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Tolerance and Efficacy of Amphotericin B Inhalations for Prevention of Invasive Pulmonary Aspergillosis in Haematological Patients

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The tolerance of aerosolised amphotericin B as prophylaxis against invasive pulmonary aspergillosis was investigated in 61 granulocytopenic periods in 42 patients treated for a haematologic malignancy. Each patient was to receive amphotericin B in doses escalating to 10 mg three times daily (t.i.d.), but only 20 (48%) patients managed to complete the scheduled regimen. One patient tolerated the full dose initially, but had to discontinue treatment when dyspnea developed as a result of pneumonia and acute respiratory distress. Another 22 patients (52%) experienced side effects, including eight (19%) who reported mild coughing and dyspnea but who tolerated the full dose and three (7%) patients whose dose was reduced to 5 mg t.i.d. Another six (14%) patients could tolerate only 5 mg t.i.d., and five (12%) others stopped treatment because of intolerance. Elderly patients ($p < 0.05$) and those with a history of chronic pulmonary obstructive disease ($p = 0.09$) were more likely to develop side effects during inhalation. Twelve (28%) patients developed proven or possible invasive fungal infections, but no correlation was established between infection and the total amount of amphotericin B inhaled. Inhalation of aerosolised amphotericin B is poorly tolerated and does not appear useful in preventing invasive pulmonary aspergillosis in granulocytopenic patients.

The use of broad-spectrum antibacterial agents has reduced the mortality associated with bacterial infections in granulocytopenic patients, but the incidence of invasive fungal infections in these patients has increased. Today, invasive fungal infections constitute a major cause of morbidity and mortality in patients treated for haematological cancer as well as in other immunocompromised patients (1). Apart from granulocytopenia and mucositis, other recognised predisposing factors include treatment with corticosteroids and broad-spectrum antibiotics and the use of central venous catheters (2).

Invasive pulmonary aspergillosis (IPA) is the most common form of invasive aspergillosis (3), but its diagnosis is difficult to establish at an early stage of infection. Consequently, it has become common practice to institute empiric therapy with amphotericin B for fever unresponsive to antibacterial treatment (4). Prophylaxis may produce better results, but parenteral treatment with amphotericin B is often complicated by side effects (5), and the drug is not absorbed when given orally. Inhalation of aerosolised amphotericin B seems to offer an alternative approach to delivering the drug to the site of infection (6–10, 12, 13), but its value has yet to be determined by formal randomised clinical trials, since the studies undertaken thus far have relied on comparison with historical controls (7, 9–13).

When the incidence of IPA increased in our department (14), we investigated the feasibility of administering aerosolised amphotericin B to our haematological patients. If proven tolerable and effective, this formulation would offer a means of

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Table 1: Patient characteristics.

No. of patients (periods of prophylaxis)	42 (61)
No. of females: males	22:20
Median age in years (range)	42 (18–68)
Haematological disorder (periods of prophylaxis)	
Acute myeloid leukaemia	22 (33)
Acute lymphocytic leukaemia	17 (25)
Aplastic anaemia	1 (1)
Autologous bone marrow transplantation	1 (1)
Chronic myeloid leukaemia	1 (1)

delivering treatment with amphotericin B without the risk of nephrotoxicity and other systemic side effects.

Patients and Methods

Patients. Forty-two patients admitted to the Department of Haematology, University Hospital Groningen, the Netherlands, were included consecutively if granulocytopenia (absolute neutrophil count < 500 cells/ml) of more than seven days' duration was anticipated as a result of treatment of their underlying disease and the patient had given written informed consent. The study was approved by the local medical ethics committee. Chemotherapy for acute myelogenous leukaemia (AML) or Chronic myelogenous leukaemia (CML) in myelogenous blast crisis consisted of a regimen of cytarabine and daunorubicine or amsacrine for the first two induction courses; a combination of mitoxantrone and etoposide was given for consolidation treatment. The treatment of acute lymphoblastic leukaemia (ALL) included two courses of prednisolone, adriamycin, and vincristine. Aplastic anaemia was treated with antithymocyte globulin. Cyclophosphamide and total body irradiation were given as conditioning treatment for autologous bone marrow transplantation. Twelve patients were nursed in single rooms equipped with laminair air flow and high-efficiency particulate air (HEPA) filtration. The remainder were housed in standard rooms, two or more persons to a room, without any air filtration.

Study Design. Patients were entered into the study as soon as the diagnosis of haematological disorder had been made and a treatment had been planned. The starting dose of amphotericin B was 1 mg t.i.d., which was increased every day by 1 mg until 5 mg t.i.d. was attained. The dose was to be increased thereafter to 10 mg t.i.d. unless severe side effects occurred in which case 5 mg t.i.d. was given. Treatment would continue until the granulocytopenia ended unless signs of bronchial obstruction appeared, severe nausea or vomiting occurred, less than 5 mg amphotericin B t.i.d could be tolerated, or the patient withdrew consent. When i.v. amphotericin B was deemed necessary, inhalations were continued.

Delivery of Amphotericin B. A sterile solution of 5 mg/ml amphotericin B desoxycholate was prepared in 5% glucose and diluted in 2 ml sterile water to obtain the desired quantity. This solution was administered as an aerosol using a Pari-InhalerBoy (Pari-Werk GmbH, Germany), which produces particles of 0.5 to 5.5 microns. This device was chosen because it is designed to deliver particles of the desired size sufficiently deep in the bronchial tree (15). Patients were instructed to inhale amphotericin B solution through a mouthpiece by inhaling as deeply as possible and to exale through the nose. The procedure took approximately 20 min.

Selective Gut Decontamination. Selective gut decontamination was started immediately before cytoreductive treatment and consisted of either 960 mg cotrimoxazole t.i.d. or 500 mg ciprofloxacin b.i.d. In addition, all patients were given 500 mg tablets of amphotericin B and 10 mg lozenges containing the drug q.i.d. Cotrimoxazole was replaced by 200 mg colistin q.i.d. when a patient developed a skin rash.

Empiric Therapy. When fever developed, a thorough physical examination was performed, radiographs of the chest and sinuses were obtained, and venous blood was drawn for cultures. Patients were then given 500 mg imipenem-cilastatin i.v. q.i.d. empirically. This regimen was complemented with 1 mg/kg/day amphotericin B i.v. when unexplained fever persisted for more than six days.

Classification of Aspergillosis. For aspergillosis to be considered proven, histologic evidence of hyphae invasion was required; for probable aspergillosis, typical pulmonary infiltrates on computed tomography scan of the thorax in combination with positive culture of sputum and/or bronchoalveolar lavage (BAL) fluid or cytological examination of BAL fluid showing septate hyphae was necessary (16).

Statistical Analyses. Frequencies were analysed by the chi-square test. Nonparametric tests were used for ordinal data; means were given along with their 95% confidence intervals, and p values below 5% were considered significant.

Results

Patients. The characteristics of the patients in the study are shown in Table 1. Twenty-nine patients received amphotericin B inhalations during one granulocytopenic period, seven (3 ALL, 4 AML) patients during two consecutive granulocytopenic periods, and six (2 ALL, 4 AML) during three episodes.

Tolerance of Amphotericin B Inhalations. Twenty-eight (67%) patients completed treatment at the full dose, although nine experienced side effects (Table 2). Another patient had no complaints and continued the regimen until he developed acute respiratory distress syndrome secondary to pneumonia. Of the remaining patients, three continued to inhale 10 mg t.i.d. until they developed severe progressive dyspnea necessitating a dose reduction to 5 mg t.i.d. One of these patients also developed a cough. Six (14%) other patients were able to tolerate only 5 mg t.i.d. because of dyspnea with signs of bronchial obstruction that were sufficiently severe to prohibit a further increase in the dose of amphotericin B. The five (12%) remaining patients withdrew because of intolerable dyspnea and signs of severe bronchial obstruction and/or vomiting that developed immediately after inhaling the

Table 2: Results with inhaled amphotericin B.

Treatment completed	No. of patients	Side effects	Action taken	No. of patients	Mean dose (95% CI)	No. with proven IPA	No. with probable IPA
Yes	28	no	none	19	795 (713–920)	0	1
		yes	none	9	776 (581–988)	2	2
No	14	yes	dose not escalated	6	736 (270–1123)	2	2
			dose reduced	3	555 (185–1170)	1	
			treatment discontinued	5		1	1

IPA, invasive pulmonary aspergillosis.

drug (2 patients at the dose of 1 mg amphotericin B, 2 at 3 mg, and 1 at 4 mg). Thus, 22 (52%) patients experienced side effects, only nine (41%) of which were able to complete the full course of treatment. A high-pressure liquid chromatography assay of patient sera showed there was no sample with more than 0.1 mg/l of amphotericin B.

Subjects experiencing side effects were 11.9 years (95% CI, 0.9–25) older than those who had no complaints (mean age, 38 years; 95% CI, 29–46) ($p < 0.05$) and had a mean age of 51 years (95% CI, 42–60). Patients with a history of chronic obstructive pulmonary disease also tended to have a lower tolerance for inhalations ($p = 0.09$). However, there was no correlation between gender, haematologic disorder, type of selective gut decontamination, or the total amount of amphotericin B inhaled and the development of side effects.

Efficacy of Amphotericin B Inhalations. Twelve (28%) patients developed a proven or probable invasive fungal infection. Histologic examination of lung tissue obtained at autopsy of four patients and from the sinus maxillaris tissue of another patient who survived the infection showed invading hyphae of a filamentous fungus; *Aspergillus fumigatus* was cultured in two cases. Two of these patients had tolerated the full dose of amphotericin B, two had been treated with 5 mg t.i.d., and one patient stopped treatment because he could not tolerate it. Seven patients were designated as having probable IPA; *Aspergillus fumigatus* was recovered from BAL fluid in two cases and repeatedly from the sputa in a third case, while septate hyphae were seen in BAL fluid in the remaining four cases. Two of these patients had tolerated the full dose, three could tolerate only 5 mg t.i.d., and one stopped treatment because of intolerance. Three of these seven patients died with progressive fungal infection despite extensive treatment. One patient who experienced no side effects during treatment with the full dose developed probable IPA and survived.

The development of proven or probable IPA was related to i) the presence of chronic obstructive lung disease (overall $\chi = 6.37$, $p < 0.025$; for proven pulmonary aspergillosis $\chi = 6.1$, $p < 0.025$); ii) increasing age, with a difference of 11.9 years ($p < 0.05$; 95% CI, 2–28); and iii) the occurrence of inhalation-related complaints ($p < 0.05$, $\chi = 5.99$). Rooms equipped with HEPA filters in combination with inhalation of amphotericin B may have offered better protection against IPA since there was one case of probable IPA during 18 courses in HEPA-equipped rooms compared with five proven and six probable cases of IPA during 43 (25.6%) courses in regular rooms. However, there were not enough patients for this difference to reach significance ($p < 0.2$, $25.58 = 3.63$, degrees of freedom = 2). The total dose of amphotericin B inhaled had no detectable impact on the occurrence of IPA, as mean total dose was 688 mg (95% CI, 585–835 mg) in patients who developed IPA compared with 720 mg (95% CI, 440–925 mg) in those who did not.

Discussion

Twenty-eight (67%) patients managed to complete prophylacxic treatment with aerosolised amphotericin B, although nine (32%) of them experienced side effects. Thus, one-third of the patients were unable to complete prophylaxis at the full dose because coughing, signs of bronchial obstruction, and nausea or vomiting occurred during and immediately after inhalation. Tolerance was poorer amongst elderly patients and those who had pre-existing signs of chronic pulmonary obstructive disease, possibly because the droplets in the aerosol contained a higher concentration of

amphotericin B or desoxycholate than used in other studies (17), which can cause or aggravate bronchial obstruction (18). Others have reported that fewer side effects were associated with a nasal spray containing concentrations of amphotericin B similar to that used by us, concentrations that seldom led to discontinuation of treatment (7, 9–11). Moreover, the occurrence of side effects showed no correlation with the total amount of amphotericin B inhaled.

Other studies have reported that inhalation of nebulised amphotericin B is not well tolerated. Beyer et al. (12), who employed the same inhalation device as we did, noted that 23% of patients receiving 10 mg amphotericin B twice daily had to stop treatment prematurely because of coughing, a bad taste, or nausea but not because of bronchial obstruction. In a study by Gryn et al. (14), all patients complained of coughing and an unpleasant aftertaste, while 69% experienced nausea and 17% developed wheezing. In another study bronchospasm affected one of 26 patients during inhalation (13). The apparent differences in the incidence of side effects in these studies might be explained by the various aerosolising devices used (20).

Extensive experience with aerosolised pentamidine in AIDS patients suggests that it is rather difficult to predict side effects due to inhaled medication (21). Nonetheless, the degree of intolerance to inhaling nebulised amphotericin B is sufficiently high to question its usefullness as prophylaxis. This form of prophylaxis does not appear effective in preventing IPA, since 12 (28.6%) patients in our study developed proven or probable IPA. Behre et al. (17) came to the same conclusion after performing an interim analysis of their randomised study. Compliance does not appear to be the only factor responsible for these disappointing results, since there was no correlation between the incidence of IPA and the total amount of amphotericin B inhaled. Rather, the dose delivered at any one time might play a much more important role. Schmitt et al. (6) showed that amphotericin B might protect rats infected experimentally with *Aspergillus* spp. from developing disease, provided that the drug is delivered to the alveolar space in a sufficient amount. However, a dose of 1.6 mg/kg of amphotericin B was required, which is equivalent to 112 mg for a 70 kg man or a dose tenfold higher than the one we used. Such a high dose is clearly out of the question, given the poor tolerance to 10 mg.

In conclusion, amphotericin B inhaled at a dose of 10 mg t.i.d. is so poorly tolerated by granulocytopenic patients, particularly those most at risk of acquiring IPA, such as the elderly and those with chronic obstructive pulmonary disease, that it has little to offer as a means of prophylaxis.

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