ONKOLOGIE

Original Article · Originalarbeit

Onkologie 2011;34:254–258
DOI: 10.1159/000327802

Published online: April 26, 2011

Prophylactic Application of Nebulized Liposomal Amphotericin B in Hematologic Patients with Neutropenia

Annette Hullard-Pulstinger[a] Ernst Holler[a] Joachim Hahn[a]
Reinhard Andreesen[a] Stefan W. Krause[b]

[a]Abteilung für Hämatologie und Internistische Onkologie, Klinikum der Universität Regensburg,
[b]Medizinische Klinik 5, Universitätsklinikum, Erlangen, Germany

Keywords

Amphotericin · Antifungal agents · Antibiotic prophylaxis · Neutropenia · Inhalation

Summary

Background: Pulmonary invasive fungal infections (IFI) are well-recognized complications with high morbiditiy and mortality in patients with hematologic malignancies. **Patients and Methods:** Aerosolized liposomal amphotericin B (lipAmB) was evaluated as an antifungal prophylaxis in patients with an expected neutropenia of more than 10 days due to intensive chemotherapy or stem cell transplantation, in a prospective phase II trial. **Results:** 98 treatment episodes were included in the study and compared to 105 historical control patients. Inhalation was performed between 0 and 103 days. No severe side effects of therapy occurred. 40 patients considered inhalations as unpleasant, 2 as very unpleasant, mostly due to bad taste or cough. Few cases of definite or probable IFI were recorded, whereas a large number of patients were treated with systemic antifungal therapy for pneumonia or fever of unknown origin without a significant difference between study patients and controls. In a predefined subgroup analysis of 48 patients with newly diagnosed acute myeloid leukemia (AML), significantly more patients survived for 1 year in the AmB prophylaxis than in the control group (80% vs. 54%, $p < 0.01$). **Conclusions:** Inhalations of lipAmB are feasible and safe. Results in the subgroup of patients with AML together with data from other trials suggest further evaluation of effectiveness.

Schlüsselwörter

Amphotericin · Medikamente, pilzwirksame · Antibiotikaprophylaxe · Neutropenie · Inhalation

Zusammenfassung

Hintergrund: Pulmonale invasive Pilzinfektionen (IFI) sind bekannte Komplikationen mit hoher Morbidität und Mortalität bei Patienten mit hämatologischen Neoplasien. **Patienten und Methoden:** In einer prospektiven Phase-II-Studie untersuchten wir den prophylaktischen Einsatz von vernebeltem liposomalem Amphotericin B (lipAmB) bei Patienten mit einer erwarteten Neutropeniedauer von mehr als 10 Tagen aufgrund einer intensiven Chemotherapie oder einer allogenen Stammzelltransplantation. **Ergebnisse:** Patienten in 98 Behandlungsepisoden wurden in die Studie eingeschlossen und mit 105 historischen Kontrollen verglichen. Schwere Nebenwirkungen wurden nicht beobachtet. 40 Patienten empfanden die Inhalationen als unangenehm, 2 als sehr unangenehm, überwiegend aufgrund von schlechtem Geschmack oder Husten. Wir registrierten nur wenige Fälle einer sicheren oder wahrscheinlichen Pilzinfektion, trotzdem erhielten viele Patienten eine systemische pilzwirksame Therapie aufgrund von Pneumonien oder Fieber unklarer Ursache. In einer vorab definierten Subgruppenanalyse an 48 Patienten mit neu diagnostizierter akuter myeloischer Leukämie (AML) beobachteten wir ein besseres 1-Jahres-Überleben im Vergleich zum Kontrollkollektiv (80% vs. 54%, $p < 0,01$). **Schlussfolgerungen:** Eine Inhalation mit lipAmB kann sicher durchgeführt werden. Aufgrund der positiven Ergebnisse in der Subgruppe von AML-Patienten und Ergebnissen anderer Studien sollte eine weitere Evaluation dieser Strategie erfolgen.

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Prof. Dr. med. Stefan W. Krause
Medizinische Klinik 5
Universitätsklinikum
Krankenhausstr. 12, 91054 Erlangen, Germany
Tel. +49 9131 8535-957, Fax -958
www.medizin5.uk-erlangen.de/

Introduction

Invasive fungal infections (IFI) are well-recognized complications in patients with hematologic malignancies [1–3]. Individual risk of IFI increases with profound neutropenia of prolonged duration, i.e. more than 10 days. Clinical outcomes in leukemia patients with additional clinically apparent IFI are significantly inferior to outcomes in patients suffering from leukemia alone. *Candida* infections as one type of IFI take their origin mainly from gastrointestinal infections and can be effectively reduced by prophylaxis with fluconazole, although this strategy has only formally been shown to be effective in patients after allogeneic transplantation. The majority of IFI develop in the lung, with *Aspergillus* as the most frequent pathogen, and it can be assumed that these infections originate from inhaled conidia.

A prophylactic strategy using nebulized antifungal agents therefore seems to be a rational approach. Amphotericin B deoxycholate (AmBd) was tested earlier for this purpose. Although initial phase II studies showed promising results, in a formal phase III study, AmBd did not improve outcomes of high-risk patients in comparison to placebo [4]. However, based on preclinical models, liposomal AmB (lipAmB) may be better tolerated and more effective than AmBd due to the following reasons: Unlike AmBd, lipAmB does not foam during nebulization, resulting in a more uniform formation of small droplets; in addition, lipAmB does not inhibit the function of pulmonary surfactants as reported for AmBd [5] and, in animal models of pulmonary aspergillosis, lipAmB or AmB lipid complex was more effective than AmBd [6–9]. Furthermore, nebulized lipAmB or AmB lipid complex was successfully used in previous small published case series in patients [10, 11] and further unpublished studies and was therefore systematically tested in our single-institution phase II study described below.

Patients and Methods

Study Design

Nebulized lipAmB was evaluated in a phase II trial in patients with expected neutropenia (< 0.5 G/l) of more than 10 days due to induction and/or consolidation chemotherapy for acute leukemias and/or allogeneic stem cell transplantation. The patients did not have to be included upfront before entering their first chemotherapy cycle but could also be included during subsequent cycles, provided that the next chemotherapy would lead to profound neutropenia and that no systemic mould-active drug had already been given. Fluconazole prophylaxis was allowed and was used in the vast majority of patients according to local common practice at 400 mg/day throughout the whole study period, in patients and controls. A patient could enter the study for a second time as a 'new' patient (e.g. during therapy of a relapse of his/her leukemia) if more than 3 months had passed since the last lipAmB inhalation.

Prophylaxis consisted of lipAmB (Ambisome®, Gilead, Foster City, CA, USA) nebulized by the use of jet stream nebulizers (LC Star, Pari, Starnberg, Germany). LipAmB was reconstituted with distilled water as for regular intravenous (i.v.) use and applied undiluted in doses of 12.5 mg on 4 consecutive days and then twice weekly until neutrophil recovery. Inhalations were supervised by physiotherapists, a single application taking 10–20 min. Treatment was paused with recovery of neutrophils > 1 G/l and resumed during neutropenic episodes following subsequent chemotherapy cycles. If systemic mould-active antifungals were started due to suspected IFI in an individual patient, per-protocol lipAmB inhalations were stopped, but inhalations could be continued at the discretion of the treating physician. Management of suspected IFI was not specified in the protocol, although some recommendations for diagnostic workup were given. Computed tomography (CT) scans of the chest and serum *Aspergillus* antigen tests were recommended in fever refractory to antibacterial therapy. The patients were interviewed for possible side effects of inhalations in regular intervals.

The protocol was approved by the ethics committee of the University of Regensburg. All patients gave their written informed consent before inclusion in the study.

Patient Population

Patients were screened for eligibility from 07/2003 until the target number of 100 treatment episodes was reached. Patients fulfilling the same entry criteria and treated in the same institution in the years 2000–2002 served as historical controls [12].

Study Analysis

Evaluation of toxicity and a possible reduction in the incidence of proven or probable fungal infections according to published consensus criteria [13] were defined as primary outcome parameters. Predefined secondary outcome parameters were incidence of fever of unknown origin refractory to antibacterial therapy for more than 72 h, delays of chemotherapy in patients with AML, and 1-year survival within the subgroup of newly diagnosed AML patients.

Results

From 07/2003 to 10/2005, 101 courses of lipAmB prophylaxis in 96 independent patients were included in the study. 3 of these were not eligible because systemic mould-active antifungal therapy had already been initiated at study entry. The remaining 98 eligible cases were analyzed by intent-to-treat principles. The majority of patients received chemotherapy for acute leukemia; 43 patients were treated by transplantation of allogeneic bone marrow or blood stem cells. A corresponding sample of patients that would have met inclusion criteria was analyzed for control purposes. Further patient characteristics are given in table 1. Baseline characteristics for age, sex, and fraction of allogeneic transplants were rather similar between study patients and controls, with the exception of myeloproliferative diseases (mainly chronic myeloid leukemia (CML)) that were only present in the control group.

10 patients never started inhalation due to general fatigue, patient request, or start of systemic antifungal therapy; 46 patients remained on therapy per protocol until neutrophil recovery after the last planned chemotherapy cycle or until start of systemic antifungal therapy, and in 41 patients inhalations were either stopped prematurely or not restarted in subsequent chemotherapies (13 patients), mainly due to the request of patients feeling uncomfortable during the inhalation procedure. In 1 patient, the exact timing and reason for discontinu-

Table 1. Patient characteristics

	Patients	Controls
Individual patients recruited	96	105
Recruited twice (more than 3 months apart)	5	13
Treatment episodes	101	118
Excluded from analysis (did not meet inclusion criteria)	3	–
Evaluated for effectivity (ITT population)	98	118
Never started study treatment	10	–
Evaluated for toxicity	88	–
Demographics		
Men/women	66/32	65/53
Median/mean age, years	48/49	52/49
Disease (ITT population)		
AML, AUL or MDS	75	70
Newly diagnosed AML	48	52
ALL	16	13
Lymphoma or multiple myeloma	6	17
Myeloproliferative disease	–	16
Aplastic anemia, PNH	1	2
Therapy during observation period (ITT population)		
Chemotherapy only	55[a]	65
Allogeneic transplantation only	17	34
Both	26	19
Therapy during 'at risk' period (before initiation of systemic antifungal therapy)		
Chemotherapy only	68	74
Allogeneic transplantation only	17	34
Both	13	10

[a]Plus autologous transplantation in 1 patient.

ITT = Intention-to-treat, AML = acute myeloid leukemia, AUL = acute undifferentiated leukemia, MDS = myelodysplastic syndrome, ALL = acute lymphoblastic leukemia, PNH = paroxysmal nocturnal hemoglobinuria.

Table 2. Results in the study population

		Patients	Controls
Execution of therapy during period at risk	mean/median number of chemotherapy, cycles in pts. without transplantation	1.7/1	1.6/1
	mean/median number of chemotherapy, cycles in pts. with transplantation	2.0/1	1.3/1
	never started inhalations, n	10	–
	premature stop of inhalations, n	41	–
	mean/median duration of inhalations in patients with premature stop, days	26.7/23	–
	inhalations per protocol, n	46	–
	mean/median duration of inhalations in per-protocol patients, days	43.6/33	–
	missing data, n	1	–
Side effects, n	none	40	–
	minor/some discomfort[a]	33/7	–
	severe discomfort[b]	2	–
	missing data of self-assessment	6	–
	any side effects requiring medical intervention	0	–
Effectiveness	mould-active systemic antifungals (for the following reasons), n	68	68
	definite IFI (*Aspergillus*)	0	3
	probable IFI (*Aspergillus*)	2	1
	pulmonary infiltrates of any type	33	27
	persisting fever	25	34
	Aspergillus antigen without focus	3	0
	suspected non-pulmonary fungal infection	3	3
	intensified prophylaxis	2	0

n = Number of patients.

[a]In descending order: cough, laborious, bad taste, nausea/vomiting.

[b]1 patient: bad taste; 1 patient: cough plus nausea/vomiting.

ation was unfortunately not documented. Discounting breaks of study therapy during periods of neutrophil recovery, inhalation was performed between 0 and 103 days with a mean duration of 32 days, and a median duration of 26 days (table 2).

No severe side effects of therapy like fever or acute dyspnea occurred and no pulmonary infiltrates or other symptoms during further follow-up where considered to be provoked by lipAmB inhalations. 40 patients considered inhalation as unpleasant, 2 as very unpleasant, mostly due to bad taste or cough. All side effects can therefore be specified as grade I according to common toxicity criteria (CTC). Few cases of definite or probable IFI were recorded, whereas a large number of patients were treated with systemic antifungal therapy for possible IFI, unspecific pneumonia, or fever refractory to antibacterial drugs, without a significant difference between study patients and controls (table 2). In an analysis

Table 3. Results in the subgroup of de novo AML patients

	Patients	Controls
Newly diagnosed AML, number of patients	48	52
Age, years (median/mean)	48/50	57/54
Lost to follow-up	3	0
Evaluable for survival	45	52
Allogeneic transplantation within study period	15	8
Death within 1 year	9[a]	24

[a]$p < 0.01$ compared to controls by Fisher's exact test.

conducted ex post, we analyzed possible differences in outcome between patients strictly adhering to the inhalation protocol and others: Of the 10 patients never starting inhalation, systemic antifungals were initiated in all 10 (100%), of 41 patients prematurely terminating inhalations, antifungals were started in 28 (68%), and in per-protocol patients, antifungals were used in 29 (63%), pointing to a possible protective effect in protocol adherers.

We evaluated the 1-year survival of newly diagnosed AML patients in a predefined subgroup analysis (table 3). Study patients were, on average, somewhat younger and transplanted more frequently. In these AML patients, 80% in the lipAmB prophylaxis group survived for 1 year, significantly more than in the control group (54%). 3 AML patients were lost to follow-up for this analysis because they had moved out of our area. Of the deceased 9 patients in the prophylaxis group, 3 had not taken up inhalations and 3 had terminated inhalations prematurely, whereas of the surviving 36 patients 4 had never inhaled lipAmB and 19 had terminated prematurely. In 4 out of 9 deceased patients in the treatment group and 8 of 24 in the control group, evidence of pneumonia in the last weeks before death was reported in response to our inquiries; however, course of disease and causes of death during this extended observation period were difficult to evaluate since many patients received further care outside of our hospital and, therefore, for many patients only the life status and limited clinical data were available. The exact definition of treatment delays due to infections in AML patients turned out to be very much prone to individual interpretation, and the analysis of this point was therefore omitted although initially planned in the protocol.

Discussion

In our study, we could confirm that prophylactic inhalations of nebulized lipAmB are feasible without significant toxicity. A concern is early termination of prophylactic inhalation in a substantial fraction of patients; however, this occurred because patients considered this treatment unpleasant, and not because of organ toxicity greater than grade I. Therefore, if a clear clinical benefit could be proven and were explained to patients, it is likely that most of them would adhere to such a strategy.

During data analysis of patients and controls, we could define only very few proven or probable IFI. Methods for the detection of IFI are not sensitive, and many cases of IFI are hidden behind 'fever of unknown origin', 'unspecific' pulmonary infiltrates or 'possible fungal infection' [14, 15]. For this reason, early antifungal interventions without clear-cut evidence of a fungal infection are common practice, because an undetected and untreated IFI may rapidly become life-threatening. This strategy is also supported by a recent publication [16]. In our patients, systemic antifungal therapy was frequently applied empirically by treating physicians in ambiguous situations. For studies of IFI prophylaxis or early intervention, stringent diagnostic workup schedules using frequent determination of serum *Aspergillus* antigen and high-resolution CT scans with defined diagnostic criteria are helpful; however, unfortunately these criteria have not been defined in such a strict manner in our protocol and diagnostics have been somewhat sparser in the earlier control group. However, even in recent clinical trials with standardized diagnostics, large patient numbers were needed to show the advantage of a prophylactic antimycotic strategy, in that case oral posaconazole solution [17]. Therefore, in retrospect, the absence of a significant difference in proven and probable IFI between the study arm and the historical control is not surprising. In addition, the results may also be biased against a positive result of our study by a somewhat increasing empirical use of antifungals in our institution over the study period. This is mainly due to the increasing use of CT scans allowing earlier and more sensitive detection of pulmonary infiltrates and due to the approval of voriconazole which is applied more generously due to fewer side effects when compared to systemic AmBd.

Data from our preplanned subgroup analysis in AML patients showing an improvement in survival compared to historical controls are more positive, but of course have to be interpreted with great caution. Confounding is possible using historical controls in a retrospective, non-randomized study. No obvious differences in the management of AML between the treated patients and historical controls were introduced in our institution, except for the use of voriconazole that was already available for the intervention group but not for the control group, as described above. AML patients in the intervention group were somewhat younger than those in the historical control cohort, and the rate of allogeneic transplantation in AML patients in the treated cohort was higher than in the controls. These differences may be indicators of bias due to different patient characteristics. Allogeneic transplantation may also have a favorable impact on mortality by itself; however, the difference in numbers of allogeneic transplantations is smaller than the difference in mortality. Analysis of the causes of death may be misleading for two reasons: Firstly, some of the patients were treated outside our institution after completion of induction and consolidation chemotherapy and information

regarding these patients is scarce. Secondly, a fungal infection may delay optimal therapy and indirectly lead to relapse, but nevertheless is not the direct cause of death in such cases. Taken together, the results of this subgroup analysis are encouraging, but clearly cannot be taken as a proof of this concept.

Recently, a similar but larger randomized phase III study conducted in parallel to our study was reported by Rijnders et al. [18]. Here, nebulized lipAmB was shown to decrease invasive aspergillosis in high-risk neutropenic patients. In this study, a comparably high rate of probable infections was detected in control patients, due to more extensive diagnostics or due to a genuinely higher baseline risk, or to a combination of both factors.

In conclusion, prophylactic inhalations of lipAmB were feasible without safety issues, but unpleasant to a considerable fraction of the patients. Due to the very low numbers of events qualifying as definite or probable IFI even in the control group and very high numbers of empirical mould-active antifungal therapies in both groups, we were not able to meet our objectives to show a reduction of IFI or use of antifungals. However, several lines of evidence indicate that such a strategy may have a clinical benefit. Firstly, we saw a trend for a better outcome in patients adhering to the inhalation scheme per protocol. Secondly, the improved survival of the AML cohort in our study is encouraging, although a significant bias due to different patient characteristics in the intervention and control group cannot be ruled out. Taking the positive results of the larger study of Rijnders et al. [18] into account, inhalations of nebulized lipAmB may be an alternative to prophylactic posaconazole [17]. Both regimens should be compared in a larger randomized study.

Acknowledgements

The excellent support of the physiotherapy team of the University Hospital Regensburg is greatly appreciated.

Disclosure Statement

Analysis of control patients was supported by an unrestricted grant from Gilead Sciences. S.W.K. obtained travel support by Gilead. Gilead was not involved in the conduction or analysis of the study presented in this paper.

References

1 Groll AH, Shah PM, Mentzel C, Schneider M, Just-Nuebling G, Huebner K: Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. J Infect 1996;33:23–32.
2 Marr KA, Carter RA, Crippa F, Wald A, Corey L: Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis 2002;34:909–917.
3 Nivoix Y, Velten M, Letscher-Bru V, Moghaddam A, Natarajan-Ame S, Fohrer C, Lioure B, Bilger K, Lutun P, Marcellin L, Launoy A, Freys G, Bergerat JP, Herbrecht R: Factors associated with overall and attributable mortality in invasive aspergillosis. Clin Infect Dis 2008;47:1176–1184.
4 Schwartz S, Behre G, Heinemann V, Wandt H, Schilling E, Arning M, Trittin A, Kern WV, Boenisch O, Bosse D, Lenz K, Ludwig WD, Hiddemann W, Siegert W, Beyer J: Aerosolized amphotericin B inhalations as prophylaxis of invasive *Aspergillus* infections during prolonged neutropenia: Results of a prospective randomized multicenter trial. Blood 1999;93:3654–3661.
5 Griese M, Schams A, Lohmeier KP: Amphotericin B and pulmonary surfactant. Eur J Med Res 1998;3:383–386.
6 Allen SD, Sorensen KN, Nejdl MJ, Durrant C, Proffit RT: Prophylactic efficacy of aerosolized liposomal (ambisome) and non-liposomal (fungizone) amphotericin B in murine pulmonary aspergillosis. J Antimicrob Chemother 1994;34:1001–1013.
7 Ruijgrok EJ, Vulto AG, Van Etten EW: Efficacy of aerosolized amphotericin B desoxycholate and liposomal amphotericin B in the treatment of invasive pulmonary aspergillosis in severely immunocompromised rats. J Antimicrob Chemother 2001;48:89–95.
8 Ruijgrok EJ, Fens MH, Bakker-Woudenberg IA, van Etten EW, Vulto AG: Nebulization of four commercially available amphotericin B formulations in persistently granulocytopenic rats with invasive pulmonary aspergillosis: Evidence for long-term biological activity. J Pharm Pharmacol 2005;57:1289–1295.
9 Cicogna CE, White MH, Bernard EM, Ishimura T, Sun M, Tong WP, Armstrong D: Efficacy of prophylactic aerosol amphotericin B lipid complex in a rat model of pulmonary aspergillosis. Antimicrob Agents Chemother 1997;41:259–261.
10 Purcell IF, Corris PA: Use of nebulised liposomal amphotericin B in the treatment of *Aspergillus fumigatus* empyema. Thorax 1995;50:1321–1323.
11 Palmer SM, Drew RH, Whitehouse JD, Tapson VF, Davis RD, McConnell RR, Kanj SS, Perfect JR: Safety of aerosolized amphotericin B lipid complex in lung transplant recipients. Transplantation 2001;72:545–548.
12 Altmannsberger P, Holler E, Andreesen R, Krause SW: Amphotericin B deoxycholate: No significant advantage of a 24 h over a 6 h infusion schedule. J Antimicrob Chemother 2007;60:180–182.
13 Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, Denning DW, Donnelly JP, Edwards JE, Erjavec Z, Fiere D, Lortholary O, Maertens J, Meis JF, Patterson TF, Ritter J, Selleslag D, Shah PM, Stevens DA, Walsh TJ: Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: An international consensus. Clin Infect Dis 2002;34:7–14.
14 Subira M, Martino R, Rovira M, Vazquez L, Serrano D, De La Camara R: Clinical applicability of the new EORTC/MSG classification for invasive pulmonary aspergillosis in patients with hematological malignancies and autopsy-confirmed invasive aspergillosis. Ann Hematol 2003;82:80–82.
15 Borlenghi E, Cattaneo C, Capucci MA, Pan A, Quaresmini G, Franco F, Grazioli L, Carosi GP, Rossi G: Usefulness of the MSG/IFICG/EORTC diagnostic criteria of invasive pulmonary aspergillosis in the clinical management of patients with acute leukaemia developing pulmonary infiltrates. Ann Hematol 2007;86:205–210.
16 Schiel X, Link H, Maschmeyer G, Glass B, Cornely OA, Buchheidt D, Wilhelm M, Silling G, Helmerking M, Hiddemann W, Ostermann H, Hentrich M: A prospective, randomized multicenter trial of the empirical addition of antifungal therapy for febrile neutropenic cancer patients: Results of the Paul Ehrlich Society for Chemotherapy (PEG) Multicenter Trial II. Infection 2006;34:118–126.
17 Cornely OA, Maertens J, Winston DJ, Perfect J, Ullmann AJ, Walsh TJ, Helfgott D, Holowiecki J, Stockelberg D, Goh YT, Petrini M, Hardalo C, Suresh R, Angulo-Gonzalez D: Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med 2007;356:348–359.
18 Rijnders BJ, Cornelissen JJ, Slobbe L, Becker MJ, Doorduijn JK, Hop WC, Ruijgrok EJ, Lowenberg B, Vulto A, Lugtenburg PJ, de Marie S: Aerosolized liposomal amphotericin B for the prevention of invasive pulmonary aspergillosis during prolonged neutropenia: A randomized, placebo-controlled trial. Clin Infect Dis 2008;46:1401–1408.