Prevention of Bacterial and Fungal Infections in Acute Leukemia Patients: A New and Potent Combination of Oral Norfloxacin and Amphotericin B

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The effect of a combination regimen using norfloxacin (NFLX) and amphotericin B (AMPH-B) for prevention of infections in patients with acute leukemia being treated by remission-induction chemotherapy in a randomized, controlled trial was studied. One hundred and six consecutive, evaluable patients were randomly assigned to receive orally 200 mg of norfloxacin two or four times daily and 200 mg of amphotericin B four times daily, or amphotericin B only. A smaller percentage of patients with bacteriologically-documented infections was observed in the study group compared with the control group (34.6% vs 56.9%; $P<0.05$). The mean number of days that the patients received empirical antibiotic therapy was shorter in the study group (23 days vs 30 days; $P<0.05$). The percentage of patients with a gram-negative bacterial infection (9.6% vs 27.5%; $P<0.05$) or a fungal infection (17.3% vs 37.3%; $P<0.05$) was decreased in the study group. This new combination antimicrobial regimen is safe and effective for prevention of gram-negative bacterial as well as fungal infections in patients with acute leukemia being treated with cytotoxic remission-induction chemotherapy. (Internal Medicine 32: 710–715, 1993)

Key words: infection prophylaxis, gram-negative bacterial infection, fungal infection

Introduction

Infections remain the most important complications in patients with acute leukemia during cytotoxic therapy in spite of the recent development of supportive therapies such as potent antibiotics and colony-stimulating factors (CSFs). Many of these infections are caused by gram-negative bacteria that colonize in the alimentary tract. Thus, selective or total decontamination of the alimentary tract is a useful approach for prevention of infections in these patients (1).

Norfloxacin is an oral quinolone structurally related to nalidixic acid, and its antibacterial spectrum is broader than those of nalidixic acid (2) and trimethoprim/sulfamethoxazol (3), which have been used previously for prevention of infections in granulocytopenic patients (1, 4, 5). It was reported that oral norfloxacin preserves the anaerobic gastrointestinal flora, thereby maintaining colonization which is resistant to the acquisition of new aerobic bacteria (6). Patients administered this agent for a long period showed no significant hematologic toxicity, gastrointestinal irritability, or other serious adverse effects (7). For these reasons, several controlled trials of

norfloxacin prophylaxis in granulocytopenic patients were initiated (3, 6–8).

In the randomized, controlled study, we investigated the effect of norfloxacin plus amphotericin B in the prevention of bacterial and fungal infections in patients with acute leukemia being treated by remission-induction chemotherapy.

Patients and Methods

All adult patients with acute leukemia, newly diagnosed or in relapse, who were admitted to our hospital between 1 January 1988 and 31 March 1991 were randomized to a control group or a group receiving norfloxacin prophylactically. Informed consent was obtained from all patients before starting the study. In the norfloxacin group, 200 mg of norfloxacin was given orally two times or four times daily. In both groups, oral amphotericin B (800 mg/day) was added to prevent colonization of the alimentary tract by yeasts. The prophylactic treatment was started shortly before cytotoxic treatment was initiated and ended when the granulocyte count recovered to more than 1,000/ μ l. The chemotherapeutic agents used were com-

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binations of doxorubicin, vincristine and prednisolone for acute lymphoblastic leukemia, and combinations of behenoyl cytosine arabinoside, daunorubicin, 6-mercaptopurine and prednisolone for acute myelogenous leukemia.

Clinical evaluations and complete blood counts were performed daily, and chemical studies were repeated as needed. The onset of fever or signs of infection prompted institution of intravenous antibiotics empirically. Febrile episodes were defined as the development of a fever above 38°C without an allergic reaction due to transfusion or drugs. Infectious complications were defined as bacteriologically and clinically documented infections or septicemia. Episodes accompanied by definite clinical signs and symptoms of infection plus microbiologic confirmation were termed "bacteriologically-documented infections." Episodes accompanied by definite clinical signs and symptoms of infection without microbiologic confirmation were termed "clinically-documented infections." The criteria for diagnosis of infectious diseases were as follows. Pneumonia was diagnosed on the basis of a fever and cough with infiltrates on chest roentgenograms, excluding those with the appearance of atelectasis or pulmonary edema. Tracheobronchitis or upper respiratory tract infection were diagnosed on the basis of a fever and cough without infiltrates on chest roentgenograms. Urinary tract infection was defined as pyuria of greater than 10⁵ colony-forming units of bacteria per milliliter of urine. Gastrointestinal infection was diagnosed on the basis of a fever with persistent watery diarrhea which was not due to antibiotics or cytotoxic agents and the absence of any other site of infection. Septicemia was defined as at least one blood culture yielding an organism considered to be a pathogen. Clinical diagnoses of perianal abscess, phlegmon, oral candidiasis, phlebitis, and otitis media were made by the attendant physicians. We also recorded unexplained febrile episodes with a body temperature of greater or equal to 38°C without any clear site of infection.

Statistical methods

Mean values were compared by Student's t-test. Differences in proportions between groups were analyzed by the chi-square test with Yates' correction. The times to the first febrile episode were compared by Kaplan-Meier plots and analyzed by the generalized Wilcoxon test.

Results

Patient characteristics

One hundred and eleven patients were randomly assigned to the norfloxacin group (norfloxacin plus amphotericin B) or the control group (amphotericin B only). Patients without cytotoxic therapy and patients who died within a week after starting the chemotherapy were excluded, resulting in 106 patients being evaluated (53 patients in each group).

The characteristics of the patients are shown in Table 1. There were no differences between the two groups in terms of the age, sex, subtype of leukemia, status of disease, antileukemic chemotherapy, duration of neutropenia (<1,000/ μ l), granulocyte

Table 1. Characteristics and Outcome of 106 Eligible Patients with Acute Leukemia Receiving Induction Chemotherapy

		Norfloxacin	Control
Patient number		53	53
Median age		49	39
Female	n.	21	26
Male	n.	32	27
Diagnosis			
ALL	n.	12	11
AML	n.	31	32
hybrid	n.	1	2
AML/MDS	n.	9	8
Disease status			
initial diagnosis	n.	36	28
relapse	n.	17	25
Mean duration of prophylaxis	day	34	38
Mean duration of neutropenia with granulocyte count			
< 100	day	17	18
< 500	day	26	27
<1,000	day	31	34
Complete remission	n.	25	28
Death during treatment	n.	11	10

ALL: acute lymphoblastic leukemia, AML: acute myelogenous leukemia, AML/MDS: AML transformed from myelodysplastic syndrome

count or study duration. Although there were more relapsed patients in the control group, the incidence of infection was not significantly different among the initially diagnosed patients and relapsed patients, in either the control group or the norfloxacin group. The outcome, in terms of the rate of complete remission and the number of deaths during the study period, was similar in both groups. The characteristics of the patients were similar in the two groups of patients given 400 mg/day and 800 mg/day of norfloxacin. We combined and analyzed these two groups of patients, since the prophylactic effect was not significantly different between these two groups.

Febrile episodes and frequency of infection

One patient in the norfloxacin group and two in the control group were febrile at the time of initiating remission-induction chemotherapy, and the fever persisted throughout the study period. For this reason, these three patients were excluded from the analysis of febrile episodes. Three patients in the norfloxacin group and five in the control group who were febrile at the time of initiating the chemotherapy and became afebrile soon after were included in the analysis. The data on febrile episodes are compiled in Table 2. Febrile episodes occurred in 38 of 52 patients in the norfloxacin group and 44 of 51 in the control group. Febrile episodes occurred 52 times in the norfloxacin

Table 2. Febrile Episodes

	Norfloxacin		Control	
	Number of patients	Number of episodes	Number of patients	Number of episodes
Patients analyzed	52		51	
All patients with fever	38	52	44	74
Clinically-documented infection	23	29	24	28
Bacteriologically-documented infection ^{a)}	16	20	22	36
Septicemia ^{b)}	3*	3	10*	10
a) or b)	18 [†]	23	29 [†]	43

* $P < 0.10$; determined by the chi-square test with Yates' correction, [†] $P < 0.05$; determined by the chi-square test with Yates' correction.

group compared with 74 times in the control group. The mean number of febrile episodes in one patient during the study period was 1.0 in the norfloxacin group and 1.4 in the control group, with a statistically significant difference ($P < 0.05$).

In the norfloxacin group, febrile episodes occurred in 26 of 36 previously untreated patients and in 12 of 16 relapsed patients. In the control group, febrile episodes occurred in 22 of 28 previously untreated patients and 22 of 23 relapsed patients. There were no statistical differences in the incidences of infection between each disease status in the norfloxacin group as well as in the control group. Septicemia tended to occur more frequently in the control group compared with the norfloxacin group (10 versus 3 patients), but this was not statistically significant ($P < 0.10$). Bacteriologically-documented infection or septicemia occurred in 18 and 29 patients ($P < 0.05$), and the numbers of those episodes were 23 and 43 times, in the norfloxacin group and the control group, respectively. In Table 2, afebrile episodes of oral candidiasis were excluded from the category of "bacteriologically-documented infection."

The cumulative percentages of afebrile patients after induction chemotherapy are shown in Fig. 1. Four patients in the norfloxacin group and six in the control group who were febrile at the time of initiating chemotherapy were excluded from this analysis, although three and four of them in the respective groups became afebrile soon after initiating the chemotherapy. Patients were considered censored if they went off the study without a fever. Febrile episodes occurred more rapidly in the patients not receiving norfloxacin, with a median time to the first febrile episode of 11 days compared to 18 days in the patients receiving norfloxacin. There was a statistically significant difference between the curve of the norfloxacin group and that of the control group when analyzed by the generalized Wilcoxon test ($P < 0.001$). The mean numbers of days with a fever were 8.7 days and 11.9 days, and the proportions of febrile time in the study period were 24.1% and 34.9% in the norfloxacin group and control group, respectively. There was a statistically

Fig. 1. Cumulative percentage of acute leukemia patients without fever after initiation of remission-induction chemotherapy. The difference in the overall curves between the norfloxacin group (norfloxacin plus amphotericin B) and the control group (amphotericin B only) was statistically significant ($P < 0.001$; generalized Wilcoxon test). Patients who were free of fever during the study period were censored ($\perp$).

significant difference between these percentages of febrile days ($P < 0.05$). The mean numbers of days on which patients received empirical antibiotic therapy were 23 and 30 in the norfloxacin and control groups, respectively, and the difference was statistically significant ($P < 0.05$).

Sites of infection

The sites of infection are shown in Table 3. Forty-eight and 73 sites of infection were observed in the norfloxacin group and the control group, respectively. Infections of the skin and soft tissue, stomatitis and septicemia were observed more often in the control group, but the difference was not statistically sig-

Table 3. Sites of Infection

Site	Norfloxacin		Control	
	Number of patients	Number of episodes	Number of patients	Number of episodes
Lung	12	12	12	13
Upper respiratory tract	12	13	13	15
Urinary tract	3	3	4	4
Anorectal	3	3	4	4
Skin and soft tissue	4	5	7	9
Stomatitis	5*	5	13*	15
Gastrointestinal tract	2	2	2	2
Septicemia	3 [†]	3	10 [†]	10
Phlebitis			1	1
Otitis media	1	1		
No site found ^{a)}	10	10	8	8
Totals	38	58	44	81

^{a)}Febrile episodes without any detectable site of infection. * $P < 0.10$; determined by chi-square test with Yates' correction. [†] $P < 0.10$; determined by chi-square test with Yates' correction.

nificant. Life-threatening infections, including pneumonia and septicemia, were observed in 15 patients in the norfloxacin group and 19 patients in the control group.

Bacteriologically-documented organisms

Table 4 summarizes the organisms causing infections in the patients during the study period. Gram-positive infections occurred in 14 of 52 patients in the norfloxacin group and 17 of 51 in the control group, with no significant difference. Two patients with norfloxacin and four without norfloxacin developed gram-positive septicemia. *Staphylococcus aureus*, coagulase-negative staphylococci and *Enterococcus* sp. were the principal gram-positive pathogens in each group. In the cases of pneumonia and other sites of infection, there were no predominant gram-positive pathogens in either patient group.

Gram-negative infections occurred in only five of 52 patients in the norfloxacin group compared with 14 of 51 in the control group. There was a statistically significant difference between these two groups ($P<0.05$). Gram-negative septicemia or pneumonia developed in only two patients given norfloxacin and in seven patients without norfloxacin. *Pseudomonas aeruginosa* and *Enterobacter cloacae* were the principal gram-negative pathogens in each group.

We detected fungal infections, including *Candida* stomatitis, in nine of 52 patients in the norfloxacin group and in 19 of 51 in the control group. The difference between these two groups was statistically significant ($P<0.05$). In the norfloxacin and control groups, disseminated or invasive fungal infections

occurred in four and six patients, respectively. Fungemia occurred only in the control group (two patients), and the pathogen was *Trichosporon* sp.

Compliance and adverse reactions

Compliance was similar in the two groups of patients, with ingestion of more than 95% of the total prescribed doses. Norfloxacin and amphotericin B were well tolerated, and there were no adverse effects which interrupted the study. Only a few patients complained of nausea after taking amphotericin B. Prolonged bone marrow suppression was not observed in the norfloxacin group.

Discussion

The results of the present study clearly showed the effectiveness of norfloxacin plus amphotericin B for prophylactic use in patients with acute leukemia since this combination 1) decreased the number of patients with bacteriologically-documented infections, including septicemia, 2) decreased the incidence of infections per patient during the study period, 3) prolonged the time to the first febrile episode, 4) reduced the proportion of time in a febrile state during the study period, 5) decreased the number of days on which patients received empirical antibiotic treatment, 6) decreased the number of patients with gram-negative bacterial and 7) fungal infections. Previous reports showed that the number of patients with bacteriologically-documented infections in norfloxacin-treated

Table 4. Identified Causative Organisms of Infection

	Norfloxacin		Control	
	Patients with infection (n)	Total number of infections	Patients with infection (n)	Total number of infections
Gram-positive bacteria	14	17	17	24
<i>Staphylococcus aureus</i>	6	7	7	9
Coagulase-negative staphylococcus	5	5	5	6
<i>Streptococcus</i> sp.			1	1
<i>Enterococcus</i> sp.	4	5	6	7
<i>Branhamella catarrhalis</i>			1	1
Gram-negative bacteria	5*	6	14*	21
<i>Pseudomonas aeruginosa</i>	3	3	8	9
<i>Pseudomonas cepacia</i>			1	1
<i>Escherichia coli</i>			1	1
<i>Enterobacter cloacae</i>	1	2	4	5
<i>Acinetobacter calcoaceticus</i>			2	2
<i>Flavobacterium</i> sp.			1	2
<i>Moraxella</i> sp.	1	1		
<i>Hemophilus parainfluenza</i>			1	1
Fungi	9 [†]	10	19 [†]	21
<i>Candida</i> sp.	9	9	17	19
<i>Trichosporon</i> sp.	1	1	2	2

* $P<0.05$; determined by chi-square test with Yates' correction, [†] $P<0.05$; determined by chi-square test with Yates' correction.

patients was lower (24% to 44%) (3, 6, 7, 9) compared with the control group (57%) (7) and the group which received non-absorbable antibiotics (67%) (6). Moreover, gram-negative bacterial infections occurred significantly less often in the norfloxacin-treated group (0% to 11.4%) (6–8) compared with the control group (24%, 39%) (7, 8) and the group which received non-absorbable antibiotics (16.7%) (6). In the report of Karp et al (8), the mean number of days from starting the chemotherapy to the first febrile episode was longer in the norfloxacin group, and the mean number of days with a fever during the study period was lower in the norfloxacin group compared with the control group. These findings resulted in a lower overall cost of medical care. Karp et al also showed the prophylactic effect of norfloxacin on the time to the first febrile episode in terms of the mean number of days, while we showed this in terms of the median number of days using Kaplan-Meier plots. Because the absorbance of norfloxacin is poor, it can be present at high concentrations in the gastrointestinal tract without necessarily achieving therapeutic levels in the serum (2). Thus, the drug effect is localized to the gastrointestinal tract, a major entry route of gram-negative organisms that can disseminate during intensive chemotherapy. This may explain why the incidence of respiratory tract infections and the number of patients with respiratory tract infections were similar in both groups. There may be other reasons for the very good results of the present study, such as the facts that norfloxacin has a broad antimicrobial spectrum, is poorly absorbed in spite of its high concentration in the gastrointestinal tract, and preserves colonization resistance. Although we didn't have enough data on the surveillance culture and bacteria resistant to norfloxacin, previous reports showed a low prevalence of norfloxacin-resistant aerobic bacteria in stool specimens obtained throughout the course of granulocytopenia and indicated that such bacteria appeared to be independent of norfloxacin administration (7, 8). Acquired infections in the norfloxacin group were not refractory to the empirical antibiotic treatment, and norfloxacin had no impact on the frequency of gram-positive (including MRSA, Methicillin resistant *Staphylococcus aureus*) bacterial infections in the present study or in the study of Karp et al (8).

There is substantial evidence in the literature that fungal infections increase in frequency in granulocytopenia (11, 12). Because invasive fungal infections are life-threatening in neutropenic patients, their prevention is important. There have been several reports on the prophylactic use of nystatin (10, 13–15) or amphotericin B (16–18), but their effects have been controversial. Since nystatin is of questionable value in decreasing the incidence of fungal infections (14, 15, 19) and shows poor patient compliance (11), we decided to use amphotericin B for prophylaxis of fungal infections. In the present results, the number of patients who suffered from fungal infections, including oral candidiasis, was significantly lower in the norfloxacin group. This is the first report showing the effectiveness of a combination regimen consisting of norfloxacin plus amphotericin-B in the prophylaxis of fungal infections. Although the reasons why a smaller number of patients with

fungal infections was seen in the norfloxacin group are unclear, it may have been due to the smaller number of days with a fever and the less frequent administration of empirical antibiotic treatment in the norfloxacin group or, it may have been due to the fact that norfloxacin preserves colonization resistance, which protects the anaerobes and suppresses the growth of pathogenic aerobes as well as fungi (20). Norfloxacin has no intrinsic antifungal activity, but it has been reported to augment the *in vitro* antifungal activity of amphotericin B, nystatin and other antifungal agents (6, 21). Thus, synergistic activity between norfloxacin and amphotericin B may have been another factor in the prophylactic effect on fungal infections seen in the present study.

Various disadvantages of regimens using oral non-absorbable antibiotics, such as high cost, adverse gastrointestinal effects and poor patient compliance, and a tendency to develop aminoglycoside resistance, have been reported (22). In the present study, there were no adverse side effects from norfloxacin, such as neurotoxicity, which required discontinuation of the study. Norfloxacin and amphotericin B were both well tolerated and enjoyed a high degree of patient compliance. Combination use of norfloxacin and amphotericin B for patients with acute leukemia during remission-induction chemotherapy is more useful for the prophylaxis of gram-negative bacterial and fungal infections, and it reduces the cost of medical care compared with regimens using non-absorbable antibiotics. Recently, it was reported that cytokines, such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), shorten the neutropenic period during chemotherapy for hematological malignancies. It is interesting that recent advances in cytokine therapy have aided in the prophylaxis of infection in patients with neutropenia, and this therapy is considered to be useful for prevention of infections when used in combination with an oral prophylactic regimen.

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