Chlorine Dioxide and Chlorhexidine Mouthrinses Compared in a 3-Day Plaque Accumulation Model

Spiros Paraskevas,* Nanning A.M. Rosema,* Paula Versteeg,* Übele Van der Velden,* and G.A. Van der Weijden*

Background: The aim of this study was to investigate the inhibiting effect of a chlorine dioxide mouthrinse as opposed to a mouthrinse containing chlorhexidine (0.20%) during 3 days of plaque accumulation.

Methods: At baseline, all participants (N = 77) received a professional prophylaxis and were randomly assigned to the test (chlorine dioxide) or (positive) control (chlorhexidine) group. On the following 3 days, both groups rinsed twice daily for 1 minute with 10 ml test or control solution. At the end of the experimental period, plaque was assessed, and the panelists filled out a questionnaire.

Results: Chlorhexidine inhibited plaque growth significantly more than the mouthrinse containing chlorine dioxide (plaque index = 1.39 versus 1.96, respectively; P < 0.001). The results of the questionnaire showed that the panelists found chlorhexidine easier to use and more effective. However, they preferred the taste of the chlorine dioxide mouthrinse and experienced less taste alterations.

Conclusion: Chlorine dioxide mouthrinse seems to be a less potent plaque inhibitor than chlorhexidine. J Periodontol 2008;79:1395-1400.

KEY WORDS
Chlorhexidine; chlorine dioxide; clinical trial; dental plaque.

Plaque is a biofilm with layers of microorganisms contained in a matrix that forms on oral surfaces and is continuously bathed by saliva.¹ The relationship between plaque and periodontal inflammation was established decades ago.²,³ Furthermore, several studies⁴-⁶ demonstrated the importance of plaque as an etiologic factor of periodontitis. This led to the concept that strict plaque control is a prerequisite for a stable and healthy periodontal condition.⁷ Since that time, several approaches have been developed to control plaque growth.

Plaque removal by mechanical means (mostly a toothbrush combined with dentifrice) seems to be a common way of controlling plaque.⁸ However, factors, such as dexterity and motivation, can limit the effectiveness of daily self-performed oral hygiene.⁹

A chemical approach has been introduced to deal with the potential deficiencies of daily self-performed oral hygiene.¹⁰ The challenge with chemical plaque control is to develop an active antiplaque agent that does not disturb the natural flora of the oral cavity. The use of an antibioticum could be a tempting idea to control the growth of microorganisms, but it has severe limitations (risks of bacterial resistance, hypersensitivity reactions, or superinfections).¹¹ Additionally, the incorporation of a pathogen in a biofilm can protect this organism from concentrations of antimicrobials that

* Department of Periodontology, Academic Center for Dentistry Amsterdam, Amsterdam, The Netherlands.

would normally kill or inhibit those organisms freely suspended in water (planktonic bacteria). The effectiveness of antimicrobials, as measured with in vitro tests, is dramatically reduced in vivo because of the properties of the microbial community. Mature, intact biofilms are less sensitive to such agents because the exopolymer matrix, bacterial enzymes, and low growth rate hinder the action of chemotherapeutic agents.

For many years, researchers have searched for an effective chemical agent that would prevent oral plaque growth and, thereby, could replace mechanical plaque control. Chlorhexidine (CHX) seems to be the most effective chemical agent in short- and long-term use. Because of its established use and efficacy, CHX is the gold standard against which other chemical factors should be compared when claims of efficacy are attempted.

Although CHX has a relatively low toxic effect following oral use, it is not without side effects. The most common are staining of the tooth surfaces and taste alteration, but dryness, bitter taste, soreness, burning sensation, and numbness are also reported. Taste impairment may occur as soon as after the first day of rinsing and can last as long as regular rinses are applied. These are reversed after cessation of the CHX rinses. It is not uncommon for patients, particularly children and poorly motivated adults, to refrain from using CHX for this reason.

A study by Helms et al. indicated that CHX reduced the perceptual intensity of sodium chloride and quinine-HCl solutions. This was also found in a study by Marinone and Savoldi, who reported that hypogeusia induced by CHX concerns specifically the salt and bitter taste components. Such side effects may negatively influence the compliance of patients.

Because of the side effects reported, new chemical agents that would exert the same efficacy as CHX without its side effects are being researched. Chlorine dioxide (ClO₂) is a chemical agent with known antimicrobial properties. It is increasingly used in different industries, including the dairy, beverage, and food industries, to control microbiologic growth and for the removal of biofilms. It is an oxidizing biocide, indicating that it kills microorganisms by disruption of the transport of nutrients across the cell wall. ClO₂ is a gas at room temperature. The relatively stable free radical species ClO₂ is a chemical oxidant with powerful bactericidal, viricidal, sporidical, cysticidal, algicidal, and fungicidal properties. The oxidative consumption of critical biomolecules by ClO₂ is primarily responsible for its wide range of biocidal activity, and its single-electron reduction product (ClO⁻) can also act as a reactive oxidant toward many electron-donating biomolecules (e.g., methionine, pyruvate, urate, and endogenous thiols, such as cysteine).

Therefore, the aim of the present study was to assess the plaque growth inhibition of a ClO₂-containing mouthrinse compared to a CHX-containing mouthrinse during a 3-day de novo plaque-accumulation model.

**MATERIALS AND METHODS**

**Study Population**
The study population consisted mainly of university students from Amsterdam and surrounding areas. The subjects were recruited by advertisements. All candidates were screened for suitability by the research team. Seventy-seven healthy subjects (43 females and 34 males; age range, 18 to 48 years; mean age: 23.2 years) participated in the study. Selection criteria were a dentition with ≥20 evaluable teeth (minimum of five teeth per quadrant), no oral lesions, no severe periodontal problems (no probing depth ≥5 mm), and no removable prostheses or orthodontic bands or appliances. Persons allergic to several mouthrinse components were excluded from the study.

All eligible subjects were given oral and written information about the products and the purpose of the study and were asked to sign an informed consent. The study was conducted from June 10, 2004 to July 15, 2005 in the Department of Periodontology, Academic Center for Dentistry Amsterdam, in accordance with the ethical principles originating in the Declaration of Helsinki and consistent with good clinical practices.

**Study Design**
The study was designed as an examiner-masked, two-group parallel experiment. At baseline, plaque was disclosed, and all participants received a thorough supragingival scaling and polishing to remove all plaque, stain, and calculus. This was performed using hand instruments, mechanical scalers, and rotating brushes with polishing paste. Special attention was paid at interproximal areas where dental floss was used. To ensure that all deposits had been removed, a second disclosing episode was carried out after which any remaining plaque was removed.

Subjects were randomly assigned to the test or control group. Randomization was performed using computer-generated random numbers. The allocation of test or control products was carried out by a person not directly involved in the research project.

During a 3-day experimental non-brushing period, subjects in the test group used a ClO₂ mouthrinse† twice daily, whereas subjects of the (positive) control group used a 0.2% CHX mouthrinse.† All participants

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† 10 Quist-forte (containing 100-ppm free ClO₂). The rinse is activated when 5 ml base solution is mixed with 5 ml activator solution. De Witte Tanden Winkel, Rotterdam, The Netherlands.

‡ Corsodyl (containing 0.20% chlorhexidine digluconate, ethanol, polyoxyl hydrogenated castor oil, sorbitol, E-125, purified water), GlaxoSmithKline, Zeist, The Netherlands.
were instructed to refrain from using any other means of oral hygiene during the experimental period.

The subjects in the test group received two bottles (5 ml in each bottle) of mouthrinse that had to be freshly mixed for each rinsing episode. The positive control group received one bottle of mouthrinse containing 200 ml 0.2% CHX. All subjects were instructed to rinse twice a day, in the morning and in the evening, with 10 ml solution for 60 seconds, after which they expectorated. Subsequent rinsing with water was not allowed. Written instructions were provided explaining how to use the mouthrinse. Rinsing was performed at home without supervision. To check for compliance, subjects were asked to note the times of day when they rinsed.

After 3 days, subjects returned to the clinic for the plaque assessments. The dentition was disclosed with a disclosing solution.§ Subsequently, the level of plaque assessments. The dentition was disclosed with

The present study was designed to determine the plaque-inhibiting effect of ClO₂ compared to 0.2% CHX. It used a 3-day non-brushing model that allowed for plaque accumulation. This design was previously used to assess the effect of various mouthwashes.19,28,29 Zee et al.30 and Simonsson31 also used this 3-day model to discern between “rapid” and “slow” plaque formers. It was a convenient model with which to assess the plaque-inhibitory capacity of the test product per se and determine its relative activity in relation to the well-established action of CHX. Using such a 3-day

§ Mira-2-Tone, Hager & Werken, Duisburg, Germany.
plaque accumulation model provides a general indication of how the product in question would perform under actual conditions, insofar as significant plaque reduction is a prerequisite for the reduction in gingivitis.\(^{32}\)

ClO\(_2\) exists as synthetic greenish-yellow to orange gas with a characteristic pungent chlorine-like odor at room temperature. It is a neutral chlorine compound. ClO\(_2\) is different from elemental chlorine in its chemical structure and behavior. ClO\(_2\) is a small, volatile, and very strong molecule. It was discovered in 1814 by Humphrey Davy.\(^{24}\) He first produced this gas by pouring sulfuric acid (H\(_2\)SO\(_3\)) on potassium chlorate (KClO\(_3\)). Next, he replaced sulfuric acid with hypo-chlorous acid (HOCl). This reaction is used to produce chlorine gas (Cl\(_2\)), oxygen gas (O\(_2\)), and heat. One of the most important qualities of ClO\(_2\) is its high water solubility, especially in cold water. It is \(~10\) times more soluble in water than chlorine. ClO\(_2\) is a free radical in diluted, watery solutions but is quite stable if kept cool and in the dark. Once the gas is absorbed in water, it has a low volatility. For this reason, inhalation exposure is anticipated to be minimal.\(^{33}\) At high concentrations it reacts strongly with reducing agents.

When ClO\(_2\) is photo-oxidized by sunlight, it falls apart. The end products of ClO\(_2\) reactions are chloride (Cl\(^-\)), chlorite (ClO\(^-\)), and chlorate (ClO\(_5^-\)).

The existing literature provides information on mouthrinses containing acidified sodium chlorite solution (ASC) that can be activated at low pH by mixing it with a certain acid. A reaction by-product is ClO\(_2\), which is probably present at a low concentration. Conversion rates for ClO\(_2\) range from 1% to 10% and are typically proportional to the amount and strength of the acid added to the sodium chlorite solution. ClO\(_2\) has been used in mouthrinse form for the treatment of oral malodor. The ability of ClO\(_2\) to reduce malodor is well documented.\(^{34}\) A single use of ClO\(_2\)-containing mouthrinse significantly improved mouth odor pleasantness and reduced mouth odor intensity and volatile sulfur compound concentrations in mouth air for \(\geq 8\) hours after use.\(^{35}\) ClO\(_2\) substantially reduced salivary \textit{Streptococcus mutans} and lactobacilli levels; as expected, mineral water exerted no influence on the salivary levels of each of these microorganisms.\(^{16}\)

Little is known about the influence of ClO\(_2\) on plaque accumulation. A pilot double-masked, cross-over study\(^{36}\) examined the effect of a metastabilized chlorous acid/ClO\(_2\) mouthrinse on plaque accumulation and salivary bacteria. Eighteen subjects were recruited and rinsed twice daily with the test (high or low concentration of ASC)\(^{\dagger}\) or placebo mouthrinse for 5-day periods separated by washout periods of 9 days. The results demonstrated significant plaque reductions for the test (high and low concentrations of ASC) mouthrinses compared to the placebo product. These reductions in plaque were not accompanied by significant reductions in salivary bacterial counts. According to the investigators, this may be the result of the low substantivity of ClO\(_2\) as opposed to CHX. However, the outcomes of another study\(^{37}\) are not in concordance to this. This particular study was also designed in a double-masked, cross-over manner and tested the effect of three different formulations of ASC\(^{\ddagger}\) compared to the CHX and placebo mouthrinses. The three test solutions demonstrated equivalent antibacterial activity to CHX for plaque as well as for bacterial salivary counts. The investigators concluded that the test products had comparable substantivity to CHX. These outcomes are not in line with the results of the present study, in which CHX was more effective in reducing plaque levels than was ClO\(_2\). The reason may be attributed to differences in study methodology, such as the rinsing time (30 seconds versus 60 seconds) and supervised versus unsupervised rinsing. Certainly, more research is necessary to gain

### Table 1.

**Total Plaque Index (mean ± SD) for the Test and Control Groups and Exploratory Analysis for Different Jaws, Surfaces, and Tooth Categories**

<table>
<thead>
<tr>
<th>Category</th>
<th>0.2% CHX (n = 37)</th>
<th>P Value*</th>
<th>ClO(_2) (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1.39 ± 0.42</td>
<td>&lt;0.001</td>
<td>1.98 ± 0.40</td>
</tr>
<tr>
<td>All upper</td>
<td>1.32 ± 0.47</td>
<td>&lt;0.001</td>
<td>1.94 ± 0.42</td>
</tr>
<tr>
<td>All lower</td>
<td>1.48 ± 0.44</td>
<td>&lt;0.001</td>
<td>2.01 ± 0.44</td>
</tr>
<tr>
<td>All vestibular</td>
<td>1.59 ± 0.48</td>
<td>&lt;0.001</td>
<td>2.43 ± 0.46</td>
</tr>
<tr>
<td>All lingual</td>
<td>1.19 ± 0.42</td>
<td>0.002</td>
<td>1.49 ± 0.41</td>
</tr>
<tr>
<td>All approximal vestibular</td>
<td>1.80 ± 0.44</td>
<td>&lt;0.001</td>
<td>2.54 ± 0.42</td>
</tr>
<tr>
<td>All mid-vestibular</td>
<td>1.18 ± 0.61</td>
<td>&lt;0.001</td>
<td>2.21 ± 0.59</td>
</tr>
<tr>
<td>All mid-lingual</td>
<td>0.70 ± 0.52</td>
<td>0.007</td>
<td>1.02 ± 0.50</td>
</tr>
<tr>
<td>Front</td>
<td>1.27 ± 0.42</td>
<td>&lt;0.001</td>
<td>1.88 ± 0.40</td>
</tr>
<tr>
<td>Premolars</td>
<td>1.35 ± 0.48</td>
<td>&lt;0.001</td>
<td>1.92 ± 0.40</td>
</tr>
<tr>
<td>Molars</td>
<td>1.62 ± 0.46</td>
<td>&lt;0.001</td>
<td>2.13 ± 0.48</td>
</tr>
</tbody>
</table>

* Mann-Whitney test.

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\(\dagger\) Alcide, Redmond, WA.

\(\ddagger\) Alcide.
CONCLUSIONS

ClO₂ mouthrinse was a less potent plaque inhibitor than CHX. However, subjects preferred the taste of the ClO₂ mouthrinse and experienced less taste alterations compared to CHX.

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Table 2.

Questionnaire Responses (mean ± SD) Determined by VAS

<table>
<thead>
<tr>
<th>Question</th>
<th>Response</th>
<th>0.2% CHX (n = 37)</th>
<th>ClO₂ (n = 40)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) How was the taste of the product?</td>
<td>Very bad</td>
<td>3.6 ± 2.1</td>
<td>5.5 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2) How long did the taste remain in the mouth after rinsing?</td>
<td>Very long</td>
<td>3.8 ± 1.9</td>
<td>6.4 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3) How was your taste of food and drinks affected?</td>
<td>Negative change</td>
<td>4.0 ± 1.3</td>
<td>4.6 ± 5.4</td>
<td>0.048</td>
</tr>
<tr>
<td>4) Was the use of the mouthrinse convenient?</td>
<td>Not convenient</td>
<td>7.5 ± 2.4</td>
<td>4.7 ± 2.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5) What is your opinion about the rinsing time?</td>
<td>Very long</td>
<td>5.7 ± 2.0</td>
<td>5.3 ± 1.9</td>
<td>0.336</td>
</tr>
<tr>
<td>6) What was your perception of the plaque reduction?</td>
<td>Insufficient</td>
<td>6.8 ± 2.0</td>
<td>4.0 ± 2.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Mann-Whitney test.

Correspondence: Dr. S. Paraskevas, Department of Periodontology, Academic Center for Dentistry Amsterdam, Louwersweg 1, 1066 EA Amsterdam, The Netherlands. Fax: 31-0-20-5188512; e-mail: s.paraskevas@acta.nl.

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