Phase II Dose Escalation Study of Caspofungin for Invasive Aspergillosis

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Our objective was to evaluate the maximum tolerated dose of caspofungin for invasive aspergillosis (IA). The safety and pharmacokinetics of escalating dosages of caspofungin were investigated in IA. Eight patients each received caspofungin 70, 100, 150, or 200 mg once a day (QD). Dose-limiting toxicity (DLT) was defined as the same non-hematological treatment-related adverse event of grade ≥4 in 2 of 8 patients or ≥3 in 4 of 8 patients in a cohort. A total of 46 patients (median age, 61 years; 21 female; 89% with hematological malignancies) received caspofungin (9, 8, 9, and 20 patients in the 70-, 100-, 150-, and 200-mg cohorts) for a median of 24.5 days. Plasma pharmacokinetics were linear across the investigated dosages and followed a two-compartment model, with weight as the covariate on clearance and sex as the covariate on central volume of distribution. Simulated peak plasma concentrations at steady state ranged from 14.2 to 40.6 mg/liter (28%), trough concentrations from 4.1 to 11.8 mg/liter (58%), and area under the concentration-time curve from 175 to 500 mg/liter/h (32%) (geometric mean, geometric coefficient of variation). Treatment was well tolerated without dose-limiting toxicity. The rate of complete or partial responses was 54.3%, and the overall mortality at 12-week follow-up was 28.3%. In first-line treatment of invasive aspergillosis, daily doses of up to 200 mg caspofungin were well tolerated and the maximum tolerated dose was not reached. Pharmacokinetics was linear. Response rates were similar to those previously reported for voriconazole and liposomal amphotericin.

Invasive aspergillosis (IA) remains an important cause of infectious morbidity and mortality in immunocompromised patients. It is the most common invasive fungal disease (IFD) in patients with hematological malignancies (5). Current first-line therapies with liposomal amphotericin B and voriconazole fail in approximately 50% of patients. With 12-week mortality rates as high as 28%, new approaches are urgently needed (7, 12).

High-dose liposomal amphotericin B (i.e., 10 mg/kg per day for the first 2 weeks of treatment) did not yield better outcomes than a standard dose of 3 mg/kg per day but resulted in higher rates of renal adverse events (AEs) (7). Dose escalation of voriconazole is not pursued due to the nonlinear disposition of the compound and a narrow therapeutic window. Antifungal combination therapy is another attractive strategy, but it has yet to be proven superior to monotherapy (22).

Caspofungin is generally well tolerated and exhibits favorable pharmacokinetic properties (21). Unlike the triazoles, it is not metabolized through the cytochrome P450 enzyme system (11). The drug had excellent efficacy and safety results in clinical trials of candidiasis (1, 17, 23, 24) and was effective as salvage therapy for IA after amphotericin B or itraconazole proved ineffective or toxic (15), and a large-scale study in neutropenic patients with persistent fever demonstrated an efficacy similar to that of liposomal amphotericin B but improved tolerability (26). Two recently published trials investigated the caspofungin standard maintenance doses of 50 mg once a day (QD) for first-line treatment of IA and yielded response rates of 33 and 42% (13, 25). While these response rates were below the expected outcomes (7, 12), they have been attributed to the severely ill patient groups enrolled in these trials and the rigorous enforcement of the EORTC/MSG.  

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consensus criteria (8), which may result in a delayed treatment trigger (6, 10).

Preclinical and limited clinical data support the concept of dose-dependent antifungal efficacy of caspofungin (1, 19, 23). Data from healthy volunteers demonstrate that caspofungin can be safely given at doses of up to 210 mg/day (19). In patients with candidemia and invasive candidiasis, the safety and tolerability of caspofungin 150 mg once daily were comparable to those of the 50-ng standard dose (2, 20).

Dose escalation of caspofungin for treatment of invasive aspergillosis has not been formally studied. We therefore investigated the safety, tolerability, and pharmacokinetics of higher doses of caspofungin, up to 200 mg QD, in a dose escalation study in adult patients with proven or probable invasive aspergillosis.

MATERIALS AND METHODS

This was a formal phase II dose escalation study in patients with invasive aspergillosis pursuing the definition of the maximum tolerated dose (MTD) of caspofungin in this setting and determining the pharmacokinetic properties of caspofungin given at dosages ranging from 70 to 200 mg QD. The study was registered with the European Union Drug Regulating Authorities Clinical Trials website (EudraCT 2006-001936-30) and on ClinicalTrials.gov (NCT00404092).

Study endpoints. The primary endpoints of the study were the safety and tolerability of caspofungin. Endpoint of safety and tolerability were the numbers of toxicity-related study therapy discontinuations and predefined grade ≥3 clinical- and laboratory events, as evaluated on the basis of current NCI criteria (18). Secondary endpoints included pharmacokinetic parameters for each dosage level and efficacy of caspofungin at four escalating dosages. The primary variable of antifungal efficacy was therapeutic success, defined as the complete or partial response of initial proven or probable aspergillosis at the end of caspofungin treatment. Other efficacy variables included assessments of relapse of IA at 4 and 12 weeks after the end of caspofungin study therapy (in those patients with therapeutic success at the end of therapy), the absence of drug discontinuations due to toxicity or lack of efficacy, and survival.

Patient eligibility criteria. Adults 18 years or older were eligible if they had an immunocompromising condition associated with invasive fungal disease and evidence of proven or probable IA defined by modified EORTC criteria as described previously (7). Briefly, patients were also included and considered a probable IFD case with a chest computed tomography (CT) scan positive for a halo or air crescent sign without microbiological evidence.

Female patients of childbearing age must have had a negative pregnancy test at study entry and had to take adequate contraceptive measures throughout the study. Men with normal prostatic fluid or seminal fluid data were both board-certified hematologists and infectious disease experts.

Criteria for assessment of the maximum tolerated dose were as follows: if two out of eight patients in the same dosage cohort developed the same grade ≥3 nonhematological adverse event related to the study drug, or if four out of eight patients in the same dosage cohort developed a grade ≥3 nonhematological adverse event related to the study drug, no further dose escalation was considered and enrollment in this cohort was terminated. If two patients in the same dosage cohort developed the same grade 3 nonhematological adverse event related to the study drug, a further four patients were to be enrolled in this dosage cohort. If no further drug-related grade ≥3 AE of the same type occurred with enrollment in the next-higher dosage cohort, if DLT was reached in a dosage cohort, the dosage of the next-lower dosage cohort would have been defined as the maximum tolerated dose and an additional 12 patients enrolled in that dosage cohort to collect further safety data. Similarly, if no DLT was reached at the highest dosage level of 200 mg QD, an additional 12 patients were to be enrolled in the 200-mg cohort.

Role and composition of the DSMB. Escalation to the next-higher dosage level was carried out only after the external data safety monitoring board (DSMB) (M.K., T.L., and A.U.) and the trial committee (O.A.C., A.G.) agreed that the aforementioned safety and tolerability criteria were met. Members of the DSMB were both board-certified hematologists and infectious disease experts.

Pharmacokinetic sampling and analysis. Plasma sampling was performed on day 1 and at peak and trough time points on days 4, 7, 14, and 28. Blood specimens (5 ml) were collected in heparinized tubes and immediately centrifuged for 10 min at 1,500 g. Separated plasma was stored at −80°C until assay.

Concentrations of caspofungin were measured by a liquid chromatography tandem mass spectroscopy (LC-MS/MS) method as described in detail elsewhere (9). Due to the expected high drug levels, the method was modified to allow quantification of caspofungin in the range from 84 ng/ml (lower limit of quantitation) to 84,000 ng/ml. Accuracies were within ±11.9%, and intraday variability (precision) was ±8.1%.

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Role of the funding source. Merck & Co., Inc. (Whitehouse Station, NJ), was the financial sponsor through a grant to the University of Cologne, Cologne, Germany. The study was designed by academic authors (O.A.C., D.A., A.H.G.). The legal sponsor was the University of Cologne, Cologne, Germany. Study coordination was performed by the study sponsor, which included ensuring...
adherence to good clinical practice standards, training investigators, collecting the data, monitoring and auditing clinical sites, managing the data, and performing the statistical analyses. The primary data and statistical analyses were made available to all authors. All authors were granted full access to the complete data set of the study, and the corresponding author had final responsibility for the decision to submit for publication.

**RESULTS**

**Patients.** From September 2006 until July 2009, a total of 46 patients with proven or probable invasive aspergillosis were enrolled at three German university hospitals. In the 70-mg and the 150-mg cohorts, one patient each (2.2%) was replaced because of incomplete pharmacokinetic sampling due to early discontinuation after 3 and 4 days, respectively. Both replaced patients were included in the pharmacokinetic and safety analyses with the available data. Baseline characteristics of the 46 patients, including age, gender, weight, body mass index (BMI), fever and/or neutropenia at baseline, underlying disease, and EORTC/MSG classification of invasive aspergillosis, were comparable between cohorts. Of the 46 patients, 21 were female, 27 had acute leukemia, and 31 were neutropenic as a baseline. According to the modified EORTC/MSG criteria used for the trial, one patient had proven aspergillosis, 25 had probable aspergillosis with microbiological evidence, and 20 had probable aspergillosis without microbiological evidence. The complete details are available in the supplemental files (see Table S1 in the supplemental material). All patients had the lung as the only site of infection. Demographic characteristics did not significantly differ between dosage groups.

The median duration of study drug treatment was 24.5 days. Details are provided in Table 1. Reasons for discontinuation of study treatment were completion of the maximum 28 days on study drug (n = 15), treatment failure (n = 10), sufficient treatment response prior to day 28 (n = 8), switch to oral treatment (n = 6), adverse events (n = 5), and patients’ decision (n = 2).

**Safety and tolerance.** Analysis of the cumulated reported adverse events revealed that only one patient had an adverse event which could potentially be rated as DLT criterion as defined in the protocol. This event (elevated γ-glutamyl transferase, γGT) occurred in the 200-mg group. Since no other such adverse event occurred, DLT was not achieved.

Adverse events leading to discontinuation were suspected drug fever (n = 1), elevated liver function tests (n = 1), patient death (n = 1), worsening of aspergillus pneumonia (SAE, n = 1), and not reported (n = 1). Table 2 gives an overview of grade 3 to 5 adverse events by dose group. There was no clear relationship between caspofungin dose and the incidence of adverse events. Details on the frequency of adverse events of various severity grades and serious adverse events reported for the patients in each dose cohort are provided in the supplemental files (see Tables S2 and S3 in the supplemental material). Only two events with a probable relationship to the study drug were reported (100-mg group, grade 1 loss of appetite; 200-mg group, grade 3 γ-glutamyl transferase [γGT] elevation); no events were considered definitely related. The number of grade ≥3 events with at least a possible relationship to study drug was zero in all dose cohorts except the 200-mg group, where six such events (all grade 3) occurred in six patients: hyponatremia, elevated alkaline phosphatase (3 events), alanine transaminase, and γGT.

A total of 42 serious adverse events (SAE) occurred in 26 patients, and none was related to study treatment. The mean numbers of SAE per patient were 0.2, 1.0, 1.1, and 1.1 in the 70-, 100-, 150-, and 200-mg cohorts, respectively.

**Pharmacokinetics.** Dose-normalized trough concentrations revealed dose linearity of caspofungin across the investigated dosage range. Based on population pharmacokinetic analysis, plasma data were best described by a linear two-compartment pharmacokinetic model with weight as covariate on clearance (0.401 liter/h), sex as covariate on central volume of distribution (male, 6.7 liters; female, 4.89 liters), an intercompartamental clearance of 0.815 liter/h, and a peripheral volume of distribution of 6.84 liters. Based on the final model, simulated peak plasma concentrations at steady state ranged from 14.2 to 40.6 mg/liter (28%), trough concentrations from 4.1 to 11.8 mg/liter (58%), and area under the concentration-time curve from 175 to 500 mg/liter/h (32%) for the dosage range of 70 to 200 mg QD (geometric mean, geometric coefficient of variation of variation) (Table 3).

**Treatment success.** The treatment outcome of the different dose groups is shown in Table 1. Favorable responses at end of treatment (EOT), 4 weeks follow-up, and 12 weeks follow-up, defined as complete and partial responses, were observed in 25 (57%), 25 (57%), and 23 (52%) patients, respectively. Concerning the dosage groups, favorable outcomes in the 70-mg, 100-mg, 150-mg, and 200-mg groups at EOT were observed in 4 (44%), 3 (36%), 6 (67%), and 12 (60%) patients, respectively.

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### Table 1. Treatment allocation and outcomes

<table>
<thead>
<tr>
<th>Efficacy analysis group</th>
<th>No. of patients in group</th>
<th>Days on study drug, median (range)</th>
<th>No. of patients per group*</th>
<th>Relapseb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Favourable (CR/PR)</td>
<td>Stable disease</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>24.5 (3–29)</td>
<td>25 (1/24)</td>
<td>4</td>
</tr>
<tr>
<td>70 mg</td>
<td>9</td>
<td>28 (3–29)</td>
<td>4 (0/4)</td>
<td>2</td>
</tr>
<tr>
<td>100 mg</td>
<td>8</td>
<td>24.5 (7–28)</td>
<td>3 (0/3)</td>
<td>4</td>
</tr>
<tr>
<td>150 mg</td>
<td>7</td>
<td>16 (4–28)</td>
<td>6 (1/5)</td>
<td>2</td>
</tr>
<tr>
<td>200 mg</td>
<td>20</td>
<td>21 (4–28)</td>
<td>12 (0/12)</td>
<td>8</td>
</tr>
</tbody>
</table>

* Denominator indicates no. of patients with CR or PR at EOT.

b Denominator indicates no. of patients with CR or PR at EOT.
Survival. The median observation time (ending with the end of the follow-up or patient death) was 109 days. Thirteen of 46 patients (28.3%) died during the treatment or the 12-week follow-up period. Causes of death were the underlying disease in 5 patients, pneumonia in 3, sepsis or sepsis-related complications in 4, and pulmonary hemorrhage in 1. Twelve weeks after EOT, the estimated overall survival was 73.9% (Kaplan-Meier estimate; data not shown). At 100 days, the estimated survival rate was 71% (95% confidence interval [CI], 57% to 84%). To allow better comparison with earlier trials, a post hoc analysis of survival 12 weeks after starting treatment was performed, showing a 76% overall survival probability.

**DISCUSSION**

The results of this dose escalation study evaluating caspofungin as the first-line treatment of invasive aspergillosis demonstrate acceptable safety and tolerance of daily doses of caspofungin up to 200 mg over extended periods of time. Dose-limiting toxicity was not observed, and thus, 200 mg/kg may be defined as current MTD in this setting.

While no severe adverse events with a probable or definite relationship to study treatment were observed at all dose levels, there was a tendency toward higher rates of adverse and serious adverse events in the higher-dose groups. However, we evaluated a severely ill patient population: most individuals were still under treatment for their underlying malignancy and were profoundly immunocompromised. Under these conditions, substantial variance in the number of adverse events is anticipated. Also, given the open-label character of the study, investigators may have been inclined to attribute events to study treatment in the higher dose groups. It is still possible that a 200-mg dose of caspofungin may be associated with a higher rate of adverse events.

Assessment of pharmacokinetic parameters revealed dose-proportional increases in exposure without changes in total clearance, consistent with linear pharmacokinetics across the investigated dosage range of 70 to 200 mg QD. Following administration of 100 mg QD, the steady-state estimated mean area under the concentration-time curve (AUC) values were slightly (15%) higher in the study patients relative to values following similar dosing schedules in healthy volunteers (16). Changes in plasma composition, saturation effects of OATP1B1-mediated hepatocellular uptake, and the generally higher inter-individual variability in drug disposition in critically ill patients all may account for these conditions, substantial variance in the number of adverse events is anticipated. Also, given the open-label character of the study, investigators may have been inclined to attribute events to study treatment in the higher dose groups. It is still possible that a 200-mg dose of caspofungin may be associated with a higher rate of adverse events.

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Invasive aspergillosis is a disease associated with a high rate of treatment failure and a high mortality (7, 12). The response and 12-week survival rates compare well to the findings reported from large randomized trials of voriconazole and liposomal amphotericin B for first-line treatment of IA (7, 12). Of note, recently published trials on caspofungin monotherapy for IA reported lower response and 12-week survival in patients with hematological malignancies and patients after allogeneic stem cell transplantation (13, 25). The apparently better results from our trial may have been achieved by the higher dose and the less rigid enrolment criteria. In particular, waiving the necessity of microbiological evidence in our trial may have allowed earlier treatment of invasive aspergillosis, leading to better treatment results.
of high-dose caspofungin versus voriconazole or liposomal amphotericin and voriconazole. Response rates and survival were comparable to those achieved with liposomal amphotericin and voriconazole. The next step toward a more effective treatment than current standard therapies could be a randomized comparison of high-dose caspofungin versus voriconazole or liposomal amphotericin B.

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