Serum and intraperitoneal levels of amphotericin B and flucytosine during intravenous treatment of critically ill patients with Candida peritonitis

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Objectives: To study the relation between serum and peritoneal levels of amphotericin B and flucytosine during intravenous treatment in patients with abdominal sepsis due to a perforated gut.

Patients and methods: Included were consecutive patients with abdominal sepsis due to a perforated gut, who were treated intravenously with amphotericin B and/or flucytosine after surgery if an abdominal drain was present. Amphotericin B and flucytosine were measured from simultaneously collected serum and abdominal fluid samples.

Results: Twenty-one consecutive patients were included. Five repeated samples were taken from three patients. The time interval between the start of the medication and the first sampling was median 4.0 days (range 2–7 days). The correlation coefficient ($r^2$) between serum and peritoneal levels of amphotericin B was 0.79. In nine patients (43%) with a maximum serum level of 0.28 mg/L, amphotericin B in the peritoneal fluid was undetectable. The lowest serum level that was present with a detectable peritoneal level was 0.16 mg/L. A short duration of treatment (2 days) was associated with low serum and undetectable peritoneal levels. In seven patients, flucytosine levels were measured. Peritoneal flucytosine levels did not differ significantly from serum levels. Serum and peritoneal flucytosine levels correlated well with $r^2 = 0.88$. Peritoneal amphotericin B level was inversely correlated with C-reactive protein level on the same day ($r^2 = 0.30$).

Conclusions: It is shown, during continuous infusion, that peritoneal levels of amphotericin B are lower than serum levels. The amphotericin B serum levels should exceed 0.5 mg/L to obtain peritoneal levels above MIC values. Flucytosine levels in the abdominal fluid are comparable to serum levels and within MIC ranges.

Keywords: abdominal sepsis, ascites, antifungals

Introduction

In patients with abdominal sepsis due to perforation of the gut, Candida is prevalent in abdominal fluid in 17% to 64%.1–3 Perforation of the upper digestive tract is more often associated with intraperitoneal Candida colonization compared with the ileum and appendix.1,4 Perforation of the large bowel also leads to peritoneal Candida colonization in ~60% of cases.1 The mortality of patients with abdominal sepsis is around 50%.5 Pre-emptive or early treatment of Candida is probably associated with improved outcome.5 Most studied in this respect is fluconazole. However, a shift towards a higher incidence of non-albicans species, which may be fluconazole resistant, has been described in the specific intensive care setting.6 In fact, the optimal antifungal treatment of abdominal sepsis and Candida peritonitis due to a perforated gut in an intensive care setting is unknown. Prospective studies in this specific patient population to find the most effective agent have not been performed.
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Treatment guidelines suggest the use of amphotericin B with or without flucytosine or fluconazole. Nonetheless, in line with other indications, the newer antifungal agents are increasingly preferred above amphotericin in patients with Candida peritonitis. Amphotericin B is used less frequently for reasons of toxicity. To reduce toxicity, cumulative dose should not exceed toxic limits. In addition, monitoring the serum level can be used to achieve a level above the MIC. The septic and critically ill patient is characterized by altered pharmacokinetics and pharmacodynamics. Moreover, critically ill patients should be regarded as immunodeficient due to the excessive inflammatory response (systemic inflammatory response syndrome; SIRS) and subsequent anti-inflammation (compensatory anti-inflammatory response syndrome; CARS). These considerations make it necessary to increase our knowledge of the pharmacodynamics of amphotericin B and flucytosine in the specific patient population with perforated gut and abdominal sepsis in order to find out whether the effectiveness and safety of these ‘older’ antifungal agents can be improved. In the past, case reports have been published concerning peritoneal penetration of amphotericin B and flucytosine. However, for critically ill patients, no data are available concerning peritoneal levels. In serum, minimum and maximum amphotericin B levels have been identified to gain sufficient efficacy and to limit toxic effects. MICs of amphotericin B are 0.125–1.0 mg/L and usually below 0.38 mg/L. For flucytosine, to minimize side effects, close monitoring is necessary to maintain serum levels below 80–100 mg/L. In the present study, we determined both serum and intraperitoneal levels of amphotericin B and flucytosine in critically ill patients with abdominal sepsis due to a perforated gut in order to extend our knowledge of local concentrations of these drugs in relation to serum levels.

Patients and methods

Study design

The study was prospective and observational and was performed according to Dutch and European legislation. Amphotericin B and flucytosine levels were determined simultaneously in serum and peritoneal fluid in patients treated intravenously with these agents. Samples of the peritoneal fluid were collected from abdominal drains. The setting was a 16 bed mixed medical and surgical ICU in a teaching hospital. Antibiotic treatment for abdominal sepsis was administered by continuous intravenous (iv) infusion according to Dutch and European legislation. Amphotericin B and flucytosine were included.

Patients

All consecutive patients treated intravenously with amphotericin B as monotherapy or in combination with flucytosine were included. All patients were diagnosed with peritonitis by peri-operative culture of the abdominal fluid with Candida, usually among other microorganisms. All treatments were empirically initiated awaiting microbiological culture. Excluded were patients without an abdominal drain or with a drain which did not produce any abdominal fluid. Liver failure was a contra-indication for treatment with flucytosine. Renal failure was not a contra-indication for either amphotericin B or flucytosine, as these patients received continuous renal replacement therapy by continuous venovenous haemofiltration. APACHE II and SOFA scores were recorded according to the definitions of the Dutch National Intensive Care Evaluation (www.stichting-nice.nl).

Measurements

Whole blood was collected from an indwelling arterial line. The abdominal fluid was collected from an abdominal drain. All drains were silicon drains. All samples were fresh samples collected from the drain. All samples were immediately transported to the laboratory for analysis. Amphotericin B was extracted from serum and peritoneal fluid samples and determined by HPLC using a slightly modified technique as described by Bach. Flucytosine levels were measured using a slightly modified HPLC technique with UV detection as described by Miners et al. Creatinine clearance (CLCR) was calculated on the basis of serum creatinine and bodyweight, using the Cockcroft–Gault formula.

Statistical analysis

Data were collected and analysed in an SPSS database (SPSS 13.0, Chicago, IL, USA). Data are presented as median and interquartile range (IQR). Pearson’s correlation coefficient for continuous data was computed and presented as $r^2$. The Wilcoxon test for paired data was used to analyse the difference between serum and drain values in individual patients. In all tests, a two-sided alpha of <0.05 was considered as statistically significant.

Results

In 21 patients, 26 simultaneous serum and peritoneal fluid samples were collected for amphotericin B and/or flucytosine measurement. Baseline characteristics of the patients are shown in Table 1. Of the 21 included patients, 3 had a repeated amphotericin B measurement later during their intensive care stay and in 1 patient, a third and a fourth amphotericin B measurement was performed.

Amphotericin B

The median serum amphotericin B level for the first measurement was 0.25 (IQR 0.46) mg/L. The median peritoneal amphotericin B level for the first measurement was 0.12 (IQR 0.32) mg/L. These peritoneal levels are significantly lower than the serum levels ($P = 0.001$) and below the therapeutic range (0.5–1.0 mg/L) that is defined for serum levels in our hospital laboratory.

The relation between serum and first peritoneal amphotericin B levels is shown in Figure 1. Pearson’s $r^2$ is 0.79. Pearson’s $r^2$ is 0.68 when the outlier in the highest right-hand corner (serum level 2.3 mg/L and peritoneal level 0.83 mg/L) is deleted. Apparently, a linear relation between serum and peritoneal levels exists. The penetration ratio (peritoneal/serum level) was...
The cumulative amphotericin B dose at the time of measurement was median 160 mg (IQR 115 mg) and had no relation with the serum ($r^2 = 0.04$) or peritoneal level ($r^2 = 0.10$).

In 9 of the 21 included patients (43%), amphotericin B was below the detection level in the peritoneal fluid. The highest serum amphotericin B level in these patients was 0.28 mg/L. Of the six patients with sampling after 2 days of treatment, five had an undetectable peritoneal level of amphotericin B. The other four patients with an undetectable level were sampled after 3, 3, 4 and 4 days, respectively. For the 21 patients, the median time from the start of treatment to first sampling was 4 days (range 2–7 days).

The repeated measurements in three patients showed a median value for the difference between serum and drain amphotericin B levels of 0.22 mg/L (range 0.64 mg/L).

Median 0.41, 95% CI 0.0–0.58. The cumulative amphotericin B dose at the time of measurement was median 160 mg (IQR 115 mg) and had no relation with the serum ($r^2 = 0.04$) or peritoneal level ($r^2 = 0.10$).

Flucytosine

In seven patients, simultaneous measurement of serum and peritoneal flucytosine levels was performed. In two patients a second, and in one patient a third, fourth and fifth measurement, was performed. The median serum flucytosine level for the first measurement was 64.6 (IQR 50.9) mg/L. The median peritoneal flucytosine level for the first measurement was 54.5 (IQR 73.4) mg/L. Flucytosine levels in the peritoneal fluid were not significantly lower than serum levels ($P = 0.39$) and within the therapeutic range (50–80 mg/L). The relation between serum and peritoneal flucytosine levels is shown in Figure 2. Pearson’s $r^2$ is 0.88. The penetration ratio (peritoneal/serum level) was median 0.96, 95% CI 0.07–1.2.

As shown, a linear relation between serum and peritoneal levels exists for flucytosine as well as for amphotericin B.

Serum and peritoneal levels of either amphotericin B or flucytosine were not related to ICU or hospital mortality. Only the peritoneal levels of amphotericin B showed a trend towards a higher amphotericin B concentration ($P = 0.07$) in patients who died in the ICU. However, these patients had a non-significant difference in the SOFA score ($P = 0.26$). An inverse relation was found for C-reactive protein (CRP), as a measurement of severity of inflammation, and peritoneal amphotericin B level ($r^2 = 0.30$).

The cumulative dose of amphotericin B until the samples were taken was 160 mg (SD 69 mg). This was not correlated with serum amphotericin B level ($r^2 = 0.004$) or with peritoneal amphotericin B level ($r^2 = 0.10$).

Discussion

In our study on the relation between serum and peritoneal levels of amphotericin B and flucytosine in 21 patients, we showed that flucytosine penetrates well in abdominal fluid. In contrast,
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Amphotericin B peritoneal levels are significantly lower than serum levels. Flucytosine and amphotericin B have different pharmacokinetic properties. Flucytosine penetrates well into most body sites, including peritoneal fluid, because it is a small and highly water-soluble molecule. Moreover, 2% to 4% is bound by serum proteins, the distribution volume is low (0.7 L/kg) and the serum half-life is 3–6 h. Amphotericin B has a poor water solubility, a volume of distribution of 4 L/kg, is highly protein bound in serum (91%–95%) and when bound in serum it circulates in a complex of high molecular weight with a half-life of 15 days.

In 9 of the 21 patients, amphotericin B was undetectable in the peritoneal fluid, whereas serum levels were 0.16–0.28 mg/L. This might be due to the limit of quantification of the HPLC method for amphotericin B used in this study (0.1 mg/L). Moreover, the severity of local peritonitis may be important for amphotericin B to appear in the peritoneal fluid, as one can imagine that a severely inflamed peritoneum will more easily lead to diffusion from blood into the peritoneal cavity. However, the opposite was true, as CRP and peritoneal amphotericin B levels showed an inverse relation. We performed additional analysis of SOFA scores as a measurement of severity of disease, but we did not find a significant relation of peritoneal levels with the SOFA score on the day of measurement. In addition, we could not find a relation between serum or peritoneal levels and mortality in this small cohort, and the study was not powered to detect mortality effects.

The MIC for amphotericin B has been shown to range from 0.125 to 1 mg/L and usually below 0.38 mg/L. The serum amphotericin B levels that we measured were in 15 of 21 patients lower than the target range of 0.5–1.0 mg/L, as the median level was 0.25 mg/L. Apparently, in more than half of the patients, the dose was too low to be sure that effective treatment was reached. Peritoneal levels above the lowest MIC (0.12 mg/L) were present with serum levels from a minimum of 0.16 mg/L. On the other hand, serum levels up to 0.28 mg/L are shown to be present with a peritoneal level below 0.1 mg/L. This may imply suboptimal treatment, but we did not determine the MICs for our Candida strains, as that was not an objective of this study. Roughly, it may be stated that the amphotericin B serum levels should exceed 0.5 mg/L to obtain peritoneal levels above MIC values. The variation in serum levels in relation to dose does not allow firm conclusions, but a dose of 0.5–1.0 mg/kg/day (depending on renal function) is needed to reach target levels.

Flucytosine concentrations in the peritoneal fluid were more often in the therapeutic range. Apparently, the effectiveness of amphotericin B treatment may be improved with a better dosing strategy, but the abdominal concentration of flucytosine is appropriate when serum levels are within the target range.

In the current literature, only case reports have been published on the penetration of amphotericin B and flucytosine in the peritoneal fluid. Peterson et al. showed in one patient that with serum levels of 0.52 and 1.5 mg/L of amphotericin B, peritoneal fluid concentrations ranged from 0.44 to 0.78 mg/L. Muth et al. presented a patient with virtually undetectable intraperitoneal levels of amphotericin B despite substantial levels found in plasma. In that particular patient, intraperitoneal levels of flucytosine were between 60% and 100% of serum levels of the drug. Clinical studies of the efficacy of amphotericin B and flucytosine for Candida peritonitis are scarce. In one study, where amphotericin B combined with flucytosine was used to treat Candida peritonitis, this therapy was successful in 56% of the patients in contrast to 25% of the fluconazole-treated patients (non-significant result). It can be speculated that a higher success rate may be achieved when amphotericin B/flucytosine treatment is optimized according to peritoneal levels. A definite relation between adequate serum or peritoneal levels and outcome is not available in the literature, but common sense should drive us to optimize serum and peritoneal levels.

Our study has several limitations. First, the measurements were made at variable time intervals from the start of the antibiotics (2–7 days). It is to be expected that amphotericin B needs some days to achieve stable levels. Indeed, we did find that most patients with a short treatment episode (2 days) had undetectable peritoneal levels. On the other hand, one would like to achieve effective levels shortly after the start of the medication.

Second, the relevance of intraperitoneal levels can be discussed. On the one hand, the antifungal agents should reach effective levels at the site of the infection. On the other hand, the relation between peritoneal amphotericin B or flucytosine levels and outcome has not been established. One might hypothesize that despite low intraperitoneal levels, effective intracellular concentrations are present.

Third, the location of the drain can give rise to sample error. It can be speculated that the level of peritoneal inflammation at the drain site may be different compared with other locations in the abdominal cavity and may determine the antifungal level.

Fourth, the medication was administered by continuous infusion. It was shown previously that this may limit toxicity. The same study, efficacy was preserved, but the study was relatively small to detect mortality effects. What the effect of continuous infusion is on peritoneal levels in comparison with bolus administration can only be speculated.

In conclusion, we have shown that serum flucytosine levels show a fairly good correlation with intra-abdominal concentrations. In contrast, amphotericin B levels are significantly lower intra-abdominally compared with serum, which may hamper effective treatment. When serum levels are below 0.5 mg/L, one cannot rely on effective peritoneal levels.

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Transparency declarations

None to declare.

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