Caspofungin prolongs survival in transiently neutropenic rats with advanced stage of invasive pulmonary aspergillosis

Wendy W. J. van de Sande¹, Wim van Vianen¹, Marian T. ten Kate¹, Jolanda Vissers¹, John Laurijsens¹, Mehri Tavakol¹, Bart J.A. Rijnders¹,², Ron A.A. Mathot³ and Irma A.J.M. Bakker-Woudenberg¹

¹Erasmus MC
University Medical Center Rotterdam
Department of Medical Microbiology & Infectious Diseases
Dr. Molewaterplein 40
3015 GD Rotterdam
The Netherlands

²Erasmus MC
University Medical Center Rotterdam
Department of Internal Medicine, Section Infectious Diseases,
Dr. Molewaterplein 40
3015 GD Rotterdam
The Netherlands

³Erasmus MC
University Medical Center Rotterdam
Department of Hospital Pharmacy, Clinical Pharmacology Unit,
Dr. Molewaterplein 40
3015 GD Rotterdam
The Netherlands
ABSTRACT

Objectives: Evaluation of a high-dose-step-down strategy for caspofungin treatment in an experimental model of advanced stage of invasive pulmonary aspergillosis.

Methods: The therapeutic efficacy of caspofungin in relation to the severity of invasive pulmonary infection caused by *Aspergillus fumigatus* in transiently neutropenic rats was investigated using rat survival and decrease in fungal burden as parameters for efficacy.

Results: When started either at 16 h or 24 h after fungal inoculation caspofungin administered intraperitoneally at 4 mg/kg/day for 10 days was highly effective (100% and 93% rat survival, respectively). However only 27% rat survival was obtained with treatment started at 72 h when rats had advanced stage of infection. Increasing the dose from 4 to 10 mg/kg/day could compensate for the decrease in efficacy and resulted in 67% rat survival. The high dose of 10 mg/kg/day for 10 days appeared not necessary since a high-dose-step-down dosing schedule with 10 mg/kg/day for 3 days followed by 4 mg/kg/day for 7 days was equally effective. At 10 days after end of treatment with 10 mg/kg/day caspofungin both *A. fumigatus* DNA and *A. fumigatus* galactomannan in the infected left lung were not significantly decreased. In contrast, *A. fumigatus* galactomannan concentrations in serum were significantly decreased. CREAT, BUN, ALAT and ASAT were not elevated during treatment.

Conclusions: Caspofungin is effective in invasive pulmonary aspergillosis in transiently neutropenic rats even in advanced stage of infection. In this model, administering high-dose-step-down treatment was as effective as high doses during the whole treatment period.
Keywords: caspofungin, high-dose-step-down, Aspergillus fumigatus, quantitative PCR, galactomannan

Number of words: 248
INTRODUCTION

Invasive Pulmonary Aspergillosis (IPA) is a life-threatening fungal infection observed in severely immunocompromised patients. At present antifungal treatment has changed with the introduction of broader spectrum azoles and the echinocandins. In contrast to amphotericin B and the azoles, the echinocandins, with caspofungin as the first approved member, do not act on the cell membrane but on the cell wall (11, 12). The echinocandins inhibit the 1,3-β-D-glucan synthesis, an essential molecule which provides osmotic stability to fungi and is essential in growth and division (11, 12). As 1,3-β-D-glucan is not found in mammalian cells, inhibition of this synthesis in fungi is highly specific resulting in high tolerability of the echinocandins (11), which explains why until now, no serious side-effects of caspofungin have been published, and the drug seems to have an excellent safety profile (4). Caspofungin has been demonstrated to be effective as salvage therapy in patients with documented invasive aspergillosis, and as empiric therapy in patients with persistent fever and neutropenia (10, 18).

Although caspofungin seems to have a favourable therapeutic effect in neutropenic patients with invasive aspergillosis, animal studies remain necessary to evaluate the full efficacy of this drug. Our earlier study already showed the therapeutic efficacy of caspofungin in a clinically relevant *Aspergillus fumigatus* infection model in transiently neutropenic rats (17). Treatment was started at 16 hours after inoculation, when fungal hyphal growth was established. Caspofungin at 4 mg/kg/day administered for 10 days resulted in 100% rat survival, whereas only 27% of the rats survived after treatment with 1 mg/kg/day amphotericin B, being the maximum tolerated dose (17). The present study investigates the therapeutic efficacy of caspofungin in relation to the severity of fungal infection. In the clinical situation we are also often faced with patients with extensive fungal lesions and high fungal burden. In this study the efficacy of caspofungin was determined in transiently neutropenic rats with early versus advanced stage of IPA. Furthermore, the efficacy of therapy
with high doses of caspofungin throughout the entire treatment was compared to high-dose-step-down therapy.

MATERIALS AND METHODS

Aspergillus fumigatus isolate

In our infection model a clinical strain of A. fumigatus, originally isolated from a hemat-oncological patient with invasive pulmonary aspergillosis was used in all experiments. To maintain its virulence the strain was passed regularly through neutropenic rats and maintained on sabouraud agar slants. For this strain the MIC for caspofungin was determined according to the CLSI criteria in a previous study and appeared to be 8 mg/l (17). The MEC for this strain was 0.05 and was determined with the E-test (AB Biodisk, Goes, The Netherlands) according to the manufactures instructions.

Infection model of invasive pulmonary aspergillosis and antifungal treatment

The rat model of aerogenic left-sided invasive pulmonary aspergillosis in neutropenic rats was used as described previously (2, 3) and has slightly been modified by van Vianen et al. (17). In short, neutropenia was induced with intraperitoneal administration of 75 mg/kg cyclophosphamide (Endoxan®, Baxter, Utrecht, The Netherlands) 5 days before fungal inoculation, followed by a dose of 60 mg/kg 1 day before inoculation and 50, 40 and 30 mg/kg, respectively on days 3, 7 and 11 after fungal inoculation. Fungal infection was established by intubation of the left main bronchus under general anaesthesia. A cannula was passed through the tube and the left lung was inoculated with $6 \times 10^4$ conidia in 20 µl PBS.

Treatment with caspofungin (Cancidas®, Merck and Company, Rahway, JY, USA) was started at either 16 h, 24 h or 72 h post fungal inoculation, time-points at which hyphal growth
was established as determined by histological examination. Caspofungin was diluted in saline and was administered intraperitoneally once daily for 10 days. Treatment regimens included 4 or 10 mg/kg/day.

The experimental protocols adhered to the rules specified in the Dutch Animal Experimentation Act (1977) and the published Guidelines on the Protection of Experimental Animals by the Council of the EC (1986). The present protocols were approved by the Institutional Animal Care and Use Committee of the Erasmus MC Rotterdam.

**Parameters for therapeutic efficacy**

To determine therapeutic efficacy several parameters were monitored. The main parameter was survival of the infected rats, monitored daily during therapy and the 10 day period after termination of therapy. The decrease in fungal burden in the infected left lung was also measured on days 1, 3, 6 after fungal inoculation for the untreated control rats and on days 6, 9, 13 and 23 for the treated rats. This was done by the quantitative detection of both *A. fumigatus* DNA and galactomannan. Galactomannan is a fungal cell-wall polysaccharide that can be released by *Aspergillus* spp. during growth. Galactomannan concentrations in serum were also assessed. At the indicated time intervals rats were euthanized under CO₂. Blood was obtained by puncture of the orbital plexus. Then the left lung was dissected and stored at −80 °C until analysis.

The quantitative detection of *A. fumigatus* DNA in the left lung by a TaqMan PCR and calculation of conidial equivalents (CE) was performed as described by Bowman et al. (5) Normalisation for DNA was done by adding a universal control to each sample before DNA extraction. This control consisted of a seal herpesvirus (PhHV-1) and provided a means to test the precision and reproducibility of the assays as is prescribed in other quantitative TaqMan assays (16). When the Ct-value of the internal control exceeded the mean value ± 2 standard
deviations, it was assumed that inhibition or loss of the sample had occurred either during DNA isolation or during PCR. In such cases, the DNA isolation and TaqMan analysis were repeated till the internal control was within the normal range.

The galactomannan concentrations in both serum and left lung were determined by the commercial Platelia Aspergillus system of Bio-Rad (Platelia Aspergillus, Bio-Rad, Marnes-la-Coquette, France). To obtain quantitative results this system was modified in our lab as described before (3).

Dissected organs from all deceased animals were cultured to exclude bacterial super infections.

Pharmacokinetics of antifungal agents

The pharmacokinetics of caspofungin were determined after multiple-dose administrations in uninfected neutropenic rats. In rats receiving 3 doses of 10 mg/kg/day serial blood samples were taken at 5 minutes, 1h, 2h, 4h, 6h, 8h, 12h and 24h after the third dose by retro-orbital puncture under CO₂-anaesthesia. In rats receiving 3 doses of 10 mg/kg/day followed by 7 doses of 4 mg/kg/day serial blood samples were taken at the same time-points after the tenth dose. Plasma samples were obtained from three rats at each time-point, and the concentration of caspofungin was assessed by standard large plate agar diffusion as described before (17). The area under the plasma concentration versus time curve (AUC₂₄ₕ) was calculated using the log-linear trapezoid rule.

Toxic side effects of Caspofungin

To determine toxic side effects of caspofungin doses on the kidneys or the liver, renal and hepatic functions were monitored. This was done by sampling blood on day 6 and day 13 after starting caspofungin treatment. In these serum samples serum creatinine (CREAT) and blood
urea nitrogen (BUN) levels were determined to assess the renal functions while serum alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were measured to assess the hepatic functions. The same parameters were determined for a healthy control group, consisting of 35 rats, to calculate the normal values for this rat strain. Mild toxicity was defined when levels for either one of these parameters was more than 3 times the upper limit of normal (3 times the 95 percentile boundary of the healthy control group), severe toxicity was defined as levels higher than 5 times the upper limit of normal.

<table>
<thead>
<tr>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaplan-Meier survival curves were generated and the differences in rat survival rate assessed with log rank test. Normality of ALAT, ASAT, BUN and UREUM was determined with the Shapiro-Wilk test.</td>
</tr>
</tbody>
</table>

RESULTS

Effect of antifungal treatment on rat survival

During the treatment period of 10 days rats were persistently neutropenic (granulocyte counts $<0.1 \times 10^9/L$), and the 10 day period after termination of treatment neutrophil numbers gradually rose. As shown in figure 1, untreated control rats all died between day 5 and day 9 after fungal inoculation. Treatment for 10 days with caspofungin 4 mg/kg/day started at 16 h after fungal infection, the time at which hyphal growth in the left lung was established, resulted in therapeutic efficacy, with 100% survival of rats. Delay of treatment till 24 h after fungal infection resulted in a slight but not significant reduction in therapeutic effect (p=0.3918) providing 93% rat survival rate. When treatment was further delayed until 72 h after infection when rats had advanced stage of invasive pulmonary aspergillosis caspofungin...
was still effective compared to untreated control rats \( (p<0.0001) \). However only 27% rat survival rate was obtained, which therapeutic effect was significantly less than efficacy when treatment was started at 24 h or 16 h after fungal inoculation \( (p<0.0001 \text{ for both}) \). To evaluate if a higher dose of caspofungin dosage would compensate for the limited therapeutic effect related to late start of treatment a dose of 10 mg/kg/day was administered for 10 days. We observed that an increase in caspofungin daily dose from 4 mg/kg to 10 mg/kg resulted in a significant increase in therapeutic effect \( (p=0.0159) \) being a 67% rat survival. Finally, to investigate whether this relatively high dose of 10 mg/kg/day was needed throughout the entire treatment period we investigated whether with a high-dose–step-down schedule a similar efficacy could be achieved. As shown in figure 1 a high dose of caspofungin 10 mg/kg/day for only 3 days followed by 4 mg/kg/day for 7 days did not differ from the 10 mg/kg/day continuous schedule for 10 days \( (p=0.9147) \).

Apparently a high dose of caspofungin during the first three days of the treatment schedule only was essential. The AUC\(_{24h}\) of caspofungin determined after the third dose in rats receiving 3 doses of 10 mg/kg/day caspofungin appeared to be 549.3 µg.h/ml. The AUC\(_{24h}\) of caspofungin determined after the tenth dose in rats receiving 3 doses of 10 mg/kg/day followed by 7 doses of 4 mg/kg/day was 114 µg.h/ml.

**Toxic side effects of the dosing schemes**

In order to investigate whether increase in dosage of caspofungin was well tolerated in rats we determined the renal and hepatic functions of the treated animals. Serum CREAT, BUN, ALAT and ASAT were determined. As shown in table 1, none of the applied caspofungin dosage schemes resulted in alarming high levels of either parameter, even in the severely ill infected neutropenic animals. Although kidney and liver functions were unimpaired, it was observed that animals receiving the relatively high dose of caspofungin 10 mg/kg/day became
ethargic, had respiratory distress and felt cold within the first minutes after administration. These effects were found in both the infected as the non-infected control group. Rats recovered within one or two hours. Effects were strongest after the first dose, but became less intense each following dose.

Effect of antifungal treatment on fungal burden in rats

The CE counts in infected left lungs and GM concentrations in infected left lungs and sera of surviving rats are presented in figures 2 and 3. As shown in figures 2A and B, in untreated infected rats mean log CE counts and mean log GM concentrations increased over time in the first days after fungal infection. However, the fungal burden in terms of DNA or GM did not decrease during caspofungin therapy when started at 72 h after fungal infection. Even in rats treated with caspofungin dosage schedules that resulted in a significant rat survival rate of 67%, the fungal burden in the left lung still remained high. Figure 3 shows that in untreated infected control rats the mean log GM concentration in serum increased over time from undetectable at day 1 to 0.98 at day 6. In caspofungin-treated animals GM levels further increased with peak levels at day 9 (6 days after start of treatment). From that time GM levels decreased in surviving animals.

DISCUSSION

Previous experimental studies on the treatment of invasive aspergillosis in animals have shown to be of merit in the treatment of this disease in man (1, 5, 13, 17). However, the effect of the drug in relation to the severity of Aspergillus infection was not yet evaluated. Also data on the potential benefit of treatment with a higher dose are needed. We previously showed that in a transiently neutropenic rat model of invasive pulmonary aspergillosis a therapeutic
dose of 4 mg/kg/day caspofungin resulted in 100% rat survival when treatment was started early at 16 h after fungal inoculation, the time at which hyphal growth in the left lung is established. Treatment with 1 mg/kg/day amphotericin B resulted in a survival of only 27% at day 21. With the dose of 4 mg/kg/day caspofungin, GM concentrations in the serum decreased to undetectable levels after 11 days post infection. In the current study we investigated whether caspofungin was also effective in advanced stage of invasive pulmonary aspergillosis, which may be a clinically more relevant endpoint because the diagnosis of invasive pulmonary aspergillosis is often made at a late stage of disease. To this aim antifungal treatment in rats was delayed from 16 h to 24 h or 72 h after fungal inoculation. Although efficacy of caspofungin treatment decreased with increase in severity of infection, this decrease in efficacy could be partly compensated for by increasing the dose of caspofungin from 4 to 10 mg/kg/day. A regimen of caspofungin in which the caspofungin dose was increased during only the first three days of treatment was essential to increase therapeutic efficacy.

A relatively high AUC\(_{24h}\) (549.3 µg.h/ml) was observed after the three-day high dose of 10 mg/kg/day caspofungin. The AUC\(_{24h}\) value of 114 µg.h/ml obtained after the last dose of 4 mg/kg/day at day 10 of the high-dose-step-down schedule was comparable to the AUC\(_{24h}\) value of 91.8 µg.h/ml obtained after a single 4 mg/kg/day dose as published before (17). At 1 h after administration of the last dose of 4 mg/kg/day dose at day 10 of the high-dose-step-down schedule, the plasma concentration was 2-fold higher for compared to the single 4 mg/kg/day dose (17), at 12 h after administration the plasma levels were similar. The AUCs presented here were determined in neutropenic non-infected animals which could result in an underestimation of the real AUC as demonstrated by Groll et al. for anidulafungin, another member of the echinocandin class (9). It should be noted that the AUC\(_{24h}\) value obtained after 10 mg/kg/day caspofungin was not proportionally increased, based on the increase in dosage,
Compared to the AUC$_{24h}$ value obtained after administration of 4 mg/kg/day. This can not easily be explained.

The benefit of a relatively high AUC$_{24h}$ in the first phase of the therapy was also observed in man by Stone et al. (15). They showed that a loading dose on day one generates a higher drug concentration in plasma during the initial days of therapy; without this dose the mean concentration in the first days is below the target concentration (15). This could explain why high-dose-step-down regimen was more efficacious.

The increase in efficacy with higher doses of caspofungin in severely infected rats was only observed by rat survival and lower concentrations of galactomannan in serum. At the same time decrease in fungal burden in the lung in terms of amount of DNA or GM in the left lung was not observed. Both GM, as constitute of the fungal cell wall (8), and DNA are present in viable as well as non-viable fungal mass, not yet cleared by the host. In this respect the GM amount measured in serum may be more informative than the data from the site of infection, as the detection of GM in serum may reflect the presence of an active infection.”

Caspofungin at the relatively high dose of 10 mg/kg/day resulted in toxic side effects in some animals shortly after administration. Similar observations have been reported for mice treated with the echinocandins anidulafungin (previously known as LY303366) and micafungin (6, 7, 14). In Clemons’ study (6) 90% of the non-infected mice treated with anidulafungin 50 mg/kg/day had died after 3 days of treatment. The toxicity of anidulafungin appeared to be the result of drug interactions with immunosuppressive agents like cortisone, hydrocortisone or triamcinolone (6). The nature of the toxicity for the echinocandins still remains unclear, since no histological evidence for the cause of death in the anidulafungin treated animals was found (6). In our study hepatic and renal functions were monitored, and these values all remained in the normal range even with the relatively high dosage of caspofungin. The nature of this observed side effect may be related to the rapid absorption of the drug when it is given...
intraperitoneally to experimental animals which differs from the administration of caspofungin given intravenously over 1 hour as is done in humans. Currently, clinical studies on the safety and efficacy of higher doses of caspofungin for the treatment of invasive candida infections are ongoing. The present study suggests that this approach including the high-dose-step-down treatment may be useful for the treatment of invasive pulmonary aspergillosis as well.

ACKNOWLEDGEMENTS

This study was financially supported in part by Merck Research Laboratories, Rahway, NJ, USA.

References


Table 1: Renal and hepatic functions after administration of the different dosage schemes of caspofungin started at 72 h after fungal inoculation.

<table>
<thead>
<tr>
<th></th>
<th>CREAT (µmol/l)</th>
<th>BUN (mmol/l)</th>
<th>ALAT (U/l)</th>
<th>ASAT (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 6</td>
<td>day 13</td>
<td>day 6</td>
<td>day 13</td>
</tr>
<tr>
<td>uninfected control rats</td>
<td>41.9 ± 11.1</td>
<td>46.9 ± 12.5</td>
<td>6.4 ± 0.7</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>uninfected cyclo-control rats</td>
<td>34.8 ± 5.8</td>
<td>26.7 ± 6.1</td>
<td>4.4 ± 0.5</td>
<td>5.8 ± 0.4</td>
</tr>
<tr>
<td>infected cyclo-rats</td>
<td>42.3 ± 12.9</td>
<td>ND¹</td>
<td>6.5 ± 1.2</td>
<td>ND¹</td>
</tr>
<tr>
<td>infected cyclo-rats 10*4 CAS</td>
<td>44.3 ± 9.9</td>
<td>24.0 ± 14.1</td>
<td>12.2 ±10.2</td>
<td>15.7 ± 7.6</td>
</tr>
<tr>
<td>infected cyclo-rats 3<em>10 + 7</em>4 CAS</td>
<td>36.5 ± 4.3</td>
<td>31.0 ± 2.8</td>
<td>5.9 ± 0.9</td>
<td>6.9 ± 2.5</td>
</tr>
<tr>
<td>infected cyclo-rats 10*10 CAS</td>
<td>42.0 ± 10.2</td>
<td>28.4 ± 8.5</td>
<td>10.9 ± 6.6</td>
<td>11.4 ± 3.3</td>
</tr>
<tr>
<td>upper limit of normal</td>
<td>60.2</td>
<td>7.6</td>
<td>86.4</td>
<td>118.5</td>
</tr>
<tr>
<td>3 times upper limit boundary (mild toxicity)</td>
<td>180.6</td>
<td>22.8</td>
<td>259.1</td>
<td>355.5</td>
</tr>
<tr>
<td>5 times upper limit boundary (severe toxicity)</td>
<td>301.0</td>
<td>38.0</td>
<td>431.9</td>
<td>592.5</td>
</tr>
</tbody>
</table>

¹ND: the renal and hepatic functions could not be determined as all rats had died.

Serum creatinine (CREAT), blood urea nitrogen (BUN), alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were determined at day 6 and day 13 after fungal inoculation in uninfected control rats, cyclophosphamide (cyclo)-induced neutropenic rats and cyclo-induced neutropenic infected rats receiving various doses of caspofungin (CAS). Doses were 10 days 4 mg/kg/day (10*4), 3 days 10 mg/kg/day followed by 7 days 4 mg/kg/day (3*10 + 7*4) or 10 days 10 mg/kg/day (10*10). Mild toxicity was defined as parameter levels exceeding the 3 times upper limit boundary, severe toxicity when levels exceeded the 5 times upper limit boundary. For reference the upper limit is stated as well.
Efficacy of Caspofungin (i.p.) in neutropenic rats with invasive pulmonary aspergillosis

Treatment with CAS
Persistent neutropenia

Neutrophil rise

<table>
<thead>
<tr>
<th>Time after fungal inoculation (days)</th>
<th>CAS 10^4 start 16h (n=11)</th>
<th>CAS 10^4 start 24h (n=15)</th>
<th>CAS 10^4 start 72h (n=12)</th>
<th>CAS 3^10 + 7^4 start 72h (n=12)</th>
<th>Controls (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

P-values figure 1, comparison of the various dosage schemes:

- CAS 10^4 Start 16h: 0.3918
- CAS 10^4 Start 24h: 0.0001
- CAS 10^4 Start 72h: 0.0398
- CAS 3*10 + 7*4 Start 72h: 0.9147
- CAS 10*10 Start 72h: <0.0001
- Controls: <0.0001
Treatment with CAS

Persistent neutropenia

Neutrophil rise

Mean log CE/gram lung tissue

Start treatment

Time after fungal inoculation (days)

Mean log GM (ng/gram lung tissue)

Start treatment

Time after fungal inoculation (days)
Figure 3

![Graph showing treatment with CAS and neutrophil levels over time.](graphic)

- **Controls (n=6)**
- **CAS 10*4 (n=6)**
- **CAS 3*10+7*4 (n=6)**
- **CAS 10*10 (n=6)**

**Time after fungal inoculation (days)**

- **Start treatment**
  - 1.0: 6
  - 3.0: 6
  - 6.0: 6, 6
  - 9.0: 4, 6
  - 13.0: 4, 4
  - 23.0: 2, 3, 4

**Mean log GM (mg/mL serum)**
Legends to figures

Figure 1:
Therapeutic efficacy of caspofungin (CAS) in transiently neutropenic rats with invasive pulmonary aspergillosis. CAS was administered intraperitoneally and consisted of 10 days 4 mg/kg/day (10*4) or 10 days 10 mg/kg/day (10*10) or 3 days 10 mg/kg/day followed by 7 days 4 mg/kg/day (3*10+7*4). Treatment once daily was started either 16 hours, 24 hours or 72 hours after fungal inoculation, and continued for 10 days. Statistical significance was determined with the logrank test.

Figure 2:
Number of conidial equivalents (CE) (A) and concentration of galactomannan (GM) (B) in the infected left lung of surviving transiently neutropenic rats with invasive pulmonary aspergillosis treated with caspofungin (CAS). CAS treatment was started 72 hours after fungal inoculation and consisted of: 10 days 4 mg/kg/day (10*4) or 3 days 10 mg/kg/day followed by 7 days 4 mg/kg/day (3*10+7*4) or 10 days 10 mg/kg/day (10*10). Numbers indicated above bars are numbers of surviving rats out of groups of 6 rats at each time interval. The error bars represent standard deviation.

Figure 3:
Concentration of galactomannan (GM) in serum of surviving transiently neutropenic rats with invasive pulmonary aspergillosis treated with caspofungin (CAS). CAS treatment was started 72 hours after fungal inoculation and consisted of: 10 days 4 mg/kg/day (10*4) or 3 days 10 mg/kg/day followed by 7 days 4 mg/kg/day (3*10+7*4) or 10 days 10 mg/kg/day (10*10). Numbers indicated above bars are numbers of surviving rats out of groups of 6 rats at each time interval. The error bars represent standard deviation. In performing calculations any...
sample with a value below the limit of detection (LOD) was assigned as the highest possible value below LOD (GM ≤1 ng/mL, log GM = 0 ng/mL).