Low dose intravenous infusion of amphotericin B for prevention of fungal infection in patients with acute leukemia

Akihiro Takeshita, Kaori Shinjo, Sadahiro Tamashima, and Ryuzo Ohno

Third Department of Internal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho Hamamatsu-shi, 431-31, Japan

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The effect of amphotericin B (AMPH) on the prevention of fungal infection in twenty-eight patients with acute myeloblastic leukemia (AML) was studied. The patients received a BHAC-DP regimen as consolidation and intensification chemotherapy; Enocitabin (BH-AC) 250 mg/day for 7 days, Daunorubicin (DNR) 40 mg/day for 3 days, and Prednisolone (PSL) 30 mg/day for 7 days. Intravenous infusion (i.v.) of AMPH (0.15 mg/kg/day) was dosed, in addition to oral administration of AMPH (2,400 mg/day), in 41 treatment periods in patients with AML (i.v. infusion group). Oral AMPH alone was administered, in 38 treatment periods, to patients with AML (oral administration group). The period of apyrexia, the period of fever, C reactive protein (CRP), erythrocyte sedimentation rate (ESR), culture data, incidence of adverse effects, and the quantity of endotoxin were compared between the two groups. The i.v. infusion group showed significantly better results than the oral administration group; the period of fever was significantly shorter (2.7 ± 3.5 days, $p < 0.05$), and serious systemic fungal infection was less offen detected (0 cases) than in the oral administration group (3 cases). No serious adverse events were observed.

Key words: Amphotericin B, Prevention of fungal infection, Leukemia, β-glucan

Introduction

Patients with acute leukemia who become granulocytopenic during and after chemotherapy are prone to many bacterial and fungal infections[1,2]. These infections are the cause of death in some patients, and reflect their prognosis. In patients with leukemia, once they develop a fungal infection, it is very difficult to achieve a complete cure because of prolonged granulocytopenia, immunodeficiency, and mucosal injury induced by antitumor agents[3] The prevention of infections therefore remains extremely important in these patients.

Advances in antibiotics and antifungal agents and development of various hemopoietic factors has led to a gradual decrease in these complications. AMPH is one of the most effective antifungal agents with respect to its spectrum and potency. Oral administration of AMPH for the prevention of fungal infections in patients with acute leukemia has been reported in many centers[4~6]. However in severely granulocytopenic patients with hematologic disorders, not all types of fungal infections can be prevented[7] Intravenous infusion of AMPH is useful, but its adverse effects of fever, hypokalemia and renal toxicity, for example, are potentially serious problems associated with its use. Various methods of administering this drug to decrease its toxicity have been described[8~11]. We used a low dose (maintenance dosage, 0.15 mg/kg/day) i.v. infusion of AMPH in patients with acute leukemia, and discuss the utility of this regimen for the prevention of fungal infection in these patients.

Patients and methods

Background of patients

Twenty-eight patients with acute myeloblastic leukemia (AML) admitted to the hospital of the Hamamatsu University School of Medicine from January 1986 to January 1992 were studied. They were treated with a BHAC-DP regimen, composed of BHAC 250 mg (day 1—7), DNR 40 mg (day 1—3), and PSL 30 mg (day 1—7) in a laminar air flow room, as consolidation and intensification therapies (Fig. 1). We analysed a total of 79 treatment periods in 28 patients. Patients with other types of leukemia and those receiving remission induction therapy were excluded from this study because of variations in their chemotherapy regimens. On admission for chemotherapy, the treatment periods were randomized to either (1) oral administration of AMPH (2,400 mg/day) or (2) oral administration and i.v. infusion of AMPH (2,400 mg/day and 0.15 mg/kg/day, respectively) for prevention of fungal infections. The former treatment periods numbered 41, the latter 38. The cases were allocated to one of the two administration groups by the blind envelope method in each of 79 courses. No significant differences were observed between the two administration groups in sex, age, WBC count at nadir, and CRP value before the administration of AMPH (Table 1). All patients were given polymixin B (300×10^4 U/day) and sulfamethoxazole-trimethoprim (ST) (1,200 mg and 2,400 mg/day, respectively) for intestinal decontamination, but recombinant human granulocyte colony stimulating factor (rhG-CSF) was not administered. All of the patients had pancytopenia with circulating granulocyte counts of less than 500/μl, and were at risk of bacterial and fungal infections. The patients who had obvious bacterial infections (ex sepsis) were excluded.

Administration method of AMPH

For the i.v. infusion technique, AMPH was started at a daily infusion dose of 1 mg/day, and gradually escalated in 1 mg/day increments to reach a maintenance dose of 0.15 mg/day. AMPH was infused over 6 hours once a day in 250 ml of a 5% glucose solution.

Evaluation of the effect of the therapy

Evaluation of the therapy was performed using the following data; maximum value of CRP, the period during which the CRP value was greater than 1 mg/dl, the period of apyrexia after chemotherapy, the period of fever, the periods during which ESR was greater than 30 and 50 mm/h, the positivity of fungal cultures sampled from the pharynx, sputum, urine, stool, and blood, and endotoxin

Components of BHAC-DP regimen	Day	1	2	3	4	5	6	7
BHAC (170 mg/m²) drip infusion		↑	↑	↑	↑	↑	↑	↑
DNR (30 mg/m²) drip infusion		↑	↑	↑				
PSL (20 mg/m²) oral administration		↑	↑	↑	↑	↑	↑	↑

Fig. 1. Details of administration of the BHAC-DP regimen.

Table 1. Details of patients (age, sex, WBC count in nadir and CRP value before the administration of amphotericin B)

	Oral administration group (n=38)	i.v. infusion group (n=41)
Age (y. o.)	40.8±14.0	42.0±15.3
Sex (male/female)	21/17	23/18
WBC count in nadir	621±286	604±230
CRP value	0.11±0.10	0.14±0.17

* Statistically different from the oral administration group, ($p<0.05$)

measurements. Adverse effects of therapy were monitored by creatinine clearance, fever, serum electrolytes, skin eruption and other clinical signs. Serum was sampled before administration of AMPH, and once every two days.

Measurement of serum concentration of AMPH

Serum concentrations of AMPH were determined in some patients. In the i.v. infusion group (n=12), serum samples were collected at minimum before (trough) and immediately after (peak) each infusion. Sera were also collected ten days after administration from patients in the oral administration group (n=10). The AMPH concentration was measured by bioassay[1,2].

Endotoxin test

Endotoxins were also studied to analyze the incidence of fungal infection. We analyzed three endotoxin parameters including the Toxicolor Test (T-test), Endospecy Test (E-test) and β-glucan. An antitumor carboxymethylated (1→3)-β-glucan (CMPS), a component of the fungal cell wall, correlates with fungal infection. The amount of β-glucan in plasma was estimated by subtracting the E-test results from those of the T-test. Plasma was sampled before administration of AMPH, during the febrile periods, and 10 days after the end of chemotherapy. Data were compared between the oral administration and i.v. infusion group[1,2]. Differences between the two groups were analysed with Student's non-paired T test.

Results

The results of maximum CRP value, the period during which CRP was greater than 1 mg/dl, the period of apyrexia after chemotherapy, the period of fever, the periods during which ESR was greater than 30 and 50 mm/h, and the positivity of fungal cultures sampled from the pharynx, sputum, urine, stool, and blood were compared between the oral administration group and the i.v. infusion group (Table 2). The periods of fever greater than 37.5°C were 4.8±4.3 days and 2.7±3.5 days, respectively, significantly shorter days ($P<0.05$) in the i.v. infusion group. The maximum CRP values were 7.8±7.3 and 4.8±4.1 mg/dl, respectively, with a significantly lower value in the i.v. infusion group than in the oral administration group ($P<0.05$). No significant differences were observed in the other parameters.

The results of fungal cultures are summarized in Tables 3 and 4. If at least one sample culture from

Table 2. Period of fever, period of apyrexia, CRP and ESR values in the oral administration and i.v. infusion groups (mean±S. D.)

	Oral administration group (n=38)	i.v. infusion group (n=41)
Period of fever (>37.5 C) (days after chemotherapy)	4.8±4.3	2.7±3.5*
Period of apyrexia (<37.0 C) (days after chemotherapy)	14.6±5.9	16.4±5.8
Maximum CRP value (mg/dl)	7.8±7.3	4.8±4.1*
Period of CRP less than 1.0 mg/dl (days after chemotherapy)	7.4±5.2	4.9±4.4
Period of ESR greater then 30 mm/h (days)	9.2±8.5	8.6±7.7
Period of ESR greater then 50 mm/h (days)	7.2±8.8	6.8±5.1

* Statistically different from the oral administration group, ($p<0.05$)

Table 3. Results of fungal cultures in patients in the i.v. infusion (n=41) and oral administration groups (n=38)

Fungal culture	Positive	Negative	Total
i.v. infusion group	4 (9.6%)*	37	41
Oral administration group	11 (28.9%)	27	38
Total	15	64	79

* Statistically different from oral administration group, ($p<0.05$)

Table 4. Detailed results of fungal cultures in the i.v. infusion (n=4/41) and oral administration groups (n=11/41)

No.	Sites of positive cultures	Fungus	Method of AMPH administration
01	stool	*Candida albicans*	i.v. infusion
02	pharynx	*Candida albicans*	i.v. infusion
03	pharynx	*Candida albicans*	i.v. infusion
04	stool	*Torulopsis glabrata*	i.v. infusion
05	stool	*Candida albicans*	oral
06	pharynx	*Candida tropicalis*	oral
07	pharynx	*Candida albicans*	oral
08	sputa	*Aspergillus fumigatus*	oral
09	blood	*Candida albicans*	oral
10	pharynx	*Candida albicans*	oral
11	pharynx	*Candida albicans*	oral
12	stool	*Candida albicans*	oral
13	blood	*Candida tropicalis*	oral
14	stool	*Aspergillus fumigatus*	oral
15	stool	*Candida albicans*	oral

AMPH, amphotericin B.

the pharynx, sputum, urine, stool, or blood was positive, the case was judged as positive. The rate of positivity in the i.v. infusion group was 9.6%, while that in the oral administration group was 28.9%. *Candida albicans* was the most frequently detected fungus, while *Aspergillus* species were detected in two cases of the oral administration group, but none in the i.v. infusion group. Fungus was detected on blood cultures in two cases in the oral administration group, but none in the i.v. infusion group.

The adverse effects considered to be induced by AMPH in the i.v. infusion group are summarized in Table 5. Drug eruption was seen in two cases, while hypokalemia and mild renal dysfunction were seen in one each. No other adverse effects, such as liver dysfunction and fever, were observed in this study. No adverse effects were observed in the oral administration group except for mild nausea.

AMPH concentrations in blood were measured. The peak blood concentration of AMPH in the i.v. infusion group was $0.80\pm0.38\,\mu g/ml$ (n=10), and the trough was $0.17\pm0.08\,\mu g/ml$ (n=10). The concentration in the oral administration group was $0.11\pm0.09\,\mu g/ml$ (n=10).

The data on endotoxin changes are shown in Table 6. Values obtained by subtracting E-test from T-test results, which correlate with β-D-glucan, were significantly lower (1.0 ± 1.6 pg/ml) in the i.v. infusion group than in the oral adminis-

Table 5. Details and incidence of adverse events in the i.v. infusion group (n=14)

Adverse event	Number and incidense of adverse event	Total number of cases of AMPH administration
Drug eruption	2 (14.3%)	14
Hypokalemia	1 (7.1%)	14
Renal dysfunction	1 (7.1%)	14

AMPH, amphotericin B.

Table 6. Endotoxin levels before and after administration of amphotericin B in the oral administration group and i.v. infusion group (mean±S. D.)

Oral administration group (n=38)

Sample	Before administration of AMPH (a)	Febrile period (b)	Change (b−a)
T-test (T 1) (pg/ml)	2.7±2.0	18.8±14.8	16.1±14.9
E-test (E 1) (pg/ml)	1.9±1.4	10.7± 7.0	8.7± 7.7
(T 1)−(E 1) (pg/ml)	0.8±0.8	7.5±11.6	6.8±11.2

Intravenous infusion group (n=41)

Sample	Before administration of AMPH (a)	Febrile period (b)	Change (b−a)
T-test (T 1) (pg/ml)	2.5±1.7	12.6±8.2	10.1±8.0
E-test (E 1) (pg/ml)	2.2±1.3	11.5±8.0	9.3±8.0
(T 1)−(E 1) (pg/ml)	0.3±0.9	1.0±1.6*	0.7±1.5*

* Statistically different from oral administration group ($p<0.05$)

tration group (7.5±11.6 pg/ml) ($p<0.05$) during the febrile periods. The difference in value between the febrile period and before AMPH administration was significantly lower (0.7±6.8 pg/ml) in the i. v. infusion group than in the oral administration group (6.8±11.2 pg/ml) ($p<0.05$). Other endotoxin indexes, such as T-test and E-test results alone, showed no significant difference.

Discussion

Fungal infection is one of the most significant problems experienced by patients with hematologic malignancies, especially acute leukemia. Immunodeficiency, mucosal injury, catheterization, granulocytopenia, and the administration of broad spectrum antibacterial agents are associated with increased frequency and severity of fungal infections[1,14,15]. Oral antifungal prophylaxis has been tried in many centers, but sufficient effect has not been achieved in systemic infection[16,18]. Many centers, including our hospital, have tested oral administration of AMPH in the prevention of fungal infections associated with hematological malignant disorders[4,5,19]. Oral administration was effective, but was insufficient for the prevention of fungal infections, except for some types of fungus. One of the reasons is that AMPH was not administered in

sufficiently high doses in patients with acompanying gastrointestinal disorders. Empirical administration of AMPH is a therapy worth considering in patients with hematologic malignancy who remain febrile in spite of administration of broad spectrum antibiotics[20~22]. Other authors have suggested empiric dosing guidelines for AMPH therapy[14,23~25]. However, the toxicity of intravenous administration of AMPH has limited its use to resistant systemic infections. In one study, almost all patients who had received intravenous administration of AMPH at doses greater than 0.3 mg/kg/day had one or more adverse effects[14]. We may have to wait for the development of a more effective and less toxic drug, or devise a new administration method. It is considered that among the available antifungal agents, AMPH has a valuable effect, and thus should be administered so as to minimize its adverse effects and maximize its antifungal efficacy. We therefore investigated the preventive effect of low dose i.v. infusion of AMPH in patients with acute leukemia. In the i.v. infusion group, the period of fever was shorter, and the maximum CRP value was significantly lower than in the oral administration group. The incidence of serious fungal infection in the i.v. infusion group was less (0 cases) than that in the oral administration group (3 cases, 2 cases of suspected fungal sepsis, one of *Aspergillus* pneumonia). Moreover, only a *Candida* spp. was cultured in the i.v. infusion group. We therefore reason that administration of AMPH by i.v. infusion, in addition to oral administration, is more effective for preventing fungal infections than oral administration alone. Adverse events in the i.v. infusion group consisted of hypokalemia, renal dysfunction, and drug eruption, but these were mild and resolved shortly after the drug was stopped.

Measurement of AMPH concentration showed that the serum concentration with oral administration of AMPH was insufficient for the prevention of many kinds of fungal infection. For example, *Candida torulopsis, Corynebacterium neoformans, Aspergillus fumigatus,* and *Aspergillus niger,* with respective MIC of 0.2~0.5 μg/ml, 1.25 μg/ml, 0.2 μg/ml, 1.9 μg/ml and 1.25 μg/ml[26], were not sufficiently prevented with oral administration of AMPH. This indicates that oral administration of AMPH is not sufficient for prevention of invasion by routes other than the gastrointestinal mucosa. The serum concentration of AMPH with i.v. infusion in addition to oral administration was 7.3 times higher than that with the latter alone, which may prevent the colonization of many fungi, including those mentioned above. Some *Aspergillus* species, Mucor species, and other fungi may be insufficiently inhibited with this administration method. However, these types of fungal infection are rare in the clincal setting. We therefore conclude that the i.v. infusion dose of 0.15 mg/kg/day is appropriate for prophylactic use, in view of serum levels and toxicity. The concentration of AMPH may be affected by factors such as deoxycholation, β-lipoprotein and cholesterol levels, as well as the function of the liver and other organs.

The diagnosis of fungal infections is difficult in clinical practice. Methods which facilitate diagnosis of fungal infection include measurement of β-D-glucan, which was calculated from T-test and E-test results in this study. The value of β-glucan in the i.v. infusion group was lower than in the oral administration group. This finding also demonstrates the advantage of prevention of fungal infections. We plan to perform further studies of other diagnostic methods in the future.

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急性白血病におけるアンフォテリシンBの少量点滴投与による真菌感染の予防効果

竹下 明裕・新庄 香・玉嶋 貞宏・大野 竜三
浜松医科大学第3内科*

真菌感染の予防に関するamphotericin B（AMPH）の少量点滴静注の効果について急性骨髄性白血病の28例において検討した。患者は地固め療法と強化療法としてBHAC-DP療法を施行された。BHAC-DP療法はEnocitabin（BHAC）250 mg/日を7日間，Daunorubicin（DNR）40 mg/日を3日間，Predonisolone（PSL）30 mg/日を7日間施行した。AMPHは点滴静注群と経口投与群に分け，点滴静注群ではAMPH（0.15 mg/kg/日）の点滴静注をAMPH（2,400 mg/日）の経口投与に加えて41回の治療期間に投与された。経口投与群ではAMPH（2,400 mg/日）が38回の治療期間に投与された。無熱期間，有熱期間，CRP，血沈，培養結果，副作用，エンドトキシンが2群間にて比較検討された。点滴静注群では有熱期間が有意に短縮され，重症真菌感染の頻度（0例）も経口投与群（3例）に比較して少なかった。AMPHの少量点滴静注による重大な副作用は認められなかった。

* 浜松市半田町3600