Int. J. Radiation Oncology Biol. Phys., Vol. 50, No. 2, pp. 343–352, 2001

0360-3016/01/$–see front matter

PII S0360-3016(01)01444-4

CLINICAL INVESTIGATION **Head and Neck**

MUCOSITIS REDUCTION BY SELECTIVE ELIMINATION OF ORAL FLORA IN IRRADIATED CANCERS OF THE HEAD AND NECK: A PLACEBO-CONTROLLED DOUBLE-BLIND RANDOMIZED STUDY

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Purpose: The aim of the study was to test the hypothesis that aerobic Gram-negative bacteria (AGNB) play a crucial role in the pathogenesis of radiation-induced mucositis; consequently, selective elimination of these bacteria from the oral flora should result in a reduction of the mucositis.

Methods and Materials: Head-and-neck cancer patients, when scheduled for treatment by external beam radiation therapy (EBRT), were randomized for prophylactic treatment with an oral paste containing either a placebo or a combination of the antibiotics polymyxin E, tobramycin, and amphotericin B (PTA group). Weekly, the objective and subjective mucositis scores and microbiologic counts of the oral flora were noted. The primary study endpoint was the mucositis grade after 3 weeks of EBRT.

Results: Seventy-seven patients were evaluable. No statistically significant difference for the objective and subjective mucositis scores was observed between the two study arms ($p = 0.33$). The percentage of patients with positive cultures of AGNB was significantly reduced in the PTA group ($p = 0.01$). However, complete eradication of AGNB was not achieved.

Conclusions: Selective elimination of AGNB of the oral flora did not result in a reduction of radiation-induced mucositis and therefore does not support the hypothesis that these bacteria play a crucial role in the pathogenesis of mucositis. **© 2001 Elsevier Science Inc.**

Head-and-neck tumors, Radiation therapy, Mucositis, Oral flora, Antibiotics.

INTRODUCTION

Mucositis of the oral mucosa is a cumbersome acute side effect in the case of radiation and/or chemotherapy of tumors in the head and neck (1–4). Mucositis has a major impact on patients' well-being and can lead to treatment interruptions, with ultimately potentially detrimental effects on tumor control (and survival) (5–14). So far, there is no generally accepted and effective means to prevent or reduce treatment-induced mucositis. The most commonly suggested measures to alleviate part of the consequences of the treatment-induced mucositis are frequent oral rinsing with, for example, saline and maintaining good dental hygiene (3, 15–21). Unfortunately, none of the frequently used oral rinses such as chlorhexidine, benzydamine, and sucralfate seem to be effective when tested in a randomized trial (15, 22–34).

With regard to the pathogenesis of mucositis, it is felt that apart from loss of the cellularity in the mucous membranes as a direct consequence of the toxic agent, microorganisms, especially the aerobic Gram-negative bacteria (AGNB), can come into play and have an important impact on the progression of mucositis beyond the phase of erythema (35–45). Sonis describes the evolution of mucositis biologically as a complex of 4 consecutive interacting phases (44). In one of the latter phases, i.e., during the bacterial or ulcerative phase with pseudomembrane formation (phase 3), the damaged mucosa starts to be colonized with aerobic Gram-negative bacteria; the subsequent release of endotoxins and the stimulation thereby of (more) cytokine release leads to further amplification of the mucositis. Several studies were therefore conducted aimed at reducing the amount of the potential pathogenic aerobic Gram-negative bacteria (and yeast) from the oral flora. However, none of the preliminary studies proved to be efficacious, either in reducing the total bacterial count or in preventing (the latter phases of) mucositis (22–26). Foote *et al*. (26) even demonstrated a detrimental effect of chlorhexidine on the oral mucosa. Spijk-

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Accepted for publication 11 December 2000.

ervet *et al.* (35, 36), on the other hand, were the first to recognize the potential benefit of preventing radiation-induced mucositis by selective elimination of the aerobic Gram-negative bacteria from the oral flora using lozenges containing a combination of 3 nonabsorbable antibiotics: polymyxin E, tobramycin, and amphotericin B (PTA). Based on the encouraging results of the pilot study with the PTA lozenges, we initiated in 1993 a single-institution, randomized double-blind, placebo-controlled trial using the same three antibiotics (PTA) in an oral paste to test the hypothesis that selective elimination of potential pathogenic microorganisms from the oral flora (aerobic Gram-negative strains and yeasts) leads to reduction of the severity of radiation-induced mucositis.

METHODS AND MATERIALS

All patients with a biopsy-proven malignant tumor of the head and neck, to be treated by either primary or postoperative external beam radiation therapy (EBRT) according to the treatment guidelines of the Rotterdam Head and Neck Cooperative Group, were eligible for this randomized trial. Excluded were those patients with a WHO performance score of 3 or 4 and those treated previously by EBRT of the head and neck or with adjuvant chemotherapy or systemic antibiotics less than 2 weeks before the planned EBRT. Between July 1993 and December 1997, 114 patients were randomized. After randomization, 37 patients refused further participation in the trial, either after the first (test) application of the paste ($n = 26$) or within four weeks after start of EBRT ($n = 11$). In total, 77 patients (38 placebo and 39 PTA) fulfilled the inclusion criterion of a minimum of 4 weeks of paste application and at least 3 weeks of EBRT and were therefore evaluable.

Patients who stopped after 4 study weeks ($n = 59$) were found to be equally distributed between the two study groups, i.e., 30 (PTA) vs. 29 (non-PTA). The main reasons for stopping were bad taste of the oral paste and/or the unpleasant sensation of the paste texture in the mouth. These late "dropouts" did not differ significantly from the patients who completed the study with respect to tumor characteristics, radiation therapy parameters, mucositis grade, and intensity and duration of pain, and were therefore included as protocol compliant patients.

Table 1 summarizes the pretreatment characteristics of the study patients. The largest subsets of patients (62%) were treated for tumors in the oral cavity and oropharynx. In 67% of the cases, the histology was squamous cell carcinoma. Forty-eight patients (62%) underwent surgery of the primary tumor and were irradiated postoperatively. All patients were simulated in supine position. In the majority of cases, conventional EBRT treatment techniques were used, consisting of two lateral opposed photon beams (4 or 6 MV) for the primary and the upper neck, and, if appropriate, an abutting low anterior field with or without midline shielding. The posterior neck region was taken off-cord after 40–46 Gy and supplemented with 10 MeV electrons if appropriate. Conventional fractionation schedules of 1 fraction per day and 5 fractions of 2 Gy per week were used. After an EBRT dose of 46 Gy, in 9 patients interstitial radiotherapy was implemented in the treatment as a booster dose to the primary. In the case of interstitial radiotherapy, the start of the brachytherapy marked the end of the evaluation period with regard to the (mucositis) study parameters. Before randomization, informed consent had to be obtained.

In both groups an adhesive mouth paste containing hypromellose (16%) in a mixture of white paraffine (57%) and paraffine (24%) was used as a vehiculum. Taste was improved with saccharin sodium (0.67%) and peppermint oil (0.33%). The active PTA paste contained 0.2% Polymyxin E sulfate (Colistin sulfate), 0.18% Tobramycin and 1% Amphotericin B. The placebo paste was made identical in appearance to the PTA paste by adding cellulose (1.4%), amaranth (E123), and tartrazine (E102) to the vehiculum. One gram of PTA paste contained 2 mg Polymyxin E sulfate, 1.8 mg Tobramycin, and 10 mg Amphotericin B. Labeling of the paste was blinded.

Patients were randomized to PTA or placebo paste. Both groups were instructed to apply 1 gram of paste 4 times a day starting 3 days before EBRT, and the application was continued until the end of EBRT. All patients were seen weekly by the same trained oral hygienist (A.M.G.T.) for routine oral care and assessment of the mucosa score. Mucositis-related study parameters were assessed starting 1 week before radiation until 2 weeks after completion of EBRT. The primary study endpoint was the mucositis grade after 3 weeks of EBRT. Mucositis grade was expressed on a 5-point scale using the van der Schueren scoring system, as follows: Grade 0, no effects on mucosa; Grade 1, slight erythema; Grade 2, pronounced erythema; Grade 3, patchy mucositis; and Grade 4, confluent mucositis. As subjective study parameters, the intensity of pain due to the mucositis was scored on a 5-point scale, in which Grade 1 corresponded to no pain and Grade 5 to the most severe pain level. The duration of pain was expressed on a 3-point scale (no pain, occasionally painful, or continously painful mucosa).

Bacterial cultures were taken weekly from 1 week before the start of EBRT until 2 weeks after completion of the treatment. For this purpose the mouth was rinsed with 5 cc of isotonic saline solution for 30 s; the mouthwash was collected in a special sterile container. The sample was inoculated on two bloodagar plates, on a similar bloodagar plate with 5% heated sheep erythrocytes (chocolate plate), and on a Sabouraudagar plate. One bloodagar plate was incubated anaerobically; the chocolate plate was incubated at 5% CO_2, and the two other plates were incubated aerobically. After overnight incubation, the broth was subinoculated on a bloodagar plate and a chromagar plate. Colonies with the appearance of "normal respiratory pathogens" such as *H. influenzae*, pneumococci, hemolytic streptococci, and *S. aureus*, coliforms, *Pseudomonas*, and other nonfermen-

Table 1. Patient characteristics

	Placebo number	PTA number
Age range; median	32–79; 57.4 years	20–78; 55.7 years
Gender: male / female	20/18	25/14
Tumor site		
Oral cavity	12	11
Oropharynx	12	14
Nasopharynx	2	1
Hypopharynx	2	2
Larynx	2	2
Maxillary sinus	2	0
Salivary glands	5	6
Miscellaneous	1	3
T-stage (TNM '97)		
T1	4	6
T2	15	12
T3	9	8
T4	6	9
Tx	4	4
N-stage (TNM '97)		
N0	17	19
N1	3	3
N2a	4	4
N2b	8	8
N2c	3	3
N3	1	2
Nx	2	0
Histology		
Squamous cell carcinoma	27	27
Adenocystic carcinoma	3	2
Miscellaneous / unknown	8	10
Surgery primary		
None	16	13
Local excision	8	6
Commando	8	11
Laryngectomy	1	2
Parotidectomy	4	6
Other	1	1
Neck dissection		
None	18	13
Unilateral	18	22
Bilateral	2	4
EBRT		
46–50 Gy	6	8
60 Gy	11	11
70 Gy	21	20
OTT: range; mean	31–66; 48 days	25–64; 46 days
Bilateral	28	28
Unilateral	11	11
IRT		
Number of patients	4	5
Dose in Gy	24 or 26 Gy	21,24, or 26 Gy

Abbreviations: EBRT = external beam radiation therapy; IRT = interstitial radiotherapy.

tative Gram-negative rods, as well as yeasts, were identified and quantified (colony-forming units per ml saliva: cfu/ml). Colonies belonging to the normal indigenous apathogenic oral flora, such as the viridans streptococci and the anaerobic microorganisms, were not further specified. However, for each oral specimen the total amount of microorganisms was assessed and expressed in cfu per ml saliva.

A number of radiotherapy-related parameters were analyzed: i.e., cumulative EBRT dose given in a particular overall treatment time, fraction size, estimated percentage of oral mucosa surface irradiated, and estimated percentage of major salivary gland volume irradiated. The estimated total amount of oral mucosa (100%) was defined as the area depicted on lateral simulation films bounded by the hard and soft palate (upper and posterior boundary) and the inferior border of the mandible and the teeth (lower and anterior boundaries) (Fig. 1). For each

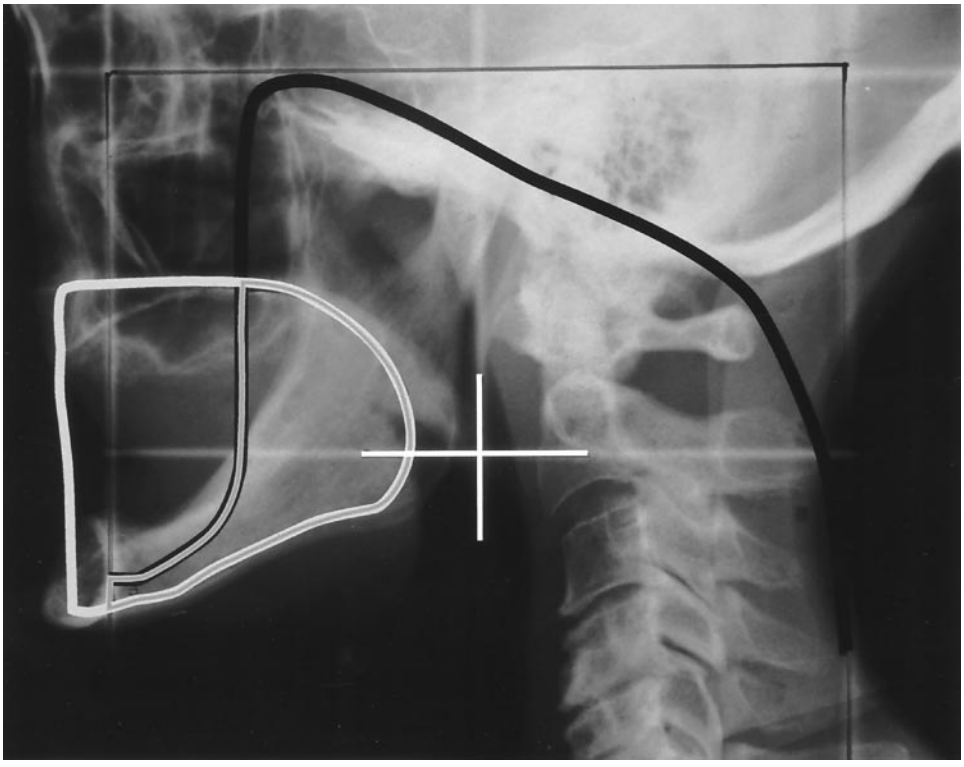

Fig. 1. Lateral simulation film with the total oral mucosal surface outlined with a white line and the irradiated mucosal surface indicated by the gray line. The black line indicates the boundaries of the lateral portal.

individual treatment field of every patient, the part of the oral mucosa within the treatment portals was delineated on the lateral simulation films and expressed as a percentage of the total oral mucosa area.

Additionally, we constructed, as a rough guide to estimate the total irradiated major salivary gland volume, a CT-based template by using the lateral beam's-eye–view projections of 10 CT-delineated (of randomly chosen patients) parotid glands and submandibular glands. We estimated the percentage of the total irradiated surface ("volume") of the delineated major salivary glands within the treatment portals for each treatment field of each patient, relative to the template contour (Fig. 2).

Statistical considerations

The sample size of the study was based on the data of the pilot study by Spijkervet *et al*. (36). Using a two-sided alpha of 5% and a power of 90% (beta = 0.10), 48 patients in both groups are necessary to detect a difference of one grade in mucositis score between the PTA and placebo group. Only 77 patients fulfilled the study criteria and were evaluable. With this number of patients, the power of the study is 83%, which is considered to be sufficient to draw conclusions. Mucositis scores and pain scores were compared with the Mann-Whitney test. The microbiologic data were compared with the chi-squared test.

RESULTS

Mucositis grade and pain scores

Table 2 summarizes the mucositis grades and pain scores. No significant differences were found between the two

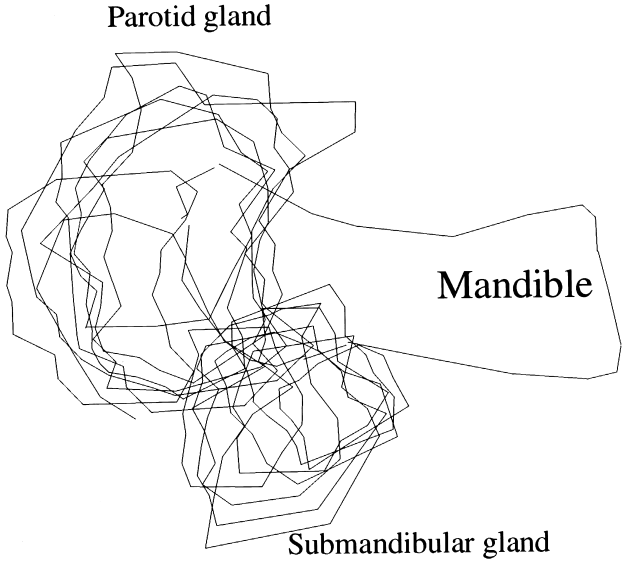

Fig. 2. Template to estimate the irradiated parotid and submandibular gland "volumes." The template is composed of 10 beam's-eye–view projections of the major salivary glands. The part of the salivary glands within treatment portals is expressed as a percentage of the standardized surface of the template.

Table 2. Mucositis study parameters

Study parameter	PTA	Placebo	*p* value
Mucositis grade Week 4			
G0	13	8	
G1	11	8	0.33
G2	7	10	
G3	3	8	
G4	5	4	
Max. mucositis grade			
G0	7	4	
G1	6	6	
G2	11	10	0.75
G3	4	12	
G4	11	6	
Moment of max. mucositis			
Week 0	7	6	
Week 1	9	9	
Week 2	12	9	0.92
Week 3	2	10	
Week 4	9	4	
Pain intensity at Week 4			
1	12	8	
2	20	18	
3	3	8	0.37
4	3	4	
5	1	0	
Max. pain intensity			
1	6	5	
2	17	16	
3	7	5	0.72
4	5	11	
5	4	1	
Moment of max. intensity			
Week 1	10	9	
Week 2	17	15	
Week 3	3	4	0.71
Week 4	7	9	
Week 5	2	1	
Pain duration at Week 4			
No pain	12	7	
Sometines pain	18	18	0.36
Continuous pain	9	13	
Maximum pain duration			
No pain	6	6	
Sometims pain	18	9	0.11
Continuous pain	15	23	
Moment of max. duration			
Week 0	8	9	
Week 1	20	13	0.44
Week 2	11	15	
Week 3	0	1	

study groups for these study parameters with *p* values (Mann-Whitney test) of 0.75, 0.11, and 0.72 for the maximum mucositis grade, maximum pain duration, and maximum pain intensity, respectively. Also, weekly evaluation of these parameters showed no significant differences between the PTA and placebo groups. Overall a Grade 3 or 4 mucositis occurred in 43% of the patients and was accompanied with moderate or severe pain, i.e., pain intensity of Grade 3 or higher by 31% of the patients. No difference was found regarding the time frame in which the maximum mucositis occurred; there was no difference in the duration of the mucositis, either. In both groups the most severe objective and subjective symptoms occurred from the fifth study week (corresponding to the fourth radiation week at a cumulative EBRT dose of 30–40 Gy) and remained at a plateau until the eighth study week.

Microbiology

The microbiology content of the oral specimens was divided into 6 groups: the normal indigenous flora, two groups of anaerobic Gram-negative strains (i.e., a group of coliforms and a group of *Pseudomonas* species), *Staphylococcus aureus*, *Candida* species, and a miscellaneous group. Table 3 summarizes the weekly microbiologic results for both treatment groups. Microbiologic analysis before start of treatment revealed an AGNB carriage rate of 33.3% for the PTA group and 24% for the placebo group. A statistically significant reduction of the carriage rate of coliforms was found in the PTA group from study Week 1 to 6 (Fig. 3); on average, in 13% of patients in the PTA group, coliforms were isolated, in contrast to 34% in the placebo group. *Pseudomonas* species were initially isolated by only 3 patients of the PTA group and persisted in 1 patient.

Mucosa and major salivary glands

For 74 patients the treatment portals were analyzed for the total area of irradiated oral mucosa and irradiated salivary gland "volume." The estimated total irradiated oral mucosal surface ranged from 0–100%, with a mean of 65%. In the great majority of patients (90%), at least 30% of the oral mucosa was within the radiation portals. For both treatment groups, a significant correlation was found between the irradiated oral mucosal surface (<25%, 25%–75%, >75%) and the maximum grade of mucositis ($p = 0.0001$, Kruskall Wallis test), the maximum duration of pain ($p = 0.004$), and maximum pain intensity ($p = 0.006$).

A variable part (9–100%, mean 77%) of the parotid glands was encompassed by the treatment portals; the submandibular glands were almost entirely (mean 93%) included within the treatment portals. Neither in the placebo nor in the PTA group could a correlation be found between the irradiated parotid gland surface ("volume") and the mucositis-related study parameters with *p* values of 0.55 for the maximum mucositis grade, 0.80 for the pain duration, and 0.88 for the pain intensity (Spearman test).

The EBRT dose groups 50 Gy, 60 Gy, and 70 Gy did not correlate with the maximum mucositis grade, either in the placebo or the PTA group ($p = 0.87$). Also the pain intensity ($p = 0.89$) and pain duration ($p = 0.48$) failed to show any correlation with the cumulative EBRT dose.

DISCUSSION

Mucositis is a common (acute) side effect of radiation therapy for head-and-neck tumors, because the oral mucosa is frequently incorporated in the beam portals. The most severe grades of mucositis are seen with intense schedules: well over 70% of patients treated with accelerated fraction-

Table 3. Oral flora content of potential pathogens per week

Species	Week 0 (38/39*)	Week 1 (38/39)	Week 2 (38/39)	Week 3 (37/39)	Week 4 (37/39)	Week 5 (36/35)	Week 6 (31/27)	Week 7 (22/23)	Week 8 (11/14)
Coliforms									
Placebo	8†	12	12	13	13	14	11	6	3
PTA	10	4	4	4	6	5	2	3	5
p value	0.65	0.03	0.02	0.01	0.06	0.01	0.005	0.27	0.31
Pseudomonas									
Placebo	0	1	2	3	2	1	0	0	0
PTA	3	1	0	2	1	0	1	1	1
p value	0.34	0.99	0.15	0.62	0.57	0.31	0.32	0.32	0.32
S. Aureus									
Placebo	0	2	3	3	3	4	3	3	1
PTA	3	2	1	0	1	1	1	0	0
p value	0.08	0.94	0.29	0.04	0.31	0.16	0.08	0.20	0.31
Miscellaneous									
Placebo	5	5	2	2	6	3	4	0	4
PTA	5	3	3	1	1	1	1	2	0
p value	0.94	0.38	0.67	0.55	0.04	0.31	0.16	0.16	0.04

* 38/39 = total number of patients in placebo/PTA group.
† Absolute number of patients in whom the specified microorganism was isolated.

ation schedules have been reported to experience confluent mucositis (9, 11, 46–57). In conventional fractionated radiotherapy, the first signs of mucositis (erythema) appear after a latent period of approximately 1 week, with Grade 3 to 4 usually at the end of the third week; the most severe amount of mucositis is scored usually around the fourth or fifth week, frequently associated with symptoms such as dysphagia, pain, and weight loss (20, 37, 57–59). When the (accelerated) regenerative response of the basal stem cells is large enough, healing can occur during the last weeks of treatment, resulting in the subsiding of signs and symptoms before the end of the radiation therapy (5, 6, 10, 13, 47, 60–62).

Microorganisms, especially the AGNB, are thought to play an aggravating role in the development of mucositis after the initial erythematous phase (20, 35, 38–40, 44, 63, 64). In a pilot study, albeit in only 15 patients, Spijkervet *et al.* (36, 37), using PTA lozenges, demonstrated a significant reduction in radiation-induced mucositis, paralleled by complete eradication of the AGNB and a significant reduction of *Candida* species from the oral flora. In the study by Spijkervet *et al.* (36), the maximum grade of mucositis was limited to erythema, and none of his patients developed pseudomembranes. In contrast, 33 of our patients (43%) experienced patchy or confluent mucositis (G3, G4) during their radiation treatments, and no significant difference was observed between the two treatment groups ($p = 0.75$, Mann-Whitney). In fact, mucositis scores in patients participating in both arms of our study are not different from what is usually seen in conventional fractionated radiotherapy, even though the oral flora of the PTA group showed a marked and statistically significant reduction of the aerobic Gram-negative strains (Coliforms) during the first six treatment weeks, i.e., 11% (PTA group) vs. 34% (placebo group). Since the initial report by Spijkervet *et al.* (36), one pilot study with matched historical controls (41) and two placebo-controlled randomized studies (42, 43) have been reported in the literature, showing a reduction in radiation-induced mucositis using PTA. However, in the randomized study of Okuna *et al.* (43), the significant difference was true only for the patient-reported subjective complaints. More importantly, no microbiologic data are available. The findings by Kaanders *et al.* (41) were based on a nonrandomized trial, and positive results were true only for the 16 oral cavity tumors that underwent postoperative EBRT; that is, the 20 patients irradiated primarily for oropharyngeal tumors failed to show any benefit from the PTA lozenges. Moreover, complete eradication of the AGNB was not achieved, and the low initial carriage rate (only 3 patients) does not allow further conclusions. In the carefully analyzed randomized study of Symonds *et al.* (42), with 224 evaluable patients, formation of intermediate and thick pseudomembranes as the primary study endpoint was not prevented, and the difference between the treatment groups for this endpoint was not statistically significant ($p = 0.12$). Statistically significant differences in favor of the PTA group were found for the maximum observed mucositis grade ($p = 0.009$) and associated subjective parameters. These results were, unfortunately, not paralleled by a complete eradication of the AGNB; i.e., no statistically significant difference was found between the two treatment groups in the overall percentage of patients with positive cultures ($p = 0.26$). However, as in our study, weekly evaluation data showed a significant reduction of the percentage of patients with positive cultures in the PTA group from Week 3 through Week 7 ($p = 0.01$). According to Symonds *et al.* (42), the insufficient drug delivery of PTA by using lozenges is to be incriminated for the failing of total eradication of the AGNB and, in fact, the use of more efficient vehicles, such as an oral paste, to deliver the antimicrobials was advocated. In our study we used PTA in an oral paste that resulted in an efficient, selective elimination of the AGNB, especially the Coliforms, from the oral flora, although complete eradication was still not achieved, and the *Candida* species was not affected. The lack of any effect of selective

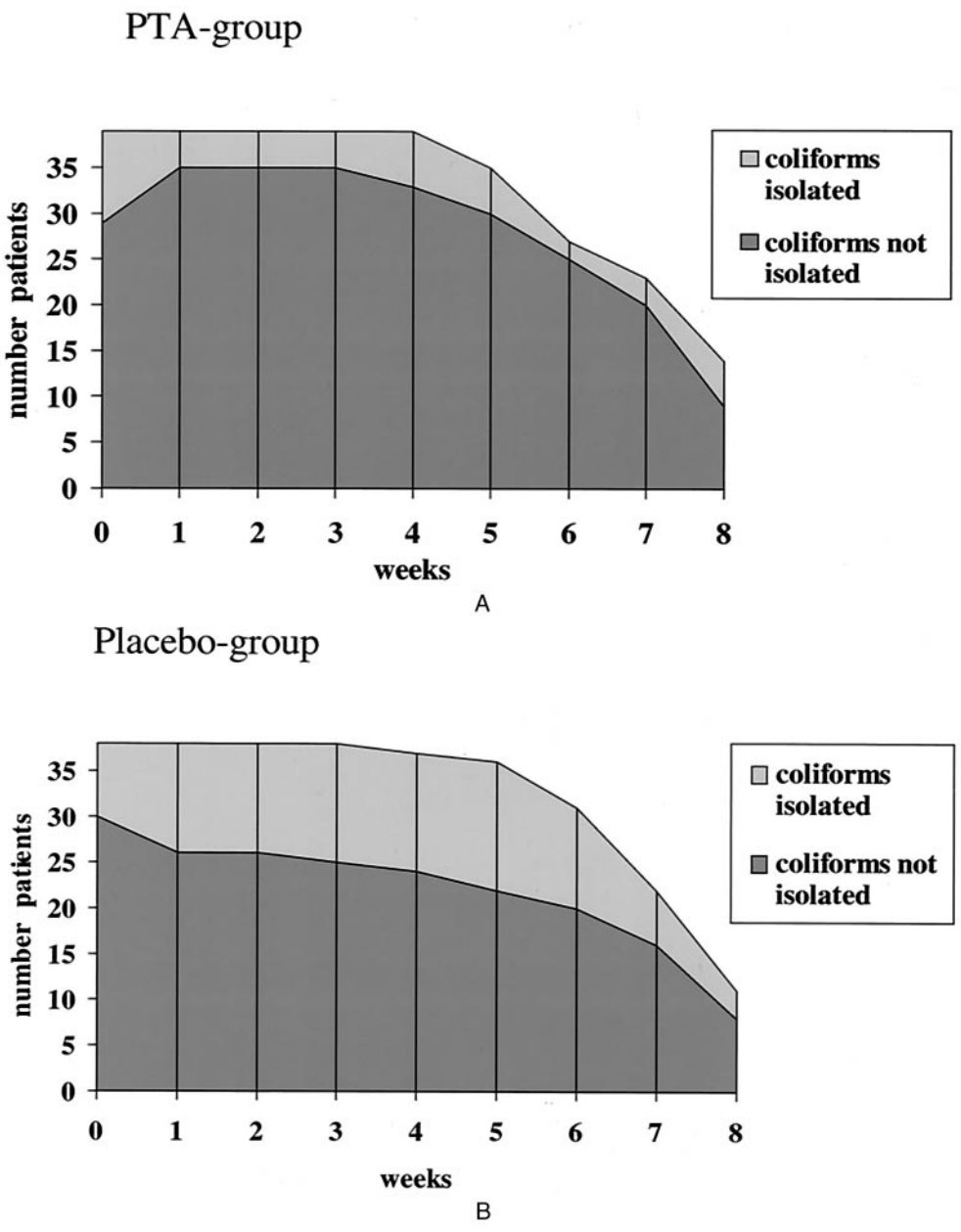

Fig. 3. (A) Coliforms isolated from the oral flora during the study weeks for the PTA group and (B) placebo group. In the PTA group a significant reduction of the percentage of patients in whom Coliforms were isolated was seen for study Week 1 through 6.

elimination of the AGNB on mucositis grade or related subjective symptoms in our study, therefore, does not support the hypothesis by Symonds *et al.* (42). Further support for rejecting the hypothesis is found in the studies of Rahn *et al.* (65) and Adamietz *et al.* (66, 67) with povidon-iodine as antimicrobial agent. Although a statistically significant reduction of the grade and severity of mucositis was found in the povidon group, this was again not paralleled by a significant reduction of the bacterial content of the oral flora. Whether microorganisms other than the AGNB play a role remains to be tested. In this respect the persistence of the *Candida* species in 30% of our patients might be an explanation of the discrepancy between our results and the results of the studies of Spijkervet *et al.* (35–37), Kaanders *et al.* (41), and Symonds *et al.* (42). Kaanders *et al.* (41) suggest that the importance of AGNB in the pathogenesis of radiation mucositis might be tumor site–dependent (different colonization patterns); moreover, in the case of postoperative EBRT, the oral defense mechanisms could be altered, and AGNB might play an even greater role in aggravating radiation mucositis. However, in our study the majority of patients were treated postoperatively, and although no relation could be found between oral flora and mucositis grade, neither was there any tumor site dependence.

Analyzing simulation films of our patients, a significant correlation was found between the estimated irradiated oral mucosal area and all study parameters, suggesting that to

reduce radiation-induced mucositis, obviously, as much mucosa from the treatment portals as possible should be eliminated (68). Other treatment-related parameters that might influence the severity and duration of mucositis, such as overall treatment time, cumulative EBRT dose, and the amount of salivary glands within the treatment portals, were evenly distributed between the study arms. None of these parameters showed a significant correlation with the mucosal study parameters in the present study.

From the present randomized study in head-and-neck cancer, analyzing the use of PTA oral paste for reducing radiation-induced mucositis, the hypothesis that aerobic Gram-negative bacteria play a crucial role in the evolution of radiation-induced mucositis could not be corroborated by our data. It remains without dispute, however, that further efforts must be undertaken to find remedies to control or reduce this important clinical problem of radiation- and/or chemotherapy-induced mucositis (17, 19–21, 64, 69–76). This is particularly relevant since (severe) mucositis can be of significant importance for quality of life endpoints such as eating, swallowing, chewing, pain, and/or the use of tube feeding. As radiation-induced mucositis is, in essence, a (radio) biologically based phenomenon, where regeneration response of the basal stem cells and cytokines play a crucial role, finding new strategies is focused on these fundamental mechanisms (6, 60, 77–81). Interesting developments in this respect are the use of growth factors, such as GM-CSF and G-CSF (82–87). An entirely different approach is the use of radioprotectors, such as amifostine (88–91).

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