Antibiotic sensitivity of bacteria on the oral mucosa

after hematopoietic cell transplantation

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We reported recently that bacterial substitution of mainly coagulase-negative staphylococci (CoNS) for streptococci occurred frequently on the oral buccal mucosa after hematopoietic cell transplantation (HCT), and other bacterial species not usually found in the normal flora were also identified [1]. We also reported that multidrug-resistant opportunistic bacteria appearing in the gingiva may be involved in fatal sepsis [2]. These observations prompted an interest in the antibiotic sensitivity of bacteria after HCT, which may explain the bacterial substitution on oral mucosa after HCT. Therefore, we performed a pilot study to determine the antibiotic sensitivity of bacteria on the oral mucosa after HCT.

We examined the antibiotic sensitivity of bacteria detected after HCT, focusing on the period from day 0 to 13, when the severity of clinically evident mucosal damage generally peaks and can cause bacteremia via the oral mucosa [3 – 5]. A total of 9 consecutive patients (M: 4, F: 5, 47.3 ± 11.0 y) receiving HCT at Okayama University Hospital were enrolled in this study. The diseases in these 9 patients were as follows: acute myelogenous leukemia ($n = 3$), myelodysplastic syndrome ($n = 1$), and malignant lymphoma ($n = 5$). Autologous HCT, conventional allogeneic HCT, and reduced-intensity HCT were administered to 2 (M: 2, F: 0, av.: 61.0 y), 5 (M: 0, F: 5, 39.4 ± 7.7 y), and 2 (M: 2, F: 0, av.: 53.5 y) patients, respectively. Informed consent for

examination of oral bacteria was obtained from each subject, and the Ethical Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences approved this study (No. 263). General infection control and oral management were performed as described in our previous report [1]. Briefly, fluoroquinolone for prophylaxis against bacterial infection was administered orally. Neutropenic fever was managed according to the guidelines of Hughes et al. [6]. A fourth-generation cephalosporin (e.g., cefepime) or carbapenem (e.g., meropenem) was administered intravenously as empirical antibiotic therapy.

Buccal mucosal swab samples were obtained from each patient twice with a one-week interval from day 0 to +13. Samples were obtained about 2 h after breakfast by swabbing from the whole surface of the buccal mucosa. All samples were plated onto agar plates under aerobic conditions, and 2 – 5 major colonies that were visibly different from each other were collected. A total of 67 colonies were collected from 9 HCT patients. Collected colonies were subjected to microbial identification and antibiotic sensitivity test. Identification of colonies thus obtained was performed using rapid ID 32 STREP API®, rapid ID 32 E API®, or ID 32 GN API® identification kits (Japan bioMérieux, Tokyo, Japan) according to the manufacturer's instructions. Due to the laboratory's capacity, almost all bacterial identification was limited to the genus level.

Antibiotic sensitivity test was performed by the broth microdilution method, and the minimum inhibitory concentration (MIC) was determined. Definitions of susceptibility, intermediate resistance, and resistance were made according to the National Committee for Clinical Laboratory Standards (NCCLS) susceptibility testing guidelines for bacterial species. Abbreviations for antibiotics are as follows: PCG, benzylpenicillin; ABPC, amoxicillin; MPIPC, mecillinam; CVA/AMPC, clavulanate/amoxicillin; CCL, cefaclor; CDTR, cefditoren; CFPM, cefepime; CEZ, Cefazolin; CTM, cefotiam; CTX, cefotaxime; CZOP, ceftizoxime; CPR, cefpirome; FMOX, flomoxef; IPM/CS, imipenem/cilastatin; MEPM, meropenem; GM, gentamicin; CAM, clarithromycin; CLDM, clindamycin; MINO, minocycline; CP, chloramphenicol; LVFX, levofloxacin.

A total of 38 *Streptococcus* spp. colonies were identified and subjected to sensitivity test for the following antibiotics: PCG, ABPC, CCL, CDTR, CFPM, CTM, CTX, CZOP, CPR, IPM/CS, MEPM, CAM, CLDM, MINO, CP, and LVFX. 7.9% – 42.1% of detected streptococcal colonies were resistant or showed intermediate resistance to penicillins (PCG, ABPC) and cephems (CCL, CDTR, CFPM, CTM, CTX, CZOP, CPR). Furthermore, 28.9% – 55.2% of these colonies were also resistant or showed intermediate resistance to macrolides (CAM, CLDM). A total of 9 CoNS spp. colonies were identified and subjected to sensitivity test for the following antibiotics:

PCG, MPIPC, ABPC, CCL, CFPM, CEZ, CTM, CZOP, FMOX, IPM/CS, CVA/AMPC, and FOM. All of the CoNS detected after HCT showed resistance or intermediate resistance to CFPM (100%), which was our first-choice antibiotic in empirical antibiotic therapy. CoNS also showed high degrees of resistance to penicillins (55.6% – 100%), *e.g.*, PCG, MPIPC, and ABPC. A total of 2 colonies of *Staphylococcus aureus* were identified and subjected to sensitivity testing; both colonies were methicillin-resistant *S. aureus* (MRSA). Sensitivity was limited to ABK, VCM, and TEIC only. One colony of *Pseudomonas* spp. was subjected to sensitivity test for the following antibiotics: ABPC, PIPC, CCL, CFPM, CEZ, CTM, CZOP, CAZ, CMZ, LMOX, IPM/CS, MEPM, AZT, GM, and AMK. This colony was resistant or showed intermediate resistance to ABPC, CCL, CFPM, CEZ, CTM, CMZ, LMOX, and AZT. This *Pseudomonas* spp. colony was sensitive to PIPC, CZOP, CAZ, IPM/CS, MEPM, GM, and AMK. Other bacteria identified were as follows: *Neisseria* spp. ($n = 9$), *Corynebacterium* spp. ($n = 5$), *Enterococcus* spp. ($n = 3$), and *Haemophilus parainfluenzae* ($n = 2$). All colonies were sensitive to most of the antibiotics tested.

The results of the present study indicated there were many antibiotic-resistant bacteria in the oral cavity after HCT, especially during the period in which the severity of oral mucositis reached its peak. Oral mucositis could be a potential route of

antibiotic-resistant infections. In our previous study, bacterial substitution mainly of CoNS for streptococci occurred frequently on the oral buccal mucosa after HCT [1]. High levels of antibiotic resistance in CoNS may explain bacterial substitution of CoNS for streptococci. On the other hand, streptococci with antibiotic resistance and/or intermediate resistance have also been detected at relatively high frequencies. Note that two colonies of MRSA and one colony of *Pseudomonas* spp. resistant to many types of antibiotic were detected. These observations are a reminder of the risk of appearance of MRSA and/or multi-drug-resistant *Pseudomonas aeruginosa* (MDRP), and the oral cavity may be a site of MRSA and/or MDRP growth. Further studies regarding the association with bacteremia/sepsis by DNA fingerprinting will yield additional insight into the clinical relevance of the present findings. Examination of specific patient-related or therapy-related risk factors for developing resistance may contribute to determination of personalized importance of oral care before and after HCT.

In conclusion, many antibiotic-resistant bacteria were detected in the oral cavity after HCT, especially during the period in which the severity of oral mucositis reaches its peak.

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