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Original Article

Potential effect of topical antibiotics administration in the oral cavity on the reduced number of bacteria entering the lower respiratory tract after oral cancer surgery

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Ventilator-associated pneumonia

Abstract *Background/purpose:* One of the causes of ventilator-associated pneumonia (VAP) is aspiration of oropharyngeal fluid containing pathogenic microorganisms into the lower respiratory tract. In this study, we aimed to investigate whether antibiotic ointment applied to the oral cavity can reduce the number of bacteria in the fluid on the cuff of a tracheal cannula. *Materials and methods:* Tetracycline ointment was applied intraorally once to a patient under endotracheal intubation by postoperative tracheostomy for oral cancer. The tetracycline concentrations in the oropharyngeal fluid and fluid on the cuff of tracheal cannula were determined by bioassay, and the total viable bacterial count was determined by delayed real-time polymerase chain reaction developed by the authors from before to 6 h after application. *Results:* A total of seven patients were enrolled. Very high antibiotic concentrations were maintained, ranging from 481 μg/ml to 2060 μg/ml in oropharyngeal fluid and from 267 μg/ml to 858 μg/ml in fluid on the cuff from 1 h to 6 h after application. Compared to the pre-application results, the inhibition rates of viable bacteria were 80.0–97.7% for oropharyngeal

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fluid and 47.6%—91.9% for fluid on the cuff at 1—6 h after application, indicating that antibiotic ointment can inhibit bacteria entering the lower respiratory tract for a long period of time in intubated patients.
Conclusion: Oral application of antibiotic ointment reduced the number of bacteria entering the lower respiratory tract, suggesting that it may be useful in the prevention or treatment of VAP.

Introduction

The saliva contains many species of bacteria and fungi. Although salivary bacteria do not enter the lower respiratory tract in healthy individuals with preserved swallowing function, aspiration of salivary bacteria can cause pneumonia in patients after highly invasive surgery such as that for head and neck cancer, upper gastrointestinal tract cancer, and lung cancer, and in elderly patients with impaired oral function. The number of commensal bacteria in the oral cavity is maintained at a certain level in healthy individuals through saliva secretion and swallowing, but the number of bacteria in the saliva increases in patients with impaired oral function and has been reported to be 100-times higher in endotracheally intubated patients than in healthy individuals.[1,2]

Ventilator-associated pneumonia (VAP) is a type of pneumonia that occurs after 48 h of endotracheal intubation and is a serious postoperative complication presenting in the intensive care unit with a low incidence but high fatality rate.[3] Daily oral care with 0.12% chlorhexidine (CHX) is recommended to prevent VAP in addition to systemic administration of antibiotics.[4] In Japan, however, the use of 0.12% CHX on mucous membranes is contraindicated, and oral care methods for the prevention of VAP have not been established.

In a randomized controlled trial, Funahara et al. found that intraoral topical tetracycline ointment administered every 6 h for 48 h after surgery significantly reduced surgical site infection rates in patients undergoing oral cancer surgery with reconstructive surgery with free flaps.[5] The purpose of the present preliminary study was to examine whether topically administered oral antibiotic ointments could suppress the bacterial counts not only in the saliva but also in the fluid on the cuff of a tracheal intubation tube. If this study shows that topical administration of antibiotics into the oral cavity is effective in suppressing bacterial counts on the cuff, we would like to conduct clinical studies to determine whether this method is effective in the prevention and treatment of VAP in the future.

Material and methods

Participants

The participants were patients undergoing oral surgery who were managed postoperatively under intubation with a tracheotomy at the Department of Oral and Maxillofacial Surgery, Nagasaki University Hospital or Hiroshima University Hospital between April 2023 and December 2023. Patients who could not be sampled due to poor general condition on the day after surgery, or patients for whom only a small amount cuff suprapubic fluid was collected due to minimal aspiration, were excluded.

Sample collection

At noon the day after the surgery, the oropharyngeal fluid and supraglottic cannula cuff fluid samples were first collected. Collection was done with a 10-ml syringe, and cases in which only 0.5 ml or less was collected due to low fluid level on the cuff were excluded. Next, 2 g of tetracycline ointment (Achromycin® Ointment 3%: Sun Pharma Japan Limited, Tokyo, Japan) was applied to the dorsum of the tongue opposite the surgical site. The oropharyngeal fluid and tracheal cannula cuff fluid were collected at 1, 2, 3, and 6 h after application. Samples were stored frozen until measurement.

Measurement of tetracycline concentration in the oropharyngeal fluid and fluid stored on the cuff

A paper disc bioassay was performed to determine the concentration of tetracycline in the samples. *Staphylococcus aureus* (clinical isolate) was inoculated with BHI agar medium adjusted to McFarland 0.5. Discs soaked in the sample were placed on the agar medium and incubated in an incubator set at 37 °C for 48 h, and the antibiotic concentration was calculated from the inhibition circle generated.

Delayed real-time polymerase chain reaction

Samples were diluted 3-fold in saline and sonicated to remove viscosity. Then, we performed our newly developed delayed real-time polymerase chain reaction (DR-PCR) to calculate the number of viable bacteria in the sample and the inhibition rate of viable bacteria by tetracycline.[6] In this method, only viable bacteria are quantified from a sample in which viable and dead bacteria are mixed, and real-time PCR is performed after growing only viable bacteria in a liquid culture for a certain period of time. Sample ratios of 10:0, 5:5, 1:9, and 0:10 of viable bacteria: dead bacteria were prepared. Then, 100 μL of these mixed samples of viable and dead bacteria were incubated in 3 ml

of BHI liquid medium at the logarithmic growth phase. A standard line was prepared with the vertical axis representing the growth rate and the horizontal axis representing the logarithm of the percentage of viable bacteria, and the percentage of viable bacteria was calculated for samples with an unknown number of viable bacteria. The reaction conditions, primer, and other details of real-time PCR were previously reported.[7]

Based on the percentage of viable bacteria, the percentage of inhibition of viable bacteria by tetracycline ointment application was calculated using the following formula.

Inhibition rate (%) = 100 (1-Mt·a/M_0)

a: percentage of viable bacteria calculated by DR-PCR (%)/100;
Mt: bacterial count after incubation for t time;
M_0: bacterial count at the beginning of incubation.

Ethics

The study was performed in accordance with the 2013 Declaration of Helsinki and approved by the Clinical Research Review Board (CRB) of Nagasaki University (#CRB7180001). This study was conducted as a specific clinical study in accordance with the Clinical Research Law enacted in April 2018 in Japan. Written informed consent was obtained from all the participants. The study protocol was registered in the Japan Registry of Clinical Trials (jRCT) on April 14, 2023 (jRCTs071230007).

Results

Participant characteristics

Ten participants consented and were enrolled in the study: two were in poor general condition and could not be sampled due to the need for management in the intensive care unit for several days; one could not be sampled due to little aspiration of fluid on the cuff. Consequently, seven patients were included in the study (Table 1).

There were six males and one female (mean age, 67.1 [48–84] years), with four cases of tongue cancer, two cases of maxillary gingival cancer, and one case of floor of mouth cancer. All of them underwent highly invasive surgery with free flap reconstruction, and the mean operative time was 682 min.

Tetracycline concentration in the oropharyngeal fluid and fluid stored on the cuff after topical application of tetracycline ointment

In the oropharyngeal fluid, a high mean concentration of antibiotic (2060 μg/ml) was detected immediately after application. Although the concentration decreased over time, it remained very high (481 μg/ml) after 6 h (Fig. 1). The minimum inhibitory concentration of tetracycline against oral commensal bacteria is approximately 0.5 μg/

Table 1 Characteristics of participants.

No	Age	Sex	Primary site	Hemoglobin	Leukocytes	Lymphocytes	Albumin	Creatinine	% VC	FEV1.0%	Surgery	Operation time (min)	Blood loss (g)	Intravenous antibiotics
1	84	female	tongue	13.2	6700	1540	4.0	0.74	78.1	49.1	TE, ND, subtotal glossectomy, ALT	662	217	ABPC/SBT 12 g/day
2	67	male	tongue	11.9	5600	1820	3.6	0.66	108.5	80.7	TE, ND, subtotal glossectomy, ALT	734	1165	ABPC/SBT 9 g/day
3	74	male	maxillary gingiva	13.7	7870	1120	3.5	0.61	120.0	82.8	TE, ND, subtotal maxillectomy, ALT	696	760	ABPC/SBT 4.5 g/day
4	69	male	floor of the mouth	12.9	4790	1740	4.4	1.05	87.6	78.2	TE, ND, marginal mandibulectomy, ALT	630	152	ABPC/SBT 7.5 g/day
5	48	male	tongue	15	4660	1920	3.8	0.68	91.6	82.3	TE, ND, hemiglossectomy, ALT	611	451	ABPC/SBT 4.5 g/day
6	69	male	maxillary gingiva	14.2	9400	2300	4.7	0.78	96.0	76.2	TE, ND, subtotal maxillectomy, segmental mandibulectomy, SO, LD	788	863	ABPC/SBT 4.5 g/day
7	59	male	tongue	11.2	5510	1480	3.3	0.56	100.0	78.5	TE, ND, hemiglossectomy, ALT	650	345	ABPC/SBT 4.5 g/day

Abbreviation: % VC: % vital capacity; FEV 1.0%: forced expiratory volume 1.0(sec) %; TE: tracheotomy; ND: neck dissection; ALT: anterolateral thigh flap; SO: scapular osteocutaneous flap; LD: latissimus dorsi musculocutaneous flap.

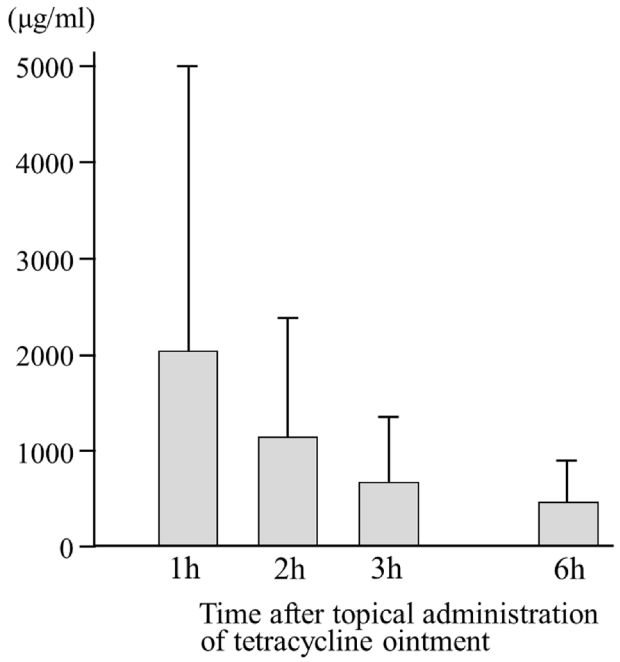

Figure 1 Concentration of tetracycline in the oropharyngeal fluid. The highest concentration of 2060 μg/ml was observed after 1 h of application, and a very high concentration of 481 μg/ml was also observed after 6 h.

ml, indicating that sufficient antibiotic concentration is maintained for 6 h after application.

The antibiotic concentration in the fluid on the cuff was 364 μg/ml at 1 h after application and reached its highest value of 858 μg/ml at 2 h, followed by a slight decrease to 267 μg/ml at 6 h, indicating that the concentration remained high enough to inhibit bacterial growth (Fig. 2).

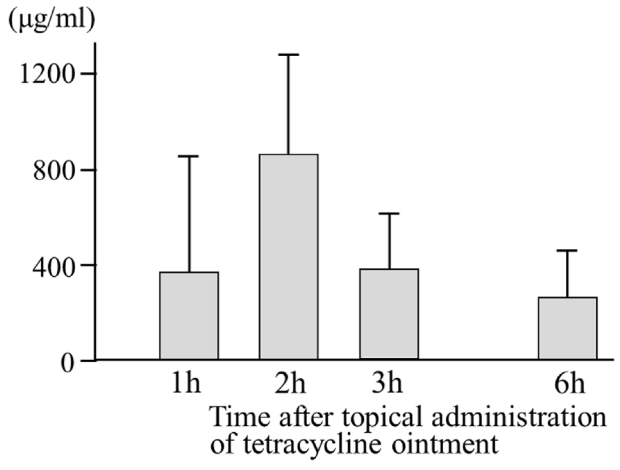

Figure 2 Concentration of tetracycline in the fluid on the cuff. After 1 h of application, the concentration was as high as 364 μg/ml, and after 2 h, the highest concentration was 858 μg/ml. Six hours later, the concentration remained high at 267 μg/ml.

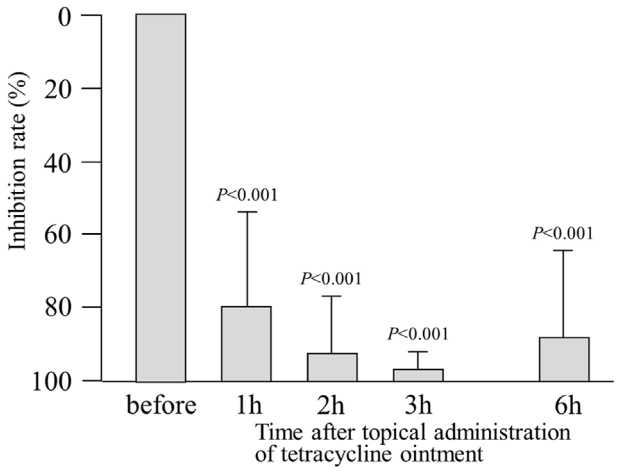

Figure 3 Inhibition rate of bacteria in the oropharyngeal fluid. Most bacteria were dead, with an inhibition rate of 80—97.7% at 1—6 h after application.

Inhibition rate of bacteria in the oropharyngeal fluid and fluid stored on the cuff after application of topical tetracycline ointment

The inhibition of bacteria in oropharyngeal fluid after topical administration of tetracycline averaged 80.0—97.7% from 1 h to 6 h, indicating that most bacteria were dead (Fig. 3). There was a significant decrease at 1, 2, 3, and 6 h, compared to before topical tetracycline administration ($P < 0.01$).

For bacteria in the fluid on the cuff, the mean percentages of inhibition after 1, 2, 3, and 6 h were 47.6%, 80.5%, 87.4%, and 91.9%, respectively, all indicating that many bacteria were dead. After 1 h, there was no significant difference compared to before topical administration ($P = 0.065$), but the percentage of dead bacteria increased

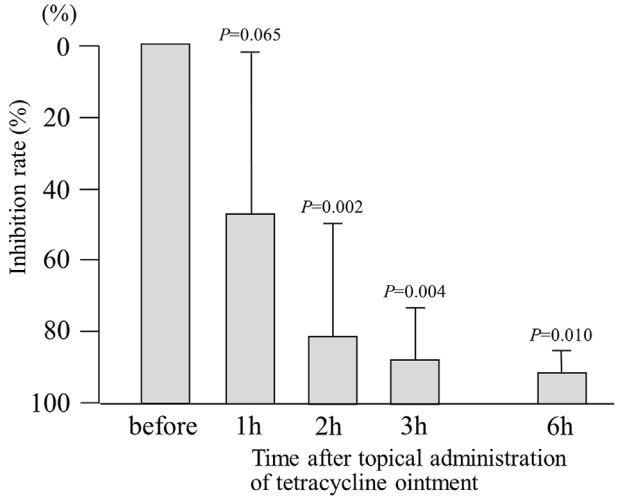

Figure 4 Inhibition rate of bacteria in the fluid on the cuff The inhibition rate was 47.6% at 1 h after application but increased over time to 91.9% at 6 h.

over time, and after 2, 3, and 6 h, the number of viable bacteria was significantly lower (Fig. 4).

Discussion

This study showed that tetracycline ointment applied to the oral cavity of patients who were intubated by tracheostomy after oral cancer surgery significantly reduced the number of bacteria not only in the oropharyngeal fluid but also in the fluid on the cuff of the intubation tube up to 6 h later.

Aspiration pneumonia can occur in intubated patients and elderly patients with impaired swallowing function. This is because aspiration is more likely to occur in patients with impaired oral function and because salivation and swallowing, which are self-cleansing functions of the oral cavity, are impaired, resulting in a significant increase in the number of oral bacteria. Patients on ventilators are at a high risk for VAP due to the influx of oropharyngeal reservoirs along the endotracheal cannula into the lower airway, combined with poor general condition.[3] Caroff et al. stated that aspiration of subglottic luminal secretions is one of the few interventions proven to significantly reduce the incidence of VAP, indicating that reducing pathogenic microorganisms in the supraglottic cuff effluent can prevent VAP.[8]

Daily oral care with 0–12% CHX is recommended by IHI to prevent VAP.[4] In Japan, however, the use of a drug on the mucous membranes, including in the oral cavity, has been banned because of reports of anaphylactic shock associated with this approach. CHX at low concentrations can be used as a mouthwash, but it does not exert antibacterial activity against oral bacteria at those concentrations.[9] There is an attempt to prevent VAP by oral care using PV-I instead of 0.12% CHX. According to the systematic review and meta-analysis by Labeau et al.,[10] CHX application may be effective, whereas the effect resulting from povidone-iodine remains unclear. Zeydi et al. reported that more rigorously designed randomized clinical trials and further evidence are required to provide better evidence on the use of povidone iodine as a suitable choice for preventing VAP among adult patients admitted to the ICU.[11]

Several attempts have been made to prevent VAP by topical administration of antibiotics to the oral cavity and gastrointestinal tract. Oropharyngeal colonization has been identified as a risk factor for the development of VAP caused by enteric gram-negative bacteria and *P. aeruginosa*. Schnabel et al. recommended topical application of paste containing amphotericin B, polymyxin E, and tobramycin in the oropharynx (selective oral decontamination: SOD), combined with intravenous cefotaxime and application of amphotericin B, polymyxin E and tobramycin in the stomach (selective decontamination of the digestive tract: SDD) every 6 h to prevent VAP.[12] Cames et al. also administered colistin sulfate, tobramycin, and amphotericin B intraorally and into the gastric tube every 6 h until 24 h after extubation, after oral care with 0.5% CHX mouthwash; in addition, mupirocin ointment was administered three times a day for 5 days. The authors concluded that these procedures reduced the risk of infectious complications including VAP.[13] In contrast, Bonten et al. reviewed 27 prospective randomized studies and six meta-analyses, concluding that SDDs were not associated with improved patient survival, reduced ventilation duration or intensive care unit stay, or reductions in antibiotic use. They advocated that as long as the benefits of SDD have not been established, its routine use for mechanically ventilated patients is not advised, given antimicrobial resistance risks.[14] IHI recommends the following five measures (Ventilator Bundle) to reduce the risk of complications in ventilator patients: 1) Elevation of the head of the bed to between 30 and 45°, 2) daily sedative interruption and daily assessment of readiness to extubate, 3) peptic ulcer disease prophylaxis, 4) deep venous thrombosis prophylaxis (unless contraindicated), and 5) daily oral care with CHX.[4] However, neither SDD nor SOD is recommended as a preventive measure.

This study showed that application of tetracycline ointment to the oral cavity can transfer high concentrations of antibiotics and strongly suppress the number of bacteria in the fluid on the cuff. However, prolonged application of antibiotics is not appropriate because it may induce bacterial resistance. Therefore, when this method is applied clinically, it is desirable to select an effective drug for the bacteria identified from the sputum bacteriological examination, and to administer it topically only during the period when antibiotics are being administered systemically.

There are several limitations to this study. First, this is a preliminary study of a small number of cases, and it is not clear whether the results obtained can be generalized. Second, the endpoint is the total bacterial count in the fluid on the cuff, not the pathogenic microorganisms. In some cases, despite a reduction in bacterial count, disease-causing bacteria may remain present and active; thus, antibiotic selection should be carefully reviewed in the clinical practice. In addition, since the development of VAP involves not only bacteria entering the lower respiratory tract but also various other factors such as the host immunity, evidence from intervention studies are required to determine whether local administration of antibiotics reduces the risk of VAP. However, this study is first to demonstrate that antibiotics administered topically to the oral cavity can adequately suppress bacteria in the fluid on the cuff. We believe that future large-scale interventional studies are needed to verify the efficacy of this method for the prevention or treatment of VAP.

Tetracycline ointment applied intraorally reduced the number of bacteria entering the lower respiratory tract for at least 6 h, suggesting that it may be useful in the prevention or treatment of VAP.

Declaration of competing interest

The authors declare no conflict of interest. This research received no external funding.

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