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Sodium Bicarbonate and Hydrogen Peroxide: The Effect on the Growth of Streptococcus mutans

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Purpose. This in vitro experiment studied the effects of sodium bicarbonate and hydrogen peroxide on the cariogenic bacteria *Streptococcus mutans* through analysis with a spectrophotometer.

Methods. The growth of *S. mutans* was analyzed using seven different environments. Twelve wells in each of the seven rows of a multi-well plate were used to incubate the test materials. In combinations of 10 µl distilled water, 100 µl broth, 10 µl 10% sucrose, 10 µl *S. mutans*, 10 µl 10% sodium bicarbonate, and 10 µl 3% hydrogen peroxide, seven different environments were created for testing. Environments had either sodium bicarbonate or hydrogen peroxide with *S. mutans*, or a combination of sodium bicarbonate and hydrogen peroxide with *S. mutans*. The plate was incubated at 37°C and measured at 0, 18, 20, 22, 24, 26, 28, 30, and 42 hours by optical density with a spectrophotometer.

Results. Results showed bacterial growth was prevented by sodium bicarbonate, hydrogen peroxide, and the combination of sodium bicarbonate and hydrogen peroxide. Although hydrogen peroxide is bacteriocidal and sodium bicarbonate is bacteriostatic, there were no significant differences among the three treatment groups in spectrophotometer readings at any of the nine readings over 42 hours.

Conclusion. There was no significant difference among the effects of hydrogen peroxide, sodium bicarbonate, or the sodium bicarbonate and hydrogen peroxide combination, as measured by optical density. The hydrogen peroxide, sodium bicarbonate, and the sodium bicarbonate and hydrogen peroxide combination prevented bacterial growth of *S. mutans*. The results show that products containing these agents have the ability to stop the growth of *S. mutans*. Products containing sodium bicarbonate and/or hydrogen peroxide may be useful to caries-prone patients. More studies are needed to confirm these results on patients.

Keywords: Gerontology, Web-based instruction, distance education, dental hygiene education

Introduction

There are many products on the market today that aim to reduce the amount of dental biofilm and bacteria in the oral cavity. Over the years, numerous different ingredients and combinations of ingredients have been utilized to accomplish this task. There exists such an abundance of oral care products that finding safe, effective, and appropriate products can be very confusing for consumers.¹ With so many products to choose from, even oral health care professionals may have difficulty making these decisions. In fact, recommending the right products for each individual patient can be a challenging task. However, being knowledgeable about the efficacy of active ingredients in oral care products is advantageous to oral

health care professionals and can lessen confusion. This study was designed to compare the antimicrobial properties of sodium bicarbonate and hydrogen peroxide, two ingredients that are commonly used in oral health care products and advertised as effective antimicrobial agents. This study is designed to solely evaluate how these agents specifically affected *Streptococcus mutans*.

A common bacterium found in dental biofilm, *S. mutans* is widely thought of as one of the main bacteria that causes dental caries.² Because previous studies have shown that high concentrations of sodium bicarbonate and/or hydrogen peroxide inhibit or slow bacterial growth, it was hypothesized that the results of this study would mirror these findings. The most effective bacteriocidal formulation was expected to be a combination of sodium bicarbonate and hydrogen peroxide, as prior research indicated.³

Literature Review

New oral hygiene products are introduced on a regular basis. Each one has different guarantees and promises, and many go untested. It is important to read the current literature on oral health care products to determine which products may be safe and effective and which ones may not. Because many consumers do not take the time to inform themselves about this topic or may not have access to such information, it is imperative that oral health care professionals take responsibility for being knowledgeable sources regarding product efficacy and safety, such as with products containing sodium bicarbonate and/or hydrogen peroxide.

Sodium bicarbonate is an ingredient in numerous dentifrices, such as Colgate Baking Soda toothpaste (Colgate-Palmolive, Piscataway, NJ), PeroxiCare Tartar Control toothpaste (Church & Dwight, Princeton, NJ), Crest Baking Soda toothpaste (Procter & Gamble, Cincinnati, OH), Mentadent Tartar Control toothpaste (Chesebrough-Pond's USA, Greenwich, CT), Sensodyne with Baking Soda (Block Drug, Jersey City, NJ), and many more.⁴ Every major dentifrice manufacturer offers some type of baking soda toothpaste.⁴ Approximately 31% of the consumer market for toothpaste consists of dentifrice combined with baking soda, or a baking soda and hydrogen peroxide combination.⁴

Hydrogen peroxide, an inhibitor of microbial growth, may be found in dentifrices with sodium bicarbonate, such as Mentadent.⁴ Hydrogen peroxide alone has other uses in dentistry as well. For example, it is the active ingredient in tooth whitening products, such as Crest White Strips (Procter & Gamble, Cincinnati, OH) or Colgate Simply White (Colgate-Palmolive, Piscataway, NJ).

Sodium bicarbonate, NaHCO₃, has particular significance in dentistry because of its ever-growing use in dentifrices and mouth rinses. Sodium bicarbonate is appealing for its safety, low cost, low abrasivity, water solubility, acid buffering properties, compatibility with fluoride, and, in high concentrations, antibacterial properties.^{1,5}

Because of its alkalinity, or buffering capacity, sodium bicarbonate has the ability to neutralize acids produced by the microbes in dental biofilm. By neutralizing the acids, the enamel matrix of the tooth is less likely to be demineralized by the effect of the acids.⁵ Another factor in sodium bicarbonate's bacteriocidal abilities comes from changes in osmotic pressure. The hypertonic sodium bicarbonate solution causes the more hypotonic microbial cell to lose water, consequently dehydrating and eventually killing the cell.⁶ Although these are all desirable outcomes, some studies have shown that the sodium bicarbonate must be allowed to interact at least 30 minutes with the bacteria cell to be fully effective. Fletcher et al. showed that sodium bicarbonate had no effect on the viability of *S. mutans* when exposed only for a short time.⁷ A study by Pihlstrom et al. also showed no benefit of using sodium bicarbonate.⁸

In many cases, sodium bicarbonate was found to be effective against periodontal microorganisms. In a study by Rams et al., a five-minute exposure to sodium bicarbonate quickly immobilized spirochetes and motile rods.⁹ Gram-positive cocci bacteria, such as *S. mutans*, were also shown to be susceptible against 4% sodium bicarbonate in a study by Drake.⁵ Additionally, in a four-week study by Legier-Vargas et al., it was found that regular use of Arm & Hammer Dental Care dentifrice (Church & Dwight, Inc., Princeton, NJ), containing 65% sodium bicarbonate, lowered the level of *S. mutans* in

saliva.¹⁰ It has also been demonstrated that brushing with a dentifrice that contains high concentrations of sodium bicarbonate may not only suppress harmful bacteria in the mouth, but may also lead to increases in healthy bacteria.¹¹

As well as affecting microbial populations, sodium bicarbonate may also neutralize the acidic environment in the oral cavity produced by the bacteria present.¹⁰ The critical pH level in human dental biofilm is from 4.5 to 5.5.¹² When the pH is at the critical level, enamel is more susceptible to decalcification, which can lead to dental caries. The ideal pH of dental biofilm is a neutral 7.0. Acting as a buffering agent, sodium bicarbonate can raise the pH from the critical pH to a safer pH closer to neutrality. Thus, in addition to the ability of sodium bicarbonate to reduce the effects of harmful bacteria in the mouth, it also increases pH levels to a safe, neutral level.

Oral health care providers have utilized hydrogen peroxide, H₂O₂, therapeutically since the early 20th century. According to Marshall et al., hydrogen peroxide was used as early as 1913 to decrease plaque development.¹³ Today, hydrogen peroxide is used to reduce the number of bacteria associated with periodontal disease and to promote healing following gingival surgery. Usually, a 3% concentration is used for these purposes. With higher concentrations of hydrogen peroxide, there is some concern about the compound being corrosive to the soft tissues as well as being precarcinogenic or carcinogenic. However, little evidence exists that cancer is a direct effect of hydrogen peroxide when used in low concentrations in the oral cavity.¹³

The antibacterial properties of hydrogen peroxide are exhibited in the elimination of gram-positive and gram-negative bacteria. When hydrogen peroxide is exposed to other compounds, it breaks down very quickly into water and oxygen. Notably, the oxygen is released in the form of a free radical and, through oxidation, destroys microorganisms, particularly those that are anaerobic.¹³ More specifically, anaerobes lack the enzymes needed to detoxify products such as hydrogen peroxide. When hydrogen peroxide reacts with oxygen, a free hydroxyl radical is formed. This radical is a very potent oxidant and can attack any organic substance in the cell.¹⁴

The effect of hydrogen peroxide on the oral cavity, like many compounds, is controversial. Previous studies have shown that rinsing with a 1% solution of hydrogen peroxide has very little effect in reducing dental biofilm and gingivitis.^{13,15} Pihlstrom et al. also found no benefits of hydrogen peroxide in patients with mild to moderate periodontitis.⁸ Conversely, a study by Marshall et al. found that when a 3% hydrogen peroxide solution was irrigated into periodontal pockets twice a week for six months, the solution suppressed or eliminated *Actinobacillus actinomycetemcomitans*, a bacterium that is a common perpetrator in periodontal diseases.¹³

Researchers have investigated the effectiveness of sodium bicarbonate in combination with hydrogen peroxide in preventing and treating oral diseases. An investigation conducted by Keyes et al. showed a reduction in spirochetes and motile rods when the mixture was used.¹⁶ Sodium bicarbonate and hydrogen peroxide have also been shown to reduce bleeding, gingivitis, suppuration, and motility, as well as decrease pocket depths.¹⁶ Also, the cariogenic bacteria *S. mutans* has been shown to be susceptible to the combination of sodium bicarbonate and hydrogen peroxide.¹³ Finally, using this combination of sodium bicarbonate and hydrogen peroxide has been shown to be more bacteriocidal than using either ingredient alone.³

Even though sodium bicarbonate and hydrogen peroxide have been shown to be effective in many studies, some studies have shown the combination of sodium bicarbonate and hydrogen peroxide to be ineffective and non-beneficial to the oral cavity.^{17,18,19} One study showed that a sodium bicarbonate and hydrogen peroxide dentifrice had little to no beneficial effects on dental biofilm reduction, biofilm regrowth, and gingival scores, compared to a sodium fluoride dentifrice.²⁰ These results suggest that the utilization of sodium bicarbonate and hydrogen peroxide is no better than traditional home care methods.¹⁹ In some studies, the positive results were accredited to scaling and root planing during the experiment, and not to the sodium bicarbonate and hydrogen peroxide.¹⁹

While conflicting evidence exists on the use of sodium bicarbonate and hydrogen peroxide, the results supporting the reduction of *S. mutans* are noteworthy and suggest the need for more research. *S. mutans*, a capsular, gram-positive bacteria

found in dental biofilm, feeds off the sucrose from substances entering the oral cavity.^{2,12} The sticky, protective capsule helps the bacteria adhere to the teeth, growing stronger from each sucrose, fructose, or glucose molecule.¹⁴ The sugars are added to the capsule layer and provide energy to the bacteria. During the metabolic processing of the sugars by *S. mutans*, lactic acid is produced as a by-product. This acid causes the oral pH to lower and begins the demineralization of tooth enamel, leading to dental caries.¹⁴

Sodium bicarbonate and hydrogen peroxide have potential for reducing the effects of *S. mutans* in the mouth. Knowledge of these effects would be very beneficial for oral health care professionals, as it could affect which oral health care products are recommended to patients. The goal of this study was to test the effectiveness of sodium bicarbonate and hydrogen peroxide on the specific organism, *S. mutans*, through analysis with the spectrophotometer, and to evaluate the results.

Methods and Materials

To study the effects of hydrogen peroxide and sodium bicarbonate on *S. mutans*, a multi-well plate was used to hold 84 test environments. Growth of *S. mutans* was measured by optical density using a spectrophotometer. Wearing gloves and working under an EdgeGARD hood (Baker, Sanford, ME) to maintain a sterile work environment, investigators gave Rows A through G different environments, with 12 wells in each row. The rows were incubated for 42 hours and measured nine times at hours 0, 18, 20, 22, 24, 26, 28, 30, and 42.

Using a Labnet Labpette micropipette (Labnet International, Inc., Edison, NJ) with a different sterile tip for each constituent, Row A was filled with 100.00 µl of Brainheart Fusion broth and 10.00 µl of distilled water. Row B had 100.00 µl of broth and 10.00 µl 10% sucrose. Row C included 100.00 µl broth, 10.00 µl 10% sucrose, and 10.00 µl *S. mutans* isolated from a human cariogenic lesion (American Type Culture Collection). Row D held 100.00 µl broth, 10.00 µl 10% sucrose, 10.00 µl *S. mutans*, and 10.00 µl sodium bicarbonate. Row E contained 100.00 µl broth, 10.00 µl 10% sucrose, 10.00 µl *S. Mutans*, 10.00 µl sodium bicarbonate, and 10.00 µl 3% hydrogen peroxide. Row F was filled with 100.00 µl broth, 10.00 µl 10% sucrose, 10.00 µl *S. mutans*, and 10.00 µl 3% hydrogen peroxide. Row G had 100.00 µl broth and 10.00 µl *S. mutans*. Rows A, B, C, and G were used as controls for the experiment. Rows D, E, and F were the experimental groups. Table I illustrates the factors used in each row.

Table I: Components of Experimental and Control Groups

| | Brainheart Fusion Broth | Distilled Water | 10% Sucrose | <i>S. mutans</i> | Sodium Bicarbonate (NaHCO ₃) | 3% Hydrogen Peroxide (H ₂ O ₂) |
|--------|-------------------------|-----------------|-------------|------------------|--|---|
| Row A* | 100 µl | 10.00 µl | ----- | ----- | ----- | ----- |
| Row B* | 100 µl | ----- | 10.00 µl | ----- | ----- | ----- |
| Row C* | 100 µl | ----- | 10.00 µl | 10.00 µl | ----- | ----- |
| Row D | 100 µl | ----- | 10.00 µl | 10.00 µl | 10.00 µl | ----- |
| Row E | 100 µl | ----- | 10.00 µl | 10.00 µl | 10.00 µl | 10.00 µl |
| Row F | 100 µl | ----- | 10.00 µl | 10.00 µl | ----- | 10.00 µl |
| Row G* | 100 µl | ----- | ----- | 10.00 µl | ----- | ----- |

*These rows were used as control groups.

After Rows A through G were filled, the lid was placed on top of the well plate. The plate was tapped gently on the countertop to mix each well. No droplets remained on the side of the cylinder. Tapping was carried out until all droplets were mixed at the bottom of the cylinder.

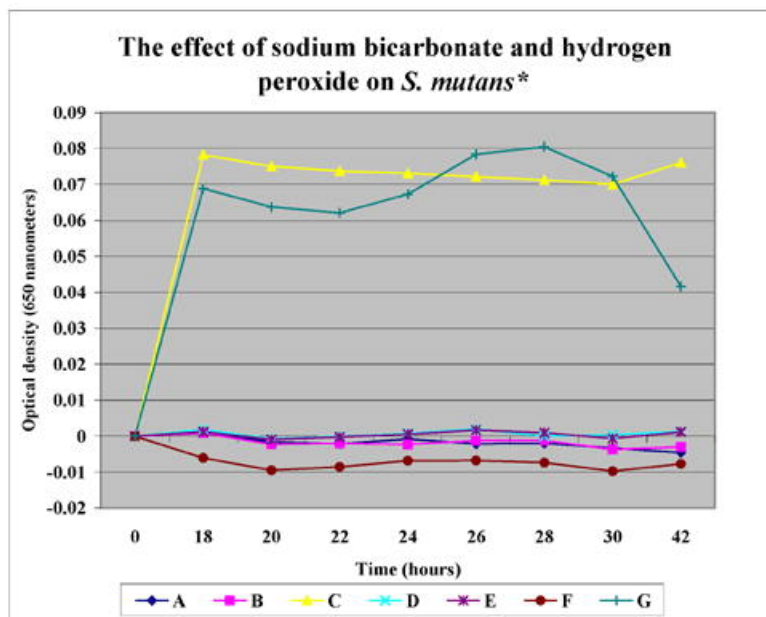
The plate was placed in the Emax Precision Microplate Reader (Molecular Devices Corp., Sunnyvale, CA) with the lid off. The initial reading of optical density was taken with the spectrophotometer at 0 hours. An increase in bacterial population causes density in the well to increase, therefore increasing the optical density measurement. A decrease in bacterial population would decrease the optical density measurement. Conditions were set with the Elisa Reader (Bio-Tek Instruments, Inc., Winooski, VT) computer program. Conditions of the spectrophotometer were placed at 650 nm wavelength to read the optical density. Immediately after the optical density reading was taken and data were collected, the lid was replaced on the plate and transferred into the Thelco Precision Scientific incubator (Precision Scientific, Winchester, VA) at 37°C

until the next reading. Data were saved in the computer through the Elisa Reader program after results were printed out. Results were analyzed using a repeated measures analysis. If the overall tests were found to be significant, the means were separated using the least significant difference test.

Results

Hydrogen peroxide, Row F, showed bacteriocidal qualities as evidenced by a decrease in optical density, indicating that initial *S. mutans* bacteria were being killed (Figure 1). Sodium bicarbonate (Row D) and the sodium bicarbonate and hydrogen peroxide combination (Row E) exhibited bacteriostatic properties because optical density remained approximately at zero. No increase or growth, as well as no decrease or loss, of *S. mutans* occurred. The bacterial growth was inhibited. There was little change when no bacteria were present for growth (Rows A and B). *S. mutans* demonstrated growth when no influencing agents, such as sodium bicarbonate and/or hydrogen peroxide, were added to prevent bacterial growth (Rows C and G).

Figure 1: The Effect of Sodium Bicarbonate and Hydrogen Peroxide on *S. mutans*



Although Row F appeared to have the most influence affecting *S. mutans*, no statistical significance was found among any wells containing sodium bicarbonate, hydrogen peroxide, or a combination of these agents (Table II). A significant statistical difference occurred between Rows D, E, and F that contained sodium bicarbonate, the hydrogen peroxide and sodium bicarbonate combination, and hydrogen peroxide from Rows C and G that contained *S. mutans* alone. After statistical analysis of each treatment row, the results revealed that there was no effect of time on treatment (Table III). The treatment remained consistent from hour to hour.

Table II: Least Square Means of Treatments

| Effect | Treatment | Estimate | Standard Error | DF | T Value | Pr > t |
|-----------|-----------|----------|----------------|----|---------|---------|
| Treatment | A* | -.00221 | .02794 | 77 | -.08 | .9372 |
| Treatment | B* | -.00186 | .02794 | 77 | -.07 | .9470 |
| Treatment | C* | .08027 | .02794 | 77 | 2.87 | .0052 |
| Treatment | D | .000583 | .02794 | 77 | .02 | .9834 |
| Treatment | E | .000448 | .02794 | 77 | .02 | .9872 |
| Treatment | F | -.08067 | .02794 | 77 | -2.89 | .0050 |
| Treatment | G* | .006717 | .02794 | 77 | 2.40 | .0186 |

*These rows were used as control groups.

Table III: The Effect of Time on Treatments

| Effect | Numerator DF | Denominator DF | F Value | Pr > F |
|----------------------|--------------|----------------|---------|--------|
| Treatment | 6 | 77 | 3.61 | 0.0033 |
| Hour | 7 | 539 | 1.05 | 0.3922 |
| Treatment times Hour | 42 | 539 | 1.00 | 0.4776 |

Discussion

Few differences were present in the results, and no statistical differences were shown among sodium bicarbonate, hydrogen peroxide, and the sodium bicarbonate and hydrogen peroxide combination. No significant statistical difference was found between *S. mutans* with sucrose and broth, and *S. mutans* with broth only. However, a significant statistical difference was present between the rows containing sodium bicarbonate and/or hydrogen peroxide and the rows not containing these agents. Differences among the groups of rows occurred because of the ability or inability to control bacterial growth. When sodium bicarbonate and/or hydrogen peroxide were present with *S. mutans*, bacterial growth was limited. When these were not present with the bacteria, significant growth occurred.

These results support the findings by Rams et al., that sodium bicarbonate is effective against periodontal pathogens.⁹ These results also agree with the results of the studies by Drake and by Legier-Vargas et al., that sodium bicarbonate is specifically effective against *S. mutans*.¹⁰ The data did not support the conclusion by Pihlstrom et al., that sodium bicarbonate was ineffective.⁸

The results also support the previous findings by Marshall et al., that hydrogen peroxide has antibacterial benefits.¹³ The findings did not reinforce studies that found hydrogen peroxide to have no benefits against oral pathogens.⁸

The combination of sodium bicarbonate and hydrogen peroxide was found to be effective against *S. mutans*. This result again agrees with the study by Marshall et al.¹³ This result also supports other studies that found the combination to be beneficial. However, the sodium bicarbonate and hydrogen peroxide combination was not found to be better than using only sodium bicarbonate or hydrogen peroxide alone and, therefore, does not confirm the findings by Miyasaki et al.³ The results from this study also do not support the studies by Beiswanger et al.,¹⁷ Cerra and Killooy,¹⁸ and Bacca et al.,²⁰ which found no benefits with the sodium bicarbonate and hydrogen peroxide combination.

Conclusion

When recommending products to patients, oral health care professionals should be aware that hydrogen peroxide and sodium bicarbonate do affect the growth of *S. mutans*, an effect which may be beneficial to patients concerned with caries and periodontal disease. Hydrogen peroxide may work more effectively by possibly eliminating *S. mutans*, while sodium bicarbonate may only inhibit *S. mutans*. Any products containing either or both of these ingredients could be recommended to patients.

These results were limited because they were studied only in vitro. Results were also limited because of the small sample size. Recommendations for future studies would be to conduct the study in vivo and to have a larger sample and longer experimental time. More studies need to be done on the effects of sodium bicarbonate and hydrogen peroxide to determine their maximum clinical significance.

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Notes

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