

## Effectiveness of oral chlorhexidine for reducing stomatitis in a pediatric bone marrow transplant population

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### Abstract

*Disruption of the oral mucosal lining and the lack of normal defense mechanisms predispose bone marrow transplant (BMT) patients to life-threatening infections, often caused by oral flora. Chlorhexidine, used as an oral antiseptic, appears promising in limiting oral bacteria and fungi, and therefore, may decrease oral complications associated with BMT. The purpose of this study was to determine in pediatric BMT recipients if a 0.12% chlorhexidine mouthrinse, used as an adjunct to normal in-hospital oral care regimens, would decrease the severity of oral mucositis as measured by oral ulcerations, bacteremia, and length of hospital stay.*

*Forty-seven pediatric BMT subjects were included in this double-blind study. Subjects were instructed to use 15 ml of a mouthrinse 3 times daily to be swished and gargled for 30 sec. Each subject had 7 oral sites scored for the percentage of ulcerated mucosa twice weekly until day +35 or hospital discharge or death. Blood was cultured daily during neutropenia. Additionally, the number of days from onset of cytoreduction to hospital discharge or death was recorded for each subject. Alpha was set at .05.*

*There was no significant difference in the severity of oral ulceration between the chlorhexidine and placebo groups ( $P = .18$ ). Chlorhexidine did not reduce the development of bacteremia ( $P > .5$ ), nor did it significantly decrease the length of hospital stay ( $P = .68$ ). According to this study the use of 0.12% chlorhexidine cannot be expected to significantly reduce oral mucositis in pediatric BMT recipients receiving normal in-hospital oral care.*

Bone marrow transplant (BMT) involves intensive chemotherapy and/or total body irradiation to kill malignant cells and blunt the response to donor antigens. Oral mucous membranes are often damaged by the nonspecific cytotoxic action of these agents (Heimdahl et al. 1985; Segreto et al. 1986), which, when combined with the resident oral flora may induce extensive oral ulceration and patient discomfort. Due to the induced bone marrow ablation, BMT patients are

immunoincompetent and therefore exceptionally prone to infection before donor cell engraftment is secure (Schimpff et al. 1972; McElroy 1984). Oral ulcers frequently become secondarily infected and may be portals for bacteremia and fungemia (Berkowitz et al. 1983; DePaola et al. 1986; Rosenberg 1986).

Previous studies indicate that proper daily oral hygiene can significantly reduce the complications of stomatitis (Hickey et al. 1982; Seto et al. 1985). However, mechanical hygiene appears to limit stomatitis only in gingival areas adjacent to the teeth with no effect on mucosa of the cheek, tongue, and floor of the mouth (Jensen 1983). Additionally, brushing and flossing during stomatitis are often too painful to perform (McGaw and Belch 1985).

Oral antiseptics, which alter bacterial adherence and have broad antimicrobial potency, appear promising for limiting oral bacteria, and may consequently decrease the oral complications frequently seen in these patients (Baker et al. 1987). Chlorhexidine is such an antiseptic, yet it has not been used widely in patients with oral ulcerations or malignancies. This study was based on the hypothesis that oral microbiologic control via a chlorhexidine mouthrinse may decrease the severity, duration, and complications of oral ulceration in pediatric BMT patients when used as an adjunct to a standard in-hospital oral care protocol.

### Materials and Methods

#### Sample and Study Design

Pediatric patients 1 year and older undergoing BMT at the University of Minnesota Hospital between September 1, 1986, and September 5, 1987, were eligible to participate in this study. The informed consent of all subjects was obtained after approval of the project by the Committee on the Use of Human Subjects in Research at the University of Minnesota. Forty-seven

subjects ranging in age from 1 year, 7 months to 21 years, 6 months were included; the median age of the 27 male and 20 female subjects was 12 years. Diagnoses of subjects included: acute lymphocytic leukemia (20 subjects); acute nonlymphocytic leukemia (8); chronic myelogenous leukemia (6); aplastic anemia (5); neuroblastoma (3); congenital metabolic disorder (1); immune deficiency (1); non-Hodgkins lymphoma (1); and other malignancies (2). Treatment regimens included 28 standard allogeneic transplants with prophylactic methotrexate for graft vs. host disease, 17 autologous transplants, and 2 T-cell depleted transplants (Table 1). All subjects received chemotherapy and total body irradiation except the 6 diagnosed with aplastic anemia and metabolic disease, who received chemotherapy only. Based on intensity of BMT conditioning schedules (radiochemotherapy), a table predicting oral mucosal injury was developed (Table 2). Mild mucosal injury was predicted for aplastic anemia treated by allogeneic transplant and acute lymphoblastic leukemia treated with autologous BMT. Moderate injury included allogeneic acute lymphoblastic leukemia, acute nonlymphocytic leukemia, and chronic myelogenous leukemia. Severe oral mucosal injury included neuroblastoma. As shown in Table 2, the expected oral injury designations were evenly divided between the chlorhexidine and control groups by the stratification and randomization schema.

**TABLE 1. Subject Randomization**

Type of BMT	Chlorhexidine	Placebo
Allogeneic	15	13
Autologous	7	10
T-cell depleted	1	1
<i>Diagnosis</i>		
ALL	10	10
ANLL	4	4
CML	4	2
Aplastic anemia	3	2
Neuroblastoma	1	2
Metabolic disorder	0	1
Immune deficiency	0	1
Non-Hodgkins lymphoma	1	0
Other malignancies	0	2

**TABLE 2. Expected Oral Mucosal Injury\***

	Chlorhexidine	Placebo	Total
Mild	9	10	19
Moderate	14	12	26
Severe	0	2	2

\* Based on radiochemotherapy schedules used for BMT conditioning.

Subjects were stratified into 2 groups based on type of transplant protocol. Group 1 consisted of autologous and T-cell-depleted BMT protocols and group 2 consisted of standard allogeneic BMT protocol. Only group 2 received methotrexate. Double-blind randomization was completed within each of the 2 groups with one arm receiving a 0.12% chlorhexidine mouthrinse (test) and the other arm receiving a placebo rinse (control-Table 1).

Subject preparation included full-mouth radiographs, dental prophylaxis, and completion of all dental restorative and surgical needs by the investigator prior to cytoreduction. Subjects/parents and nurses were instructed to use/administer 15 ml of a test or control mouthrinse to be swished and gargled for 30 sec and expectorated 3 times daily from day -8 through day +35 or hospital discharge or death. Ten ml was used for children 3 and younger. If unable to swish and gargle, an oral sponge was used to apply the rinse to all oral tissues. In addition to test or control rinse use, patients performed routine dental care consisting of twice daily tooth brushing with a dentifrice, 3 times daily salt and soda rinses for gross debridement, and either 5 times daily clotrimazole troche or 4 times daily mycostatin elixir for control of oral yeast infection. Herpes simplex seropositive subjects received prophylactic acyclovir. Flossing was discouraged due to possible gingival trauma and subsequent bacteremia.

The required observation period extended from 8 days prior to transplantation (cytoreduction period) until 35 days post-transplantation, or hospital discharge, or death (-8 to +35).

Baseline quantitation of intraoral ulceration was recorded for all subjects at the pre-BMT dental prophylaxis appointment. The study investigator subsequently assessed subjects twice weekly for the percentage of ulcerated mucosa. Seven oral sites were examined visually and scored separately including: buccal and labial mucosa, alveolar mucosa, gingiva, hard palate, soft palate and oropharynx, floor of mouth and ventral surface of tongue, and dorsal surface of tongue. Blood cultures to monitor infection were performed daily while the patients were neutropenic. Additionally, the number of days from onset of cytoreduction to hospital discharge or death was recorded.

### Statistical Analysis

Repeated-measures analysis of variance (ANOVA) was used to test for statistical significance of the average percentage of ulcerated mucosa as classified into group factors and subject factors. Group factors included treatment group (chlorhexidine vs. placebo) and transplant type (standard allogeneic vs. autologous and T-cell depleted). Subject factors included time period (day -8, -4, -1, +3, +7, +10, +14, +17, +21, +24) and

area of mucosa (keratinized vs. nonkeratinized). Therefore, a 4-way factorial ANOVA was used after classification by treatment group, transplant group, time period, and area of mucosa. Life table analysis for bacteremia and time to hospital discharge was done using BMDP software (Kaplan-Meier product-limit with 95% confidence interval) and Mantel-Cox tests of significance. *P*-values less than .05 were considered statistically significant in this study.

## Results

Graphs of the percentage of oral ulceration were generated to compare mean values and are presented in Figures 1-4 (Fig 4 on page 40). Only 10 of the 13 time points were included on the abscissa. Days +28, +31, and +35 were not included due to hospital discharges and

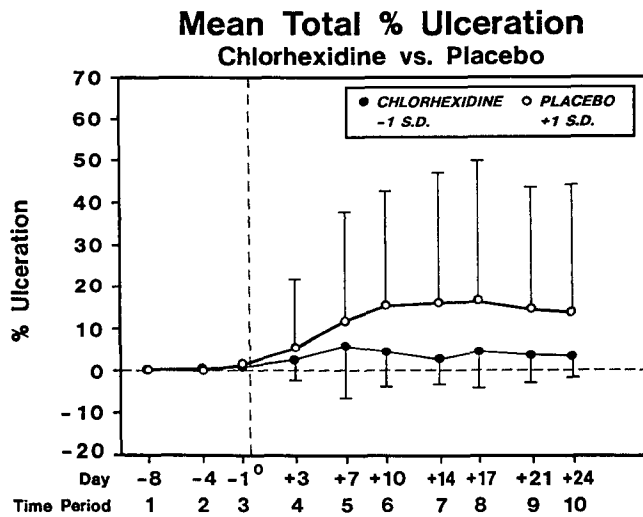


FIG 1. Mean total percentage of ulceration.

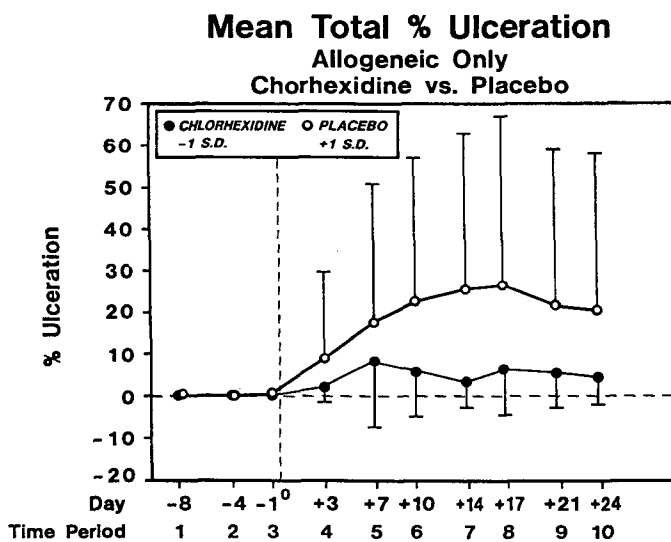


FIG 2. Mean total percentage of ulceration (allogeneic only).

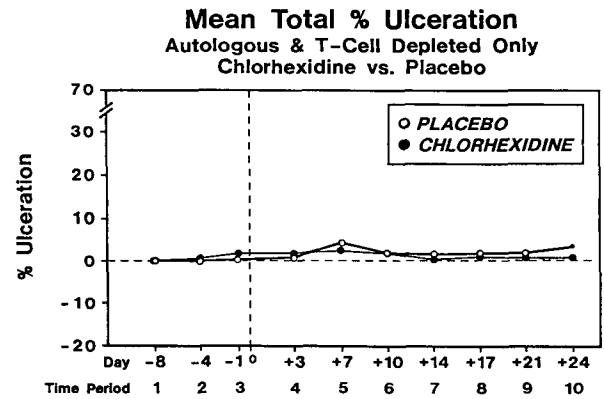


FIG 3. Mean total percentage of ulceration (autologous and T-cell depleted only).

deaths during these 3 time periods, resulting in a significant number of missing values. Standard deviations for the mean total percentage of ulceration scores were included in Figures 1 and 2. Due to the extreme similarity between autologous chlorhexidine and placebo groups at all time periods, standard deviations were not included in Figure 3.

The mean percentage of oral ulceration over time is depicted in Figure 1, comparing chlorhexidine-treated subjects vs. placebo-treated controls. As shown, oral ulceration in the control subjects became apparent earlier and peaked at day +17 post-transplant with 13.5% of the oral mucosa ulcerated. In contrast, chlorhexidine patients had more modest mean ulceration (5% maximum on day +7 post-transplant). Significant healing (reduction in area of ulcerated mucosa) was not apparent by day +24 post-transplant in either group. Although, throughout the time course chlorhexidine-treated patients had less extensive ulceration, there was no statistically significant difference in extent of ulceration between the 2 groups.

Similarly, analyzing only patients undergoing allogeneic transplant (Figure 2), chlorhexidine-treated patients developed less severe mucosal ulceration compared to placebo-treated patients. The allogeneic placebo group averaged 26% ulcerated mucosa at peak stomatitis (day +17), while the maximum value for the chlorhexidine group was 7% (day +7). The greatest difference between the allogeneic placebo and chlorhexidine group occurred at day +14, when mean values were 25 and 3%, respectively. No significant difference could be assigned to the extent of ulceration between the allogeneic test and control groups.

Autologous and T-cell-depleted mean ulceration percentages were nearly identical at all 10 time periods for both chlorhexidine and placebo (Fig 3). The modest mucositis in the autologous and T-cell-depleted BMT recipients is reflected in the low percentage scores (all less than 5%).

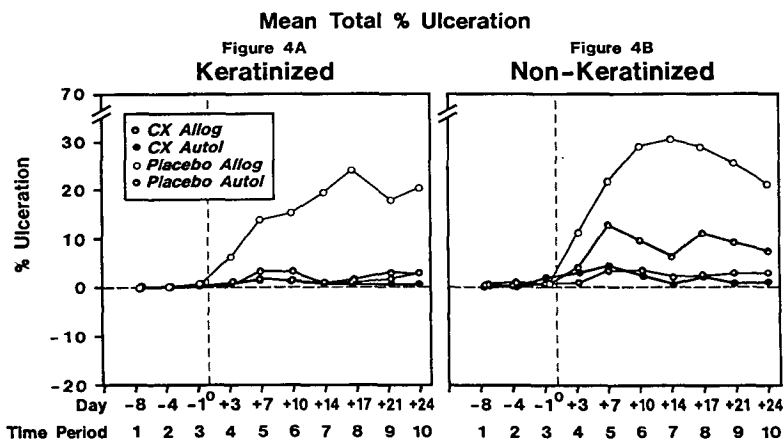
Figures 4a and b present composite data from Figures 2 and 3 stratified into keratinized and nonkeratinized mucosa. Autologous and T-cell-depleted subjects demonstrated similar mean ulceration values for keratinized versus nonkeratinized mucosa. Allogeneic placebo subjects, however, demonstrated much greater mean values for both keratinized and nonkeratinized mucosa than allogeneic chlorhexidine subjects. Allogeneic chlorhexidine subjects received mean keratinized mucosa scores similar to autologous chlorhexidine and placebo subjects at all 10 time periods (Fig 4a). Mean nonkeratinized mucosa ulceration values for allogeneic placebo and chlorhexidine subjects (Fig 4b) were greater than the corresponding mean keratinized values at all post-transplant time periods (Fig 4a).

The ANOVA analysis (Table 3) highlights, independently, the factors which may be critical variables and thus distort interpretations of overall mucositis comparisons. These *P*-values suggest that consideration of the interactive variables was either statistically associated or not associated with more severe mucositis. No significant interaction was present in group

TABLE 3. Analysis of Variance of Average % Ulceration

	<i>P</i> value
Group: chlorhexidine vs. placebo	.18
Transplant type: allogeneic vs. autologous	.06
Group x transplant type	.21
Area: keratinized vs. nonkeratinized	.004
Area x group	.84
Area x transplant type	.02
Area x group x transplant type	.69
Time: days -8 through +24	.03
Time x group	.17
Time x transplant type	.06
Time x group x transplant type	.27
Area x time	.05
Area x time x group	.50
Area x time x transplant type	.15
Area x time x group x transplant type	.84

(chlorhexidine vs. placebo) ( $P = .18$ ) or transplant type (allogeneic vs. autologous and T-cell depleted) ( $P = .06$ ). Significant interactions were present in area (keratinized vs. nonkeratinized mucosa) ( $P = .004$ ) and time (days -8 to +24) ( $P = .03$ ). There was a statistically significant 2-way interaction between area and transplant type ( $P = .02$ ). Two-way interaction between time and transplant type ( $P = .06$ ) and area and time ( $P = .05$ ) approached significance. In no analysis was the assign-



FIGS 4a & b. Mean total percentage of ulceration. Fig a (left) keratinized; Fig b (right) nonkeratinized.

ment to chlorhexidine vs. placebo predictive of less severe mucositis.

Compliance of rinsing was demonstrable based on delivered doses. The chlorhexidine and placebo groups received  $67.4 \pm 28\%$  and  $79 \pm 21\%$  of scheduled doses, respectively. These values are not corrected for death or hospital discharge before the test period was complete, indicating a higher compliance than these numbers suggest. Common reasons for missed doses included patient unavailability due to surgery, radiography, or too ill to comply (such as support with a ventilator).

Chlorhexidine use did not reduce the development of gram-positive or gram-negative bacteremia. Gram-positive bacteremia developed in 56% (34-78%) (95% confidence limits) of chlorhexidine users and 50% (29-72%) of placebo users ( $P > .5$ ). Gram-negative bacteremia was seen in 33% (13-54%) of chlorhexidine users and 39% (19-60%) of placebo users ( $P > .5$ ).

Table 4 summarizes the outcome of BMT for all subjects and profiles the 37 patients discharged from the hospital. All subjects were either discharged from the hospital or had expired by day +154. The median day of hospital discharge represents the day by which 50% of subjects were dismissed. There was no significant difference in median time to hospital discharge between chlorhexidine and placebo groups by life table analysis ( $P = .68$ ).

## Discussion

Clinical signs of ulcerated mucosa first appeared on day +3, which was 10 days after the onset of chemoirradiation therapy (Figs 1-4). Other studies consistently report that clinical signs of ulceration appear between 10 and 14 days post-BMT chemoirradiation (Lockhart and Sonis 1979; Seto et al. 1985). Peak stomatitis was

**TABLE 4. Length of Hospital Stay Summary Table**

	Allogeneic			Autologous & T-Cell Depleted			Overall Total
	Number of Chlorhexidine Subjects	Number of Placebo Subjects	Total	Number of Chlorhexidine Subjects	Number of Placebo Subjects	Total	
Discharged Before Day +154	10	10	20	7	9	16	36
Died Before Day +154	5	3	8	1	2	3	11
Total	15	13	28	8	11	19	47
Median Day of Discharge	+79	+53		+48	+48		

experienced on days +10 to +17. Seto et al. (1985) reported maximum oral ulceration in BMT recipients on days +12 to +14. Excluding the autologous and T-cell-depleted group (Fig 3), which experienced only minor stomatitis throughout the study, severity of stomatitis first decreased on day +21. This correlates well with Seto et al. (1985), who reported that the median day of BMT stomatitis resolution began on day +19.

Allogeneic subjects (Fig 2) demonstrated greater mean percentage of ulceration than autologous and T-cell-depleted subjects (Fig 3) at all post-transplant time periods. The effect of transplant type approached significance ( $P = .06$ ). Methotrexate, which was administered to all allogeneic subjects, is stomatotoxic (Lockhart and Sonis 1979) and may account for the increased severity of allogeneic stomatitis in this group.

Ulceration scores for the placebo group appear greater than the chlorhexidine group scores (Figs 1, 2, 4). However, these results must be interpreted cautiously. The standard deviations in Figures 1 and 2 for both chlorhexidine and placebo groups are large. This indicates that in both groups, some patients exhibited severe oral ulceration, while others showed no signs of stomatitis. Such a wide distribution of stomatitis experience accounts for the lack of statistical significance between test and control groups. Data analysis after excluding 4 subjects with abnormally severe ulceration still revealed a lack of statistical significance between chlorhexidine and placebo rinses.

The greater the mitotic index of a cell type, the more profoundly it is affected by cytotoxic chemotherapy drugs (Rose and Kaye 1983). Nonkeratinized oral mucosa has a faster turnover time than keratinized mucosa (Squier et al. 1975). Thus, nonkeratinized mucosa should have increased susceptibility to the stomatotoxic effects of chemotherapy, as evidenced by increased severity of ulceration. This was found to be true ( $P = .004$ ; Figs 4a, b). Nonkeratinized mucosa developed peak ulceration earlier than keratinized mucosa and also began to resolve earlier. Complete resolution of oral

mucositis was not measured in this study. However, extrapolation of lines in Figures 4a and b, combined with evidence from other studies (Squier et al. 1975) suggest that healing of nonkeratinized mucosa should be completed before keratinized mucosal healing. The difference in mitotic index likely accounts for the earlier onset, increased severity, and earlier resolution of ul-

cerations in nonkeratinized mucosa.

The significant interaction of area x transplant type indicates that the severity of keratinized or nonkeratinized mucosal ulcerations was dependent upon whether subjects received autologous and T-cell-depleted or allogeneic BMT protocols (Table 3). It is possible that the dependence of oral ulceration on area and transplant type is due to methotrexate exerting its stomatotoxic side-effect preferentially against nonkeratinized mucosa.

Time had a significant effect on experienced ulceration ( $P = .03$ ). The interaction of time x transplant type approached significance. This indicates that allogeneic ulceration scores tended to be greater than autologous and T-cell-depleted ulceration scores at post-transplant times. In this regard, an earlier study demonstrated a statistically significant increase in duration of mucositis secondary to methotrexate administration (Berkowitz et al. 1987). As only the allogeneic patients in this study received methotrexate, this factor may explain the effect of time x transplant. The interaction of area x time also approached statistical significance, indicating that overall, patients developed more extensive mucositis in nonkeratinized epithelium peaking at day +10 to +17 post-transplant.

No indirect therapeutic benefit of chlorhexidine use was demonstrable regarding development of bacteremia. Additionally, no differences were observed in bacterial species isolated from the blood in either group, including commonly found oral flora such as alpha hemolytic streptococci.

Ferretti et al. (1987) inferred that chlorhexidine-treated BMT patients may have shorter hospital stays. However, review of Table 4 shows that the median length of hospital stay for autologous chlorhexidine and placebo groups was equal (48 days). The allogeneic chlorhexidine group had a longer median hospital stay (79 days) than the allogeneic placebo group (53 days). Thus, assignment to chlorhexidine did not favorably

impact in-hospital morbidity reflected in length of hospital stay.

The results of a previous study at the University of Minnesota Hospital involving 36 adult and child BMT subjects using a 0.1% chlorhexidine mouthrinse correlate well with the results of this study (Holpuch 1985). While the mean ulceration scores for the chlorhexidine group were consistently less than control group mean scores, the difference was not statistically significant ( $P > 0.2$ ). However, Ferretti et al. (1987) reported dramatic results of a 0.12% chlorhexidine mouthrinse in decreasing the severity of oral ulcerations in BMT patients ( $P < .05$ ). One possibility for the different conclusions between our study and that of Ferretti et al. (1987) is inherent differences in study populations. It is possible that heterogeneity in patient type may have been greater in our larger study population, thus blurring a therapeutic benefit. Also, differences between hospitals may have existed regarding the ability to employ conventional oral care protocols.

According to this study, the use of a 0.12% chlorhexidine mouthrinse cannot prevent or control stomatitis in pediatric BMT patients given standard in-hospital oral care. However, this does not mean that chlorhexidine administration is without benefit for this population. Brushing and flossing during stomatitis are often too painful for patients to perform properly. Also, these conventional oral hygiene measures often are contraindicated in light of the risk of bleeding or bacteremia due to the profound thrombocytopenia and neutropenia experienced by BMT patients. Numerous studies attest to the antiplaque and antigingivitis properties of chlorhexidine use in cancer patients (Shepherd 1978; Johnson and Rozanis 1979). Therefore, a 0.12% chlorhexidine mouthrinse may be advisable as an antiplaque and antigingivitis agent in BMT patients to augment oral hygiene only.

The authors thank Mary Schwind and Robert Haake for their assistance in preparing the manuscript.

This study was supported in part by NIH grant P01CA-21737 and the Procter & Gamble Co.

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