Short Communication

Antimicrobial effects of the saliva substitute, Oralbalance®, against microorganisms from oral mucosa in the hematopoietic cell transplantation period

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Abstract

Goals: The commercially available saliva substitute Oralbalance® has been reported to alleviate symptoms of post-radiotherapy xerostomia in head and neck cancer patients. Oralbalance® may also be effective for xerostomia in patients undergoing hematopoietic cell transplantation (HCT) with high-dose chemotherapy and total-body irradiation. However, HCT patients are severely compromised, and saliva substitute must therefore not promote infection. This study was performed to determine the effects of Oralbalance® on microbial species identified during HCT.

Patients and methods: Microbial identification of oral mucosa was performed in 28 patient undergoing HCT. The antimicrobial effects of Oralbalance® against bacteria and fungi detected in the HCT period were examined *in vitro*. Briefly, bacteria and fungi were spread on agar plates, and 0.1 g of Oralbalance® gel was applied (about ϕ1 cm). After incubation at 37°C for 24 h, the presence of a transparent zone of inhibition around Oralbalance® was observed.

Main results: Not only bacterial species constituting normal flora of the oral mucosa, but also those not usually constituting normal flora, *e.g.*, coagulase-negative *Staphylococcus*, were detected. A transparent zone was observed around Oralbalance® in all bacterial species examined. No transparent zone was observed for *Candida albicans*, but growth was inhibited in the area where Oralbalance® was applied.

Conclusions: Oralbalance® does not facilitate increases in microorganisms in the HCT period. Oral care with Oralbalance® does not promote infection in patients undergoing HCT.

Key Words: hematopoietic cell transplantation, xerostomia, saliva substitute, anti-microbial activity

Introduction

High-dose chemotherapy and total-body irradiation, which are performed as the conditioning regimen of hematopoietic cell transplantation (HCT), are associated with xerostomia. Xerostomia not only results in uncomfortable oral dryness, but also may cause the oral mucositis induced by chemotherapy and/or irradiation to be more severe, because patients with xerostomia lose one of the most important factors in protecting the oral mucosa, saliva, which contains many components of the innate and acquired defense systems, and not only eliminates microorganisms from the oral cavity [1,8] but also moderates mechanical contact between the teeth and oral mucosa. Indeed, we often see the development of ulcerative mucositis on mucosa in contact with dry teeth clinically. Oral care using saliva substitute may alleviate the symptoms induced by xerostomia.

Oralbalance®, which is a commercially available saliva substitute, has been reported to alleviate the symptoms of post-radiotherapy xerostomia in head and neck cancer patients [7,9]. Therefore, this product may be effective in HCT patients. However, as these patients are in a markedly compromised condition throughout the period of HCT, saliva substitute must not promote infection.

Therefore, the present study was performed to investigate the effects of the saliva substitute, Oralbalance®, on microbial species identified during HCT.

Patients and Methods

Identification of microorganisms from oral mucosa

A total of 28 patients undergoing HCT at Okayama University Hospital (M: 17, F: 11, 38.9 ± 16.6 y) were enrolled in this study. Microbial samples were obtained from oral mucosal swabs. Culture and identification of microorganisms were performed at the Central Clinical Laboratory of Okayama University Hospital. Microbial samples from mucosal swabs were plated onto brain heart infusion agar plate, and cultured in aerobic condition at 37°C. Identification of obtained colonies was performed by rapid ID 32 STREP API®, rapid ID 32 E API® or ID 32 GN API® identification kits (Japan bioMerieux, Tokyo, Japan) according to the manufacturer's instructions. Microbial identification was performed three times (first: day –7 ~ –1; second: day 0 ~ +7; third: day +8 ~ +14) for each patient (a total of 84 examinations in 28 patients).

Antimicrobial test of Oralbalance®

The antimicrobial effects of Oralbalance® against microbial species in the HCT period, with the exception of those detected only once throughout the total of 84 examinations of microorganisms, were examined *in vitro*. Antimicrobial tests were performed against the following standard strains: *Streptococcus sanguis* ATCC 10556, *Streptococcus salivarius* JCM 5707, *Neisseria mucosa* ATCC 19695, *Stomatococcus mucilaginosus* JCM 10910, *Staphylococcus epidermidis* NBRC 12993, *Staphylococcus aureus* FDA 209, and *Candida albicans* NBRC 1385. Aliquots of these bacteria and

fungi at concentrations of McFarland turbidity standard No. 0.5 were spread on brain heart infusion agar plates (Difco Laboratories, Detroit, MI, USA) or Sensitivity Disk Agar-N plates (Nissui Pharmaceutical, Tokyo, Japan). Then, 0.1 g (about ϕ1 cm) of Oralbalance® and an equal amount of Oralbalance® that had been pre-incubated at 90°C for 30 min to denature the antimicrobial enzymes contained in the gel were applied separately to the same plates. Tetracycline disks for antimicrobial ability test (BD Sensi-Disk Tetracycline 30; BD Biosciences, Franklin Lakes, NJ, USA) or paper containing 100 μg of amphotericin B (Invitrogen, Grand Island, NY, USA) were also applied to the plates as positive controls. After incubation at 37°C in air for 24 h, bacterial and fungal growth on the plates was examined.

Results

Microorganisms identified on the oral mucosa during HCT

The microorganisms identified on the oral mucosa during HCT are shown in Table 1. No samples were obtained during 13 of the 84 examinations because of the patients' conditions. Alpha- and γ-*Streptococcus* spp. (87.3% and 29.6%, respectively), *Neisseria* spp. (43.7%), and *Stomatococcus* spp. (23.9%), which are components of normal oral flora, were identified frequently. Coagulase-negative *Staphylococcus* spp., which are not constituents of the normal flora, were also identified frequently (46.5%). The fungus, *C. albicans*, was identified at a frequency of 5.6%. *S. aureus*, *Haemophilus influenzae*, *Enterococcus* spp., *Stenotrophomonas maltophilia*, *Bacillus* spp., and *Torulopsis glabrata* were identified at low frequencies (1.4% ~ 2.8%).

Antimicrobial ability of Oralbalance®

The results of antimicrobial tests on Oralbalance® against *S. sanguis*, *S. salivarius*, *N. mucosa*, *S. mucilaginosus*, *S. epidermidis*, *S. aureus*, and *C. albicans* are shown in Fig. 1. The presence of a transparent zone of inhibition was observed around Oralbalance® for all bacterial species examined. No such transparent zone was observed around heated Oralbalance®. With regard to fungi, although there was no transparent zone on *C. albicans* cultures, growth was inhibited in the area where Oralbalance® had been applied.

Discussion

The commercially available saliva substitute, Oralbalance®, showed antimicrobial activity against the bacterial species detected during HCT. Against fungi, although there was no transparent zone observed on *C. albicans* cultures, growth was inhibited in the area where Oralbalance® had been applied *in vitro*. These result suggested that Oralbalance® would not contribute to the infection in patients undergoing HCT.

There have been some reports regarding the relationships between the bacteria that constitute the normal oral flora, *e.g.*, *Streptococcus* species [6] and *Stomatococcus* species [2,3], and bacteremia in neutropenic patients. In the present study, bacteria not usually seen in the normal flora in the oral mucosa, *e.g.*, coagulase-negative *Staphylococci* (CNS), were also detected with high frequency during HCT, probably because bacterial substitution occurred due to the use of many antibiotics against infections in patients under neutropenic conditions. CNS is the bacterium isolated most frequently from blood cultures of febrile neutropenic patients [5]. The oral mucosa should be considered a potential source of organisms, including CNS, associated with bacteremia in immunocompromised patients [4]. In our *in vitro* studies, Oralbalance® did not facilitate an increase in such microorganisms related to bacteremia. The antibacterial effect of Oralbalance® is mainly due to antimicrobial enzymes of salivary origin, *i.e.*, lactoperoxidase, lysozyme, and lactoferrin. Indeed, no transparent zone was observed around heat-incubated Oralbalance®. As Oralbalance® does not contain any antibiotics, it does not contribute to the appearance of antibiotic-resistant bacteria.

In conclusion, the saliva substitute, Oralbalance®, would not facilitate an increase in microorganisms

during the HCT period.

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Table

Table 1. Microorganisms identified from the oral mucosa and detection frequency during HCT

Microorganism	Detection frequency (%)	Number (/71)
Bacterial componens of the normal flora		
α-*Streptococcus* spp.	87.3	62
γ-*Streptococcus* spp.	29.6	21
Neisseria spp.	43.7	31
Stomatococcus spp.	23.9	17
Bacteria not usually found in the normal flora		
Coagulase-negative *Staphylococcus* spp.	46.5	33
Staphylococcus aureus	2.8	2
Haemophilus influenzae	1.4	1
Enterococcus spp.	1.4	1
Stenotrophomonas maltophilia	1.4	1
Bacillus spp.	1.4	1
Fungi		
Candida albicans	5.6	4
Torulopsis glabrata	1.4	1

The microorganisms identified on the oral mucosa are shown. Microbial identification was performed three times (first: day –7 ~ –1; second: day 0 ~ +7; third: day +8 ~ +14) for each patient (total of 84 times for 28 patients). No samples were obtained during 13 of the 84 examinations because of the patients' conditions at these time points. Findings from 71 examinations are shown.

Figure legends

Fig. 1

Antimicrobial ability test of Oralbalance® against bacterial and fungal species isolated from patients during HCT. (A): *Streptococcus sanguis*, (B): *Streptococcus salivarius*, (C): *Neisseria mucosa*, (D): *Stomatococcus mucilaginosus*, (E): *Staphylococcus epidermidis*, (F): *Staphylococcus aureus*, and (G): *Candida albicans*. Appearance of the entire plate surface; Oralbalance® was applied to the upper right portion of the plates. Heat-incubated Oralbalance® was applied to the upper left portion of the plates. Tetracycline disks (A–F) or paper containing amphotericin B (G) were applied to the lower part of the plates. There was a transparent zone of inhibition around Oralbalance® for all bacterial strains examined. Although there was no apparent transparent zone in *C. albicans* cultures, growth was inhibited in the area where Oralbalance® had been applied.

Fig. 1