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Evidence for the Microbicidal Activity of a Chlorine Dioxide-Containing Oral Rinse Formulation *In Vivo*

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Abstract

The ability of an oral rinse preparation, containing an admixture of the oxohalogen oxidants chlorite anion and chlorine dioxide, to diminish salivary levels of *Streptococcus mutans*, lactobacilli and *Candida albicans* was investigated in a group of 33 dental patients. Patients underwent oral rinsing episodes with the above product (20 ml for a period of 60 seconds, three times daily) for a total of 14 days, and subsequently repeated this exercise with mineral water in place of the oral rinse formulation. A group of 10 dental student volunteers, conducting the same oral rinsing regimens with mineral water in place of the oral health care product, served as a control group. Salivary microorganism levels were determined both prior and subsequent to the above trial period. The results demonstrated that biocidal oxohalogen oxidants present in the oral rinse formulation tested gave rise to a substantial reduction in salivary *S. mutans* and lactobacilli levels ($p < 0.001$ and 0.005 , respectively), although the decrease observed in *C. albicans* failed to reach statistical significance. As expected, mineral water employed as an oral rinsing system by the same group of patients, or the student control group, exerted no influence on the salivary levels of each of these microorganisms. The therapeutic, microbial and biochemical ramifications of the results obtained are discussed. (*J Clin Dent* 12:67-70, 2001.)

Introduction

The incorporation of chlorine dioxide (ClO_2) and/or chlorite anion (ClO_2^-) into several contemporary oral health care products represents an effective advance in terms of preventative or therapeutic measures for the purposes of: 1) shielding against or combating periodontal diseases; and 2) maintaining a high level of oral hygiene. Indeed, the relatively stable free radical species ClO_2 is a chemically reactive oxidant with powerful bactericidal,¹ viricidal,^{2,4} sporocidal,⁵ cysticidal,⁶ algicidal,⁷ fungicidal⁷ and organoleptic⁸ properties, and has previously been successfully employed as a deodorizing and bleaching agent,⁹ and for water disinfection. ClO_2 is a more effective biocidal agent than aqueous solutions of chlorine (Cl_2),⁴ and is also less hazardous to human health since it does not appear to generate a series of toxic chlorinated organic products on reaction with trace levels of organic components (*e.g.*, phenols and humic and fulvic acid adducts) present in water for human consumption.

The oxidative consumption of critical biomolecules by ClO_2 is primarily responsible for its wide range of biocidal activity, and its single-electron reduction product, ClO_2^- , can also act as a reactive oxidant toward many electron-donating biomolecules

(*e.g.*, methionine, pyruvate, urate and endogenous thiols, such as cysteine).¹⁰

Dental practices have employed ClO_2^- -containing oral rinses for many years and, in addition to exerting potent antimicrobial properties, an oral rinse formulation containing an admixture of ClO_2 (up to 20 ppm) and 0.10% (w/v) ($1.48 \times 10^{-2} \text{ mol.dm}^{-3}$) ClO_2 (RetarDEX®, Rowpar Pharmaceuticals Inc., Scottsdale, Arizona, USA) has been demonstrated to: 1) oxidatively consume volatile sulphur compounds (VSCs) responsible for oral malodor (halitosis); 2) remove residual organic solutes; 3) increase salivary and plaque O_2 tensions; and 4) inhibit the activity of bacterial proteolytic enzymes (presumably via oxidative inactivation).¹¹ Moreover, a recent clinical investigation involving the therapeutic application of this novel product in combination with a toothpaste preparation (RetarDENT, Rowpar Pharmaceuticals Inc., Scottsdale, AZ, USA) containing equivalent $\text{ClO}_2/\text{ClO}_2^-$ contents showed that 67% of 2,085 pockets diminished from 4 mm to < 3 mm within a average period of 3.4 months. Furthermore, 71.85% of bleeding observed at probing sites ceased between two dental hygiene visits within an average period of 6.9 months ($p < 0.01$).¹¹

This study was designed to evaluate the ability of RetarDEX oral rinse to control or reduce the levels of *Streptococcus mutans*, lactobacilli and *Candida albicans* in a group of elderly dental patients.

Materials and Methods

Experimental Design

The investigation involved 33 patients with a mean age of $62.3 (\pm 1.7)$ years. The patients were all receiving conservative, prosthetic and/or periodontal treatment at the time of the study. A group of 10 dental students with a mean age of $21.4 (\pm 0.8)$ years was also recruited into the study as an independent control. Consent was gained from each participant prior to their entry into the trial. The study had no subjects who had undertaken a course of antibiotic therapy within the previous 4 weeks, or were already using a mouthrinse.

Prior to each sample collection period, each participant was requested to refrain from oral activities (*i.e.*, eating, drinking,

toothbrushing, oral rinsing, smoking, etc.) for a period of at least 2 hours. Immediately prior to commencing the trial, subjects were seated and then collected all unstimulated saliva into a sterile container for a period of 10 minutes. Baseline (control) levels of *S. mutans*, lactobacilli and *C. albicans* (in counts per ml) were determined in these biofluids using chairside diagnostic kits (Vivacare Ltd., Liechtenstein). The patients were then supplied with 2 × 500 ml bottles of RetarDEX oral rinse, and received both verbal and written instructions to undertake oral rinsing episodes (20 ml of oral rinse for 60 seconds, three times daily) for a total of 14 days, and also to adhere to their normal oral hygiene regimens (*i.e.*, twice-daily toothbrushing episodes) and, of course, to avoid the use of all classes of additional oral healthcare products throughout the 14-day test period. Subsequently, all dental patient participants returned the oral rinse bottles, a further post-treatment saliva specimen was collected and the levels of *S. mutans*, lactobacilli and *C. albicans* were determined in the manner described above.

Each of the above patients was then recalled to the clinic, their saliva samples collected as described above, and baseline levels of *S. mutans*, lactobacilli and *C. albicans* were determined. These subjects were then again given both verbal and written instructions to repeat the above oral rinsing episodes for a 14-day period with mineral water (supplied) in place of the RetarDEX oral rinse, after which they returned to the clinic and supplied a post-placebo saliva specimen in which the same microbial counts were conducted. In this manner, potential interferences arising from the Hawthorne effect (*i.e.*, an increase in toothbrushing patterns and frequency engendered by participation in a dental investigation) were controlled as much as possible.

The student control group ($n = 10$) conducted oral rinsing episodes with mineral water in place of the above oral rinse (20 ml for 60 seconds at the same time points), and also supplied a saliva sample for microbiological analysis both prior to and after completion of the 14-day trial period.

Statistical Analysis

For each class of microorganism counted, a paired t-test was performed on experimental data acquired (*i.e.*, bacterial counts pre- versus post-administration of RetarDEX oral rinse) in order to test for oral rinse-mediated differences in their levels. This test was applied to both untransformed and $(x + 1)^{1/2}$ -transformed determinations, the latter transformation being appropriate for enumeration data, ensuring additivity and normality.

Results

Therapeutic administration of RetarDEX oral rinse in the manner described in the Materials and Methods section gave rise to a marked reduction in *S. mutans* and lactobacilli numbers in patients recruited into this study ($p < 0.001$ and 0.005 , respectively for untransformed data, and $p < 0.001$ and < 0.002 , respectively for $[x + 1]^{1/2}$ -transformed data). However, this oral health care product was found not to exert a microbicidal action toward *C. albicans*. Table I lists the mean differences observed and their corresponding standard errors and p-values for both untransformed and $(x + 1)^{1/2}$ -transformed experimental data ($n = 33$

in each case), and the corresponding raw data mean (standard error values for both the pre- and post-mouthrinse treatment groups) are given in Table II. Application of this statistical analysis procedure to microbial count data acquired from the same group of dental patients using mineral water in place of the $\text{ClO}_2^-/\text{ClO}_2^-$ -containing oral mouthrinse preparation revealed that there were no significant differences between the pre- and post-placebo sample groups (*i.e.*, $p > 0.10$) for *S. mutans*, lactobacilli and *C. albicans*.

Table I
Mean Difference for Paired t-Tests Conducted on Both Untransformed and $(x + 1)^{1/2}$ -Transformed Experimental Data ($n = 33$)

	Untransformed data			$(x + 1)^{1/2}$ -Transformed data		
	Mean Difference	Std Error	p	Mean Difference	Std Error	p
<i>S. Mutans</i>	1.030	0.182	< 0.001	0.343	0.061	< 0.001
Lactobacilli	2.093×10^5	6.754×10^4	< 0.005	205.57	58.26	< 0.002
<i>Candida Albicans</i>	1.727	2.043	n.s.	0.211	0.257	n.s.

The p-values represent the probabilities of the mean values obtained arising purely by chance. n.s. = not significant.

Table II
Raw Data Mean and Standard Error (SE) Values for Salivary Levels of *S. Mutans*, Lactobacilli and *C. Albicans* (Counts per ml) Prior and Subsequent to a 14-Day Period of Three Times Daily Oral Rinsing Episodes with RetarDEX Mouthrinse

	Pre-treatment	Post-treatment
<i>S. mutans</i>	2.21 (0.16)	1.18 (0.19)
Lactobacilli	$[4.47 (0.84)] \times 10^5$	$[2.38 (0.40)] \times 10^5$
<i>Candida albicans</i>	8.12 (3.37)	6.39 (3.04)

The small mean differences observed between baseline and post-test salivary levels of *S. mutans*, lactobacilli and *C. albicans* in the control group of dental students who received mineral water in place of the oral rinse formulation ($n = 10$) were found not to be statistically significant at the 5% level (paired t-test performed on both untransformed and $[x + 1]^{1/2}$ -transformed data).

Five of the 33 patients undergoing oral rinsing episodes with RetarDEX oral rinse reported a mild taste disturbance during the trial (specifically, that tea and carbonated beverages tasted bitter).

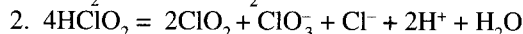
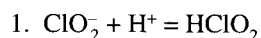
Discussion

Experimental data acquired in this investigation demonstrate that the oxohalogen oxidant-containing oral health care product tested clearly exerts a powerful bactericidal action toward salivary *S. mutans* and lactobacilli. These results are of much importance in light of previous evidence indicating that *S. mutans* is one of the most cariogenic species present in the oral environment.¹² Moreover, since low pH-resistant lactobacilli are often isolated from advanced carious lesions where the retention of acids generated by plaque bacteria is favored by topography¹³

(organic acid metabolites derived from lactobacilli would, of course, be expected to promote lesion development under these circumstances), this microorganism is also implicated in the pathogenesis of dental caries. Interestingly, the salivary lactobacillus count has previously represented a method for determining caries activity in dental patients (*i.e.*, > 10,000 lactobacilli per ml of saliva suggests a high caries activity, whereas < 1,000 per ml is indicative of caries immunity),¹³ although the diagnostic value of this test remains speculative since salivary lactobacilli levels do not necessarily reflect those of plaque. Nevertheless, although 81% of the patients taking part in this investigation had salivary lactobacilli levels of > 10,000 per ml prior to undergoing oral rinsing episodes with RetarDEX mouthrinse, after completion of the 14-day trial it was found that 57% of the participants had salivary lactobacilli levels in this "caries-active" group, *i.e.*, 8 of the participants had decreases in salivary lactobacilli levels below this "high risk" threshold. Excluding the few observations where zero microbes were detectable both prior and subsequent to treatment, coefficients of variation (*i.e.*, the sample standard deviation expressed as a percentage of the mean) for the oral mouthrinse treatment-induced decreases observed in the numbers of *S. mutans* and lactobacilli (raw, untransformed data) were as high as 95% and 168%, respectively, conforming to the non-uniformity of response to this treatment. Possible explanations for this non-uniformity in response are given in detail below. Of the control group of 10 students who undertook oral rinsing episodes with mineral water for a 14-day period, 3 were in this "high risk" group both prior and subsequent to the trial period.

The bacteriocidal properties of ClO_2 are well known,¹ and the capacity of Purogene, an aqueous solution of pH 8.6 described as containing a "stabilized" form of ClO_2 (presumably chlorite anion) to act in this context has been previously evaluated by Harakeh *et al.*¹ These researchers found a 4-log. scale of reduction in *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* Group A, *Salmonella typhimurium* and *Bacillus subtilis* test organisms when the above agent was applied at a concentration of 0.75 mg/l. Moreover, reduction of Purogene's pH to a value of 3.5 (but maintenance of the experimental medium at pH 7.0) elevated its bacteriocidal actions, an observation consistent with ClO_2 being the active disinfecting agent since this relatively stable free radical species is liberated from chlorite via the disproportionation of chlorous acid, HClO_2 (equations 1 and 2). Indeed, the pK_a value of chlorous acid is 2.31, and hence a small but nevertheless significant level of ClO_2 is expected to be generated at pH 3.5.

Equations:



ClO_2 has a virtually indiscriminate broad-spectrum oxidizing activity, and readily affects the oxidative transformation of: 1) thiols, such as the volatile sulphur compound (VSC) precursor cysteine together with inorganic and hydrogen sulphides;^{14,15} 2) phenols,¹⁶ including the amino acid tyrosine, and thiophenols (generating the corresponding disulphide, disulphoxide and sul-

phonic acid derivatives - the disulphides also react with ClO_2 to produce further disulphoxide and sulphonic acid);¹⁴ 3) secondary and tertiary amines, both aliphatic and aromatic;^{14,17} 4) 1,4-dihydroxybenzenes, which give rise to corresponding quinones without any detectable aromatic ring chlorination;^{14,18} 5) alcohols and carbohydrates (yielding carboxylic acid functional groups);^{14,19} 6) additional amino acids, *e.g.*, proline, hydroxyproline, histidine, tryptophan and methionine,²⁰ the latter also being a VSC precursor; 7) aldehydes, producing corresponding carboxylic acid adducts;¹⁴ and 8) the carbon-carbon double bonds of unsaturated aliphatic organic compounds, including unsaturated fatty acids.²¹ Many of the above biomolecules are critical for the sustenance, development and growth of cariogenic microorganisms such as *S. mutans* and lactobacilli, and selected ClO_2 -mediated oxidative inactivations (either individual ones or two or more in combination) serve as potential mechanisms for their bacteriocidal actions.

In view of these phenomena, the variable (between patient) microbicidal response to the RetarDEX oral mouthrinse observed here is explicable by: 1) variations in the salivary concentrations of $\text{ClO}_2/\text{ClO}_2^-$ -inactivating endogenous electron-donors (*e.g.*, pyruvate, urate *etc.*¹⁰); 2) variabilities in the suppression of the $\text{ClO}_2/\text{ClO}_2^-$ -mediated consumption of biomolecules essential for the growth and sustenance of these cariogenic microorganisms, a parameter presumably inversely related to the salivary levels of $\text{ClO}_2/\text{ClO}_2^-$ -reactive components not required for promoting their propagation; 3) variations in salivary pH values (both within and between participants) which, when lowered significantly, may facilitate the transformation of ClO_2^- to ClO_2 (equations 1 and 2); and/or 4) between-patient differences in the nature and preponderance of strains of *S. mutans* and/or lactobacilli, or more specifically, their potentially differing capacities to resist attack by the oxohalogen oxidants present in the oral mouthrinse preparation tested.

In conclusion, the oral rinse formulation tested here suppresses salivary levels of the cariogenic bacteria *S. mutans* and lactobacilli *in vivo*, an observation reflecting the bacteriocidal actions of oxohalogen oxidants present in this product.

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