungin group, 2.5 mg/kg BW; caspofungin group, 0.875 mg/kg BW). Micafungin (low-dose group, 3 mg/kg BW; high-dose group, 30 mg/kg BW) administration had no effect on cardiac function or survival time (12), a finding which holds true in this study using endotoxemic rats. Furthermore, ex vivo cardiotoxicity studies in rats performed with a Langendorff heart model and hemodynamic measurements in rats using echocardiography revealed similar findings (10, 13). The echinocandin doses used in our experiments reflect manufacturers’ recommendations for human use (16–18). Other authors used even higher echinocandin concentrations in murine models to take into account the higher metabolism rate, the higher heart
LPS; n = 7) versus the control group, 1 out of 7 animals died during the observation period (log-rank test, P = 0.0578). Data are mean percentages of survival.

**FIG 2** Time to hemodynamic failure in endotoxemic rats. Kaplan-Meier curves of control (0.9% NaCl + 1 mg/kg BW LPS; n = 6) and echinocandin-treated animals. (A) In the anidulafungin group (2.5 mg/kg BW + 1 mg/kg BW LPS; n = 7) versus the control group, 3 out of 7 animals died during the observation period (log-rank test, P = 0.0578). (B) In the caspofungin group (0.875 mg/kg BW + 1 mg/kg BW LPS; n = 7) versus the control group, 4 out of 7 animals died during the observation period (log-rank test, P = 0.0209). (C) In the micafungin group (3 mg/kg BW + 1 mg/kg BW LPS; n = 7) versus the control group, 1 out of 7 animals died during the observation period (log-rank test P = 0.2733). Data are mean percentages of survival.

frequency, and different plasma protein binding (13). In the absence of reliable metabolism data in rats, especially in systemic inflammatory conditions, we decided to test concentrations comparable to clinical use.

Especially in surgical ICU patients, bacterial infections are the main cause of sepsis. Nevertheless, mixed infections of bacterial and fungal pathogens are also very common in these patients. In clinical routine, the discrimination between bacteria- and fungal-induced systemic immune response is not feasible. Due to that fact, empirical echinocandin therapy in patients that may suffer from bacterial or mixed infections is common practice in ICUs. Considering this fact, and in order to use a standardized and well-known model, we chose LPS as the immunological agent to induce systemic inflammation and shock for our experiments. LPS are the major component of the outer membrane of Gram-negative bacteria and act as potent antigens for the immune system causing inflammatory reactions in mammals after recognition by the Toll-like receptor 4 (TLR4), which is expressed from many cell types but especially from monocytes, dendritic cells, macrophages, and B cells. Binding promotes the secretion of proinflammatory cytokines, nitric oxide, and eicosanoids.

Performing hemodynamic measurement in endotoxemic rats, we aimed to evaluate hemodynamic alterations following echinocandin administration in an endotoxin shock model. Central venous administration of anidulafungin plus LPS or caspofungin plus LPS caused immediate decreases in CO, SV, ABP, and EDV. These results may reflect impaired cardiac contractility together with LPS-induced peripheral vasodilatation. This fatal combination can synergistically result in a reduction of end-diastolic volume and in consecutive failure of the Frank-Starling mechanism, which leads to decreased CO. Investigating our hypothesis of a dose-dependent mechanism, we performed cytotoxicity studies using an LDH assay in freshly isolated rat cardiac myocytes. Corresponding to the results of our hemodynamic measurements, we again found a dose-dependent increase in cytotoxicity in cells treated with anidulafungin or caspofungin. Interestingly, according to our *in vivo* data, micafungin-treated cells did not show an increased rate of cytotoxic death. Regarding the LDH serum levels of our *in vivo* rat studies, we only found a trend toward elevated LDH values in anidulafungin-treated animals compared to that in control group animals. These results are in line with data from previous studies that did not observe micafungin-induced cardiac impairment (10–13). This fact may be explained by the hypothesis that micafungin, which is water-soluble compared with the other two lipophilic agents, would be unable to penetrate the tissue and cause serious cell damage.

Investigating the underlying mechanism leading to echinocandin-induced cardiac failure, we previously searched for alterations in cardiac mitochondrial function. Performing spectrophotometric measurements of rat left ventricular cardiac tissue after echinocandin treatment, we were not able to detect any altered mitochondrial enzyme activity (12). Previous studies on septic cardiomyopathy demonstrated the importance of mitochondrial dysfunction and reduced ATP generation (22, 23). Moreover, studies using isolated cardiomyocytes found that endotoxins alter or suppress the L channel-dependent calcium flow, possibly through changes in autonomic regulation of this channel (24, 25). These mechanisms caused a reduced concentration of intracellular calcium and a decrease in fiber contractility. With respect to our earlier results regarding isolated cardiomyocytes treated with echinocandins (14) and the results of our hemodynamic measurements, we would hypothesize that alterations in calcium homeostasis induced by echinocandins might be one potential harmful mechanism of action.

Besides these effects, vascular pathologies may contribute to the described results. Endotoxemia also has been described to induce capillary leakage by loosening epithelial tight junctions (26). Impaired vascular barrier function during endotoxemia may lead to elevated drug levels in the myocardium. Thus, vascular dysfunction may represent another important factor that aggravates echinocandin-induced cardiac impairment. Manufacturers recommend slow intravenous infusion of all echinocandins (anidulafungin, caspofungin, micafungin) over about 1 h (16–18). Rapid infusion of loading or maintenance doses over central venous
catheters that lead to a high echinocandin peak concentration may increase the risk for cardiac depression.

In summary, our results revealed that anidulafungin and caspofungin dosages that were similar to clinically used dosages in humans caused acute cardiac dysfunction in endotoxemic rats. In addition, we showed that anidulafungin and caspofungin induced dose-dependent cytotoxic effects in isolated rat cardiac myocytes. We conclude that patients suffering from severe infections may be at risk for a further aggravation of cardiac dysfunction following echinocandin administration.

Nevertheless, our study has some limitations. Pharmacokinetic and pharmacodynamic parameters vary between rats and humans, resulting in changed peak concentrations. Despite a higher basal rate of metabolism in rats, we found cardiac depression even after administration of comparable low dosages, which may be important information for health care professionals treating critically ill patients. At this time, we are not able to track down the harmful molecular mechanism that is responsible for the observed effects. Regarding the primary results of contractility studies in isolated cardiomyocytes, hemodynamic measurements, mitochondrial enzyme analyses, and cytotoxicity measurements, we would suggest that the described results may be triggered by alterations in calcium homeostasis or direct toxic effects. The mechanisms leading to echinocandin-induced heart failure will need further careful evaluation.

In conclusion, in our model of continuous hemodynamic measurements in endotoxemic rats, intravenous administration of anidulafungin or caspofungin was associated with acute cardiac dysfunction. Second, administration of anidulafungin or caspofungin in our model reduced survival. In addition, our studies revealed dose-dependent cytotoxicity in isolated cardiac myocytes exposed to anidulafungin or caspofungin. Further studies are needed to explore the underlying mechanism.

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