A Comparative Study of Changes in Fecal Flora in Patients Preconditioned with Either Amphotericin B or Fluconazole for Allogeneic Bone Marrow Transplantation

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Summary

The present study investigated fecal flora changes in 20 patients who received either the non-absorbable antifungal agent amphotericin B, or the absorbable antifungal agent fluconazole, with tobramycin and vancomycin as preparation for undergoing allogeneic bone marrow transplantation (BMT). The oral antibiotic regimen consisted of tobramycin (540 mg/day, three times a day), vancomycin (1,500 mg/day, three times a day) and either amphotericin B (2,400 mg/day, twice a day) (AMPH group) or fluconazole (400 mg/day, twice a day) (FLCZ group) and was designed to prevent infections from microorganisms in the gut. Aerobic bacterial colonies were not detected on the day of BMT or 1 week after BMT, except in 1 case unable to take the full antibiotic regimen due to nausea and vomiting.Anaerobic bacterial colonies were not detected on the day of BMT except in this single case. Furthermore, there were no episodes of bacterial infection. In both groups, Candida colonies were detected in some case. Candida colonies were also detected in the pharynx and urine. However, there were no fungal infections. The present report suggests that amphotericin B and fluconazole administrations, with tobramycin and vancomycin, are equally effective for protection against bacterial and fungal infections in BMT recipients.

Introduction

Fungal infections are common and are an important cause of morbidity and mortality in patients undergoing allogeneic bone marrow transplantation (BMT). It has not as yet been determined whether non-absorbable or absorbable antifungal agents are more effective for preventing fungal infections during BMT. The present study was undertaken to compare changes in fecal flora in patients receiving a non-
absorbable antifungal agent, amphotericin B, and those receiving an absorbable antifungal agent, fluconazole, in addition to tobramycin and vancomycin during allogeneic BMT.

**Patients and methods**

**Patients**

Twenty patients with acute myelogenous leukemia (AML) \( n=2 \), acute lymphoblastic leukemia (ALL) \( n=4 \), acute undifferentiated leukemia (AUL) \( n=1 \), chronic myelogenous leukemia (CML) \( n=4 \), myelodysplastic syndrome (MDS) \( n=5 \) and severe aplastic anemia (SAA) \( n=4 \) who received allogeneic BMT were enrolled in this study (Table 1). Their ages ranged from 16 to 45 (median 25) years. Ten were males and 10 were females. All patients except cases 14 and 17 received marrow grafts from HLA-identical siblings. Cases 14 and 17 underwent BMT from unrelated donors. Case 6 received a second BMT.

**Preconditioning regimen**

The conditioning regimen for patients with hematological malignancies consisted of busulfan (BU) and cyclophosphamide (CY) as the basis. Cases 2, 3, 12 and 13 received BU (4 mg/kg day for 4 days) and CY (60 mg/kg/day for 2 days). Cases 1 and 4 received ranimustine (MCNU; 200 mg/body for 1 day), cases 5, 7, 8 and 18 cytarabine (Ara-C; 2 mg/m²/day for 2 days) and cases 9, 15, 16 and 17 total body irradiation (TBI; 12 Gy. 6 fraction) in addition to BU and CY. Cases 6 and 19 received Ara-C, CY and TBI. Patients with SAA were conditioned with CY (50 mg/kg/day for 4 days) and irradiation. Cases 10 and 20 received CY and TBI (case 10; 10 Gy. 5 fractions and case 20; 7.5 Gy. 3 fractions). Case 14 received CY and total lymphoid irradiation (TLI; 6 Gy. 3 fractions), case 11 anti-lymphocyte globulin (ALG; 50 mg/kg/day for 3 days) including CY and TLI. For graft-versus-host disease (GVHD) prophylaxis, all patients received cyclosporin (CSA) for 100 days in combination with methotrexate (MTX: on days 1, 3, 6 and 11 post-BMT).

**Prophylactic antimicrobial regimen**

The oral antimicrobial regimen consisted of tobramycin (540 mg/day, three times a day), vancomycin (1,500 mg/day, three times a day) and either the non-absorbable antifungal agent amphotericin B (2,400 mg/day, twice a day) (AMPH group) or the absorbable antifungal agent fluconazole (400 mg/day).
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twice a day) (FLCZ group) (Table 2). Cases 1～10 received amphotericin B and cases 11～20 fluconazole. These oral antifungal drugs were started two weeks before BMT. Informed consent was obtained from all patients.

Isolation and decontamination

Laminar airflow rooms were used for this study. Sterilization procedures, skin cleaning and sterile diets were adapted from methods described previously.1

Stool cultures

Routine aerobic, anaerobic, and fungal stool cultures were obtained once a week before and after BMT. Microbial culturing techniques were performed according to a previous report.2 One-gram fecal samples taken from the center of the fecal specimen were immediately inserted into 10 ml of the diluent and homogenized with teflon grinders. The fecal suspensions were further diluted in the sterile diluent in tenfold steps and 0.1 ml samples of each dilution were spread on the surfaces of various selective agar culture media. The media used included: DHL Agar, TCBS Agar, NAC Agar, No. 110 Agar, Phenylethyl Alcohol Blood Agar, SF Agar, GS Agar, Potato Dextrose Agar, GAM Agar and Brain Heart Infusion Blood Agar, CW Agar, Bifidobacterium Agar, Rogosa SL Agar, Rifampin Blood Agar, Brucella Agar + 5% horse blood +75 µg Kanamycin + 2 µg Vancomycin, Bacteroides Agar, Modified FM Agar and Veillonella Agar + 7.5 µg Vancomycin +0.1% Tween 80, which were used as streak plates.

Culture of Candida colonies in pharynx, urine, blood and sputum

Pharyngeal, urine, blood and sputum samples were cultured semi-quantitatively as routine surveillance cultures.

Candida antigen and serum D-arabinitol concentration

Candida antigen (CAND-Tec test, Wako Pure Chemical Industries, Osaka) and the D-arabinitol concentration in serum were measured.

Pharmacokinetics of Fluconazole

Concentrations of fluconazole in serum and urine were measured by the HPLC-UV method as previously described.3 Serum samples were assayed 2 hours after the patients had taken fluconazole. Urine was collected for 24 hours to assess urinary excretion.

Statistical analysis

The significance of each set of values was assessed using a t-test, assuming equal variance.

Results

Changes in aerobic bacterial colonies in fecal flora

Aerobic bacterial colonies in fecal flora before administration of the drugs were examined in 6 cases from the AMPH group and all 10 FLCZ group cases. As shown in Fig. 1, aerobic bacterial colonies were detected in 5 of 6 AMPH group cases and 9 of 10 FLCZ group cases. The average number of aerobic bacterial colonies detected was 5.4×10^7 colonies/g. Enterococcus spp. was detected in 12 of 14 (85.7%) aerobic bacterial colonies before administration of antibiotics. Aerobic bacterial colonies were not detected on the day of BMT or 1 week after BMT, except in 1 case who was unable to take the full antibiotic regimen due to severe nausea (case 17). However, aerobic bacterial colonies were detected in 3 of 13 cases examined 2 weeks after BMT and in 4 of 13 examined 3 weeks after BMT. The average numbers of aerobic bacterial colonies detected were 1.6×10^6 colonies/g 2 weeks after BMT and 2.9×10^6 colonies/g 3 weeks after

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Changes in anaerobic bacterial colonies in fecal flora

Anaerobic bacterial colonies in fecal flora before administration of antibiotics were examined in 6 AMPH group cases and all 10 FLCZ group cases. As shown in Fig. 1, anaerobic bacterial colonies were detected in all 6 from the AMPH group and all 10 from the FLCZ group. The average number was $5.4 \times 10^9$ colonies/g. *Clostridium*, *Bacteroides*, *Fusobacterium*, *Eubacterium*, and *Bifidobacterium*, were detected in 15 (93.8%), 14 (87.5%), 12 (75.0%), 11 (68.8%) and 10 (62.5%), respectively. Anaerobic bacterial colonies were not detected on the day of BMT, except in the aforementioned case 17. Furthermore, anaerobic bacterial colonies were not detected from 1 to 3 weeks after BMT.

Changes in *Candida* colonies in fecal flora

BMT.

Changes in anaerobic bacterial colonies in fecal flora

Anaerobic bacterial colonies in fecal flora before administration of antibiotics were examined in 6 AMPH group cases and all 10 FLCZ group cases. As shown in Fig. 1, anaerobic bacterial colonies were detected in all 6 from the AMPH group and all 10 from the FLCZ group. The average number was $5.4 \times 10^9$ colonies/g. *Clostridium*, *Bacteroides*, *Fusobacterium*, *Eubacterium*, and *Bifidobacterium*, were detected in 15 (93.8%), 14 (87.5%), 12 (75.0%), 11 (68.8%) and 10 (62.5%), respectively. Anaerobic bacterial colonies were not detected on the day of BMT, except in the aforementioned case 17. Furthermore, anaerobic bacterial colonies were not detected from 1 to 3 weeks after BMT.

Changes in *Candida* colonies in fecal flora
Candida colonies in fecal flora before administration of antibiotics were examined in 9 AMPH group cases and 9 FLCZ group cases. Candida colonies were detected in 2 (22.2%) of 9 from the AMPH group and 1 (11.1%) of 9 from the FLCZ group (Fig. 2). The average number was $1.1 \times 10^3$ colonies/g before administration of antibiotics. Candida colonies were detected in some cases in both the AMPH and the FLCZ groups. Candida colonies in the AMPH group and the FLCZ group were detected in 50.0% (3/6) and 16.7% (1/6) 2 weeks after BMT, and in 33.3% (2/6) and 16.7% (1/6) 3 weeks after BMT, respectively. The detection rate of Candida colonies did not differ significantly between the AMPH and the FLCZ groups.

Changes in pharyngeal, urine, blood and sputum Candida colonies

No Candida colonies were detected in blood or sputum in any of the cases. In the AMPH group, case 3 had Candida albicans in the pharynx continuously during the course of BMT, and Candida grabrata, in one urine sample. In case 5, C. albicans, colonies were detected in urine and the pharynx 2 weeks after BMT. In the FLCZ group, cases 11 and 17 showed C. albicans, in the pharynx 3 weeks after and on the day of BMT, respectively. Case 15 had Candida krusei, in the pharynx 1 week after BMT. Cases 3, 5 and 15 showed Candida colonies in feces, but cases 11 and 15 were not themselves colonized.

Candida antigen and serum D-arabininol concentration

Candida antigens were measured in 100 samples in this study. Of the 100 samples, 5 were X2 titer positive and 1 was X4 titer positive for Candida antigen (Table 3). However, in 2 of 6 Candida antigen positive samples, Candida colonies were detected in the feces. Therefore, false positive cases may be included among these positive cases.

The D-arabininol concentration was measured in 83 samples in this study. The D-arabininol concentration and the D-arabininol/creatinine ratio in normal males and females are 4.3 ± 2.7 µmol/l, and 4.4 ± 3.1 µmol/l, and 0.4 ± 0.2 µmol/mg and 0.5 ± 0.3 µmol/mg, respectively. The D-arabininol concentration and the D-arabininol/creatinine ratio in this study were 4.34 ± 1.74 µmol/l, and 0.63 ± 0.28 µmol/mg, respectively.

### Table 3 Cases Positive for Candida antigen (Total number = 100)

<table>
<thead>
<tr>
<th>Case</th>
<th>Titer</th>
<th>Sampling time</th>
<th>Candida colonies (CFU/g)</th>
<th>Fever</th>
<th>Anti-fungal drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X2</td>
<td>BMT</td>
<td>Negative</td>
<td>—</td>
<td>AMPH</td>
</tr>
<tr>
<td>14</td>
<td>X2</td>
<td>2W</td>
<td>N.D</td>
<td>—</td>
<td>FLCZ</td>
</tr>
<tr>
<td>15</td>
<td>X4</td>
<td>BMT</td>
<td>$3.8 \times 10^6$</td>
<td>—</td>
<td>FLCZ</td>
</tr>
<tr>
<td>17</td>
<td>X2</td>
<td>3W</td>
<td>5.0 × 10^7</td>
<td>—</td>
<td>FLCZ</td>
</tr>
</tbody>
</table>

Sampling time shows the period of fecal specimen sampling after BMT. N.D = Not done

### Table 4 Serum D-arabininol concentration and D-arabininol/creatinine ratio (total number = 83)

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>30</td>
<td>5W</td>
<td>9.90</td>
<td>1.04</td>
<td>N.D</td>
<td>—</td>
<td>AMPH</td>
</tr>
<tr>
<td>35</td>
<td>1W</td>
<td>6.02</td>
<td>1.51</td>
<td>Negative</td>
<td>—</td>
<td>AMPH</td>
</tr>
<tr>
<td>36</td>
<td>2W</td>
<td>16.20</td>
<td>0.45</td>
<td>$5.4 \times 10^6$</td>
<td>—</td>
<td>FLCZ</td>
</tr>
<tr>
<td>41</td>
<td>2W</td>
<td>21.40</td>
<td>4.28</td>
<td>Negative</td>
<td>—</td>
<td>FLCZ</td>
</tr>
<tr>
<td>44</td>
<td>2W</td>
<td>81.00</td>
<td>11.57</td>
<td>Negative</td>
<td>—</td>
<td>FLCZ</td>
</tr>
</tbody>
</table>

Time : after BMT. N.D = Not done
The D-arabinitol concentration was remarkably high in 5 samples (Table 4). However, fecal Candida colonies were detected in only 1 sample.

Pharmacokinetics of fluconazole

Results are shown in Table 5. Concentrations of fluconazole in serum were measured in 3 samples on days -1, 4 and 5. The average serum concentration was 11.6 ± 1.8 µg/ml. Concentrations of fluconazole in urine were measured in 11 samples. Urine was collected for 24 hours to assess urinary excretion. The average concentration in urine was 180.4 ± 127.8 µg/ml. The average 24 hour urinary excretion was 247.3 ± 132.3 mg/day.

Recovery of white blood cell and neutrophil counts after allogeneic BMT

The white blood cell count reached 1,000/µl on median of day 17.9 (range, 12-25) in the AMPH group and 17.4 (range, 13-24) in the FLCZ group. The neutrophil count reached 500/µl on median day 18.9 (range, 12-28) in the AMPH group and 17.1 (range, 13-254) in the FLCZ group.

Discussion

The risk of invasive fungal infection is high after BMT because of the intensive conditioning regimen, the immunosuppressive effect of allografting, and graft-versus-host disease. In the present study, before and after allogeneic BMT the non-absorbable antifungal agent amphotericin B or the absorbable antifungal agent fluconazole, was administered for antifungal prophylaxis in addition to tobramycin and vancomycin. We quantitatively investigated changes in fecal flora in these patients.

The prophylactic regimen for bacterial and fungal infection was begun 2 weeks before BMT, and aerobic and anaerobic bacterial colonies were detected in only 1 case each, in both groups, at BMT. Since the period of neutropenia was about 18 days, it was thought that bacterial colonies in fecal flora would have disappeared by 3 weeks after BMT. In the present study, anaerobic bacterial colonies were detected for 3 weeks after BMT. Aerobic bacterial colonies were not detected 1 week after BMT, but gradually increased during the 2nd and 3rd weeks after BMT. However, there were no episodes of bacterial infection. On the other hand, suppression of Candida colonies in fecal flora was unsatisfactory in both groups. Candida colonies were detected in some cases. Candida colonies were also detected in the pharynx and urine. However, there were no episodes of fungal infection. Goodman et al. demonstrated that fluconazole at 400 mg/day significantly reduced fungal colonization, systemic infection and mortality from invasive fungal disease. In the present study, a dose of 400 mg/day was administered for prophylaxis. The amount of fluconazole excreted in urine in 24 hours was 247.3 mg, on average, which represents 61.8% of the dose administered. There was no difference between our data and that of El-Yazigi et al. (33.55 ± 41.6%). Furthermore, there were no side effects attributable to fluconazole. However, since the fluconazole dose is usually 50–200 mg/day for the treatment of Candida infections, although 400 mg/day is required for refractory fungal infections, it is necessary to determine an adequate dose of fluconazole for prophylaxis.

Our results suggest that administrations of amphotericin B and fluconazole, in addition to tobramycin and vancomycin, are equally effective for protection against bacterial and fungal infections in BMT recipients.
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References


同種骨髄移植におけるアンホテリシン B とフルコナゾールの
腸内細菌に及ぼす影響の比較

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要 旨
同種骨髄移植患者において、トブラマイシン、バノコマイシンと同時に、非吸収性の抗真菌剤のアンホテリシン B か、あるいは吸収性の抗真菌剤のフルコナゾールを投与して腸内細菌の変動および真菌感染症について検討した。好気性および嫌気性の細菌は一部の例を除いて移植時には検出されなかった。また、細菌感染症も認められなかった。一方、Candida のコロニーはアンホテリシン B、あるいはフルコナゾールの投与中においても糞便中に検出された。しかし真菌感染症は認められなかった。今回の検討では、トブラマイシン、バノコマイシンと同時に投与したアンホテリシン B、あるいはフルコナゾールは細菌および真菌感染症の予防に同様に有効であった。

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