CHLORHEXIDINE: AN INVESTIGATION OF THE GOLD STANDARD ON WOUND HEALING

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

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Chlorhexidine (CHX), the "gold standard" adjective aid in dentistry, has been used for over 60 years during and after various procedures. The purpose of this research is to investigate the full effects of CHX by reviewing classic and current research in order to update and educate dental providers and help them make an informed decision regarding its use. Research indicates that at the clinical concentration of 0.12% chlorhexidine is beneficial for biofilm and gingivitis prevention, however research suggests that if the periodontal ligament is altered, chlorhexidine may prolong wound healing by inhibiting fibroblasts, as well as other regenerative cells. This may interfere with the post-treatment goal of tissue healing and regeneration and potentially cause more harm than good. We have explored alternatives to chlorhexidine including: essential oils, delmopinol, and boric acid, and although these prospective alternatives look promising, further research is needed to investigate all of the benefits and limitations. In vitro and in vivo studies reveal statistical significance of regenerative cell inhibition, however human clinical trials are necessary to determine the full clinical significance of chlorhexidine on wound healing. It is important for dental professionals to maintain a healthy level of skepticism regarding

traditional therapies and encourage continual research to provide optimal and ethical patient treatment.

KEY WORDS

CHX: Chlorhexidine

NSPT: Non-surgical Periodontal Therapy

HGF: Human Gingival Fibroblasts

HPDLF: Human Periodontal Ligament Fibers

BOP: Bleeding on Probing

INTRODUCTION

Since the 1950's, chlorhexidine (CHX) has been revered as the "gold standard" antimicrobial adjunct used in dentistry due to its potent antimicrobial properties.[1] This narrative review will investigate the full effects of chlorhexidine, specifically related to wound healing. Perhaps, the "gold standard" is not as safe and effective as currently believed and alternative methods should be considered to provide patients with the best care achievable. Research on this topic supports the current NDHRA priority area of client level oral health care, health promotion, and treatment behavior and products. Current concerns regarding CHX include staining of the oral tissues; supra-gingival calculus formation; xerostomia; unusual or unpleasant taste; and decreased taste perversion.[2] However, more recent research indicated that chlorhexidine has more serious side effects, such as the inhibition of fibroblasts and other regenerative cells.[1] This narrative review will analyze the extent of inhibition at the clinical concentration of 0.12%. If inhibition is clinically significant, dental professionals should reconsider the use of CHX during and after invasive dental procedures.

The articles reviewed suggest that there is a significant cytotoxic effect on human gingival fibroblasts *in vitro*, demonstrating a delay in wound healing. Furthermore, *in vitro* studies demonstrate that although low concentrations (0.002%) of CHX do not exhibit cytotoxic effects, antimicrobial properties are inhibited. In addition, *in vivo* studies indicate that CHX delays wound healing on a cellular level. There are many potential alternatives that have significant antimicrobial properties while facilitating healing. Potential alternatives include essential oils, delmopinol, and boric acid. Pre-clinical studies suggest a statistically significant inhibition of fibroblasts when using CHX; however, the practical significance of fibroblast

inhibition and wound healing in humans needs to be further investigated. Pre-clinical studies suggest that there is fibroblast inhibition *in vivo*, but further research is necessary to determine to what extent. Therefore, the purpose of this narrative review is to discuss the pros and cons of using CHX, describe and investigate the adverse effects of CHX in relevant scientific literature, and identify alternatives to CHX in dental treatments and therapies to achieve the ultimate goal- provide the best care for patients.

SECTION I

BENEFITS AND HISTORY OF CHLORHEXIDINE USE IN DENTISTRY

Objective 1

In 1979, exploratory research by Loe and Schiott concluded that CHX was beneficial for reducing dental biofilm at a concentration of 0.2%.[3] The studies compared groups practicing no oral hygiene regimen to groups using different methods of CHX application with no other oral hygiene regimen. The results indicated the group using topical CHX demonstrated near complete absence of biofilm formation. However, when used as a mouth rinse, CHX was demonstrated to be less effective due to results of less clinically significant decreased biofilm levels and no effect on gingival inflammation. This study demonstrated that CHX successfully inhibits dental biofilm at 0.2% concentration when applied topically, but the effects on gingivitis and periodontitis were inconclusive. Moreover, in 1970, Lindhe and Hamp did a similar study on topical application of 2% CHX on chronic gingivitis *in vivo*. After one week of topical application of 2% CHX on the teeth and gingiva, clinically significant inhibition of biofilm and improvement in gingival indices were observed. Furthermore, researchers measured the exudate score using the styrene-butadiene block copolymer (SBC). Starting from day 0, the exudate decreased from 1.06 to 0.06 on the 42th day of the experiment. These studies suggest both statistically and clinically that CHX has antimicrobial properties and significantly reduces the formation of biofilm and prevents the formation of gingivitis.[4]

SECTION II

ADVERSE EFFECTS OF CHLORHEXIDINE USE

Objective 2

Conversely, a study conducted by Wyganowska-Swiatkowska, Kotwicka, and Urbaniak, et al. in 2016 evaluated the effects of CHX at both low and high concentrations on human gingival fibroblasts *in vivo*. The researchers evaluated the effects of 0.002%, 0.01%, 0.02%, 0.04%, and 0.2% CHX dilutions on cultured human gingival fibroblast cells. The cells treated with 0.01% and 0.02% CHX dilutions exhibited a decrease in mitosis with no significant changes in cell morphology ($p>0.05$).[5] The cells treated with the stronger dilutions of CHX (0.04 and 0.2%) exhibited a progressive decrease in mitosis, cell growth, and the researchers observed cell morphology changes- cells turning small and round versus spindle shaped. Evaluating gingival fibroblasts cell-cycle under different concentrations of CHX demonstrated dose-dependent results. The fibroblast cells exposed to the highest concentration of CHX (0.2%) had the largest amount of cells at the synthesis (S) phase.[5] Furthermore, cells exposed to the highest concentrations of CHX were found to have the highest rate of apoptosis. The results of this study are significant because the primary function of gingival fibroblasts is wound healing, and when inhibited, may prolong tissue regeneration, thus doing more harm than good post-treatment.[2] Fibroblasts are also the first cells to respond to infection in connective tissue and release interleukin 6 and interleukin 9 to recruit neutrophils. After nonsurgical periodontal therapy (NSPT) and periodontal surgeries, the goal is tissue healing and regeneration. To prevent infection, clinicians often irrigate with CHX or send patients home with a CHX prescription, however this may be counter intuitive. In 2016, a study conducted by Wyganowska-

Swiatkowska, Kotwicka, Urbaniak, et. al. indicates that at a lower concentration 0.002%, CHX does not exhibit fibroblast cell mortality or interfere with morphology.[5] However, the minimum inhibitory concentration of CHX on periodontal pathogens is 0.0012%. This implies that there is a very narrow window of CHX dosage that could be both safe and effective. Further research is needed to determine if there is a safe dosage between the minimum inhibitory concentration for periodontal pathogens and the minimum dosage that interferes with gingival fibroblasts. Additionally, in 2018 Liu, Werner, Kirsch, et al. conducted an *in vitro* study on the effects of CHX on three different human cells-fibroblasts cells, myoblasts, and osteoblasts. Results from the study demonstrated that daily application of 2% of CHX permanently stops fibroblast cells, myoblasts, and osteoblasts.[6] This indicates that not only can CHX can have cytotoxic effects in the oral cavity but can also have a detrimental effect on bone healing, which is critical for medical purposes because it could potentially delay healing. In 2018, Liu et al. study indicated that use of 2% CHX had both a significant cytotoxic ($p<0.05$) and delayed bone healing ($p<0.01$) effect on the oral cavity.[6] Researchers found that if cells are exposed to 2% CHX for 2-3 mins, the growth of fibroblasts is reduced. Conversely, the rate of cellular fibroblast reduction was not statistically significantly impacted when exposed for one minute ($p>0.05$).[6] This could potentially indicate that the most important factors when it comes to wound healing are the length of exposure and the extent of exposure to the tissues.

Another study conducted by Barbosa, Prada-López, Álvarez, et al. in 2015 analyzed the effect on CHX on Post Extraction Bacteremia (PEB).[7] A control group, which received no prophylaxis was compared to a group that rinsed with 0.2% CHX before tooth extraction (CHX-MW), a group that rinsed with 0.2% CHX and a subgingival irrigation with 1% CHX for one minute (CHX-MW/SUB_IR), and a group that rinsed with 0.2% CHX and a supragingival

irrigation with 1% CHX for one minute (CHX-MW/SUPRA_IR). The baseline for PTB was 2%. The prevalence of bacteremia was 4% for CHX-MW, 12% for CHX-MW/SUB_IR, and 6% for CHX-MW/SUPRA_IR. These results demonstrated that although the group that rinsed with 0.2% CHX mouthwash exhibited a reduction in PEB duration, the group that used subgingival irrigation for one minute actually decreased efficacy of the CHX mouthwash. Based on these results, researchers do not recommend irrigating the tooth subgingivally or supragingivally before tooth extractions because it is ineffective and can potentially decrease efficacy.[7]

SECTION III

ALTERNATIVES TO CHLORHEXIDINE

Objective 3

When analyzing the classic research that indicated that CHX is beneficial, it is important to note that the effect of CHX was being studied on biofilm formation and gingivitis. Although these effects have been demonstrated consistently over time, the effects of CHX as an irrigant and as an adjunctive therapy to non-surgical periodontal debridement remain uncertain. As mentioned above, more recent studies indicate that when the periodontal ligament is altered, CHX can interfere with wound healing by inhibiting the formation of fibroblasts as well as impairing several other vital regenerative cells. It is important to further examine the effects of CHX to determine when its usage should be indicated and when alternative methods would display greater benefits. First and foremost, treatment goals should be considered when determining the most beneficial alternative for patients. In addition, the type of procedure should be considered when selecting an adjective therapy. CHX may be contraindicated during and after invasive procedures involving the alteration of the periodontal ligament due to statistically significant cytotoxic effects on regenerative cells. As previously mentioned, alternatives to CHX include essential oils, delmopinol, and boric acid. The following section will investigate the effects of each potential alternative.

Essential oils

Research suggests that essential oils have been successful in reducing biofilm and preventing gingivitis without displaying any cytotoxic effects, and could be indicated for non-invasive procedures, such as rinsing.[8] Although it was previously thought that essential oils had

less substantivity as compared to CHX, current research indicates that CHX substantivity could contribute to its cytotoxic effects. A study by Tsourounakis, et al. in 2013 demonstrated if cells are exposed to 5% CHX for three minutes, there is a statistically significant (P= 0.03) increase in cell mortality. However, when cells are exposed to 10% essential oils for as long as five minutes, there is no statistically significant reduction in number of cells. This is an important factor when determining the length of time that patients should rinse. This study also revealed that the full-strength commercially available concentrations of essential oil rinses and CHX could display toxic effects on gingival fibroblasts. However, the effects from essential oils are short-lived and do not display any long term damage, while the effects from CHX inhibit cell migration, eventually leading to cell mortality.[8] In addition, essential oils could serve as an alternative to CHX as an at home mouth rinse after invasive dental and periodontal procedures.

Delmopinol

Another potential alternative is delmopinol. Delmopinol has an anti-inflammatory effect and is effective in reducing dental biofilm and gingivitis. In 2016, an *in vitro* study by Luis compared CHX (0.2%), essential oils, and delmopinol. This statistical data indicated that while delmopinol mouth rinse demonstrated a similar inhibition of anaerobic bacteria as aerobic bacteria, it was indicated that delmopinol had a weaker inhibitory effect on biofilm as compared to essential oils and CHX.[2] These results suggest that delmopinol could be a potential alternative for invasive treatments such as NSPT because of demonstrated safety and effectiveness when used subgingivally.[9] However, it must also be taken into consideration that anaerobic bacteria are often the gram-negative bacteria which is more pathogenic than aerobic gram-positive bacteria. Additionally, a classic study by Hase, Attstrom, Edwardsson, et al. in 1998 compared 0.2% delmopinol with 0.2% CHX over a six month period. The results indicated that 0.2% delmopinol

reduced bleeding on probing (BOP) at similar rates. 0.2% CHX; P-value was less than 0.05.[2] The staining index was found higher in delmopinol than CHX, however, the delmopinol staining was easier to remove compared to CHX. CHX staining has been known to be difficult to remove, even with professional prophylaxis. Furthermore, the researchers demonstrated higher biofilm scores were found in patients using delmopinol in comparison with CHX.[9] To explain the difference, CHX is considered to be bacteriostatic and delmopinol limits antibacterial activity. Therefore, it is indicated that delmopinol can attach to dental biofilm more efficiently. In addition, patients demonstrated better compliance due to less staining and taste tolerance while using delmopinol than CHX. According to table 3 (Hase, Attstrom, Edwardsson, et al. in 1998), statistical data demonstrated there were nine patients withdrawn from the study in the CHX group, where as only three patients withdrawn in the delmopinol group. Due to patient compliance, inhibition of harmful gram negative bacteria, similar biofilm inhibition and inflammation reduction properties, delmopinol should be considered as a potential to CHX.[2] However, further studies on the effect of delmopinol on regenerative cells are needed to determine whether there are cytotoxic effects.

Boric acid

Boric acid is the final potential alternative to CHX discussed in this narrative review. A randomized clinical trial conducted by Saglam, et. al. in 2013 suggested that boric acid could be used after NSPT as an antimicrobial and anti-inflammatory agent.[10] The study compared the effects of three post-NSPT irrigation adjunctive aids: boric acid, CHX, and the control, saline. The outcomes studied were probing depth; clinical attachment loss; bleeding on probing; gingival and biofilm index; and periodontal pathogens, specifically *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsynthia*. The outcomes listed above were evaluated at

baseline, 1 month post-therapy, and 3 months post-therapy. The researchers utilized ANOVA and Tukey HSD tests to evaluate statistical differences among all treatment groups for each of the parameters tested. The alpha values were all set to 0.05. The results suggested that boric acid was just as effective as CHX in reducing probing depth and clinical attachment loss with no statistically significant differences between the two treatment groups. The results of probing depth and clinical attachment loss reduction however, were clinically significant between boric acid and CHX groups. Regarding probing depth reduction from baseline and 1 month post-treatment, the mean probing depth was reduced by 0.73mm in the control group, 0.65mm in the CHX group, and 0.80mm in the boric acid group. There was a probing depth reduction difference of 0.15mm between the CHX group and boric acid group, suggesting that between baseline and 1 month, there may be a slight delay in healing in the CHX group while the boric acid group may promote faster healing as compared to the control. At 3 months post-treatment, the three results were comparable for probing depth reduction. The control showed a final reduction of 0.88mm, the CHX group showed a reduction of 0.78mm and the boric acid group showed a reduction of 0.90mm. In regards to clinical attachment loss, the boric acid group demonstrated clinically significant CAL reduction at both 1 month and 3 months post-treatment compared with CHX, but the results were clinically similar to the control group. At 1 month post-treatment, the CAL was reduced by 0.74mm in the control group, 0.66mm in the CHX group, and 0.81mm in the boric acid group. At 3 months post-treatment, the CAL was reduced by 0.88mm in the control group, 0.80mm in the CHX group, and 0.90mm in the boric acid group. These results suggest that boric acid group demonstrated higher, clinically significant reductions in CAL and PD compared with the control and CHX groups. The research described a lack of statistically significant differences in periodontal pathogen reductions among all three groups: boric acid,

CHX, and saline. At 1 month post-treatment and 3 months post-treatment, the *P* values were greater than the alpha value which was set at 0.05. For *P. gingivalis,* the *P* value was 0.14 at 1 month and 0.663 at 3 months. For *T. forsynthia,* the *P* value was 0.338 at 1 month and 0.277 at 3 months. Similarly, for *T. denticola,* the *P* value was 0.688 at 1 month and 0.739 at 3 months. Therefore, there were no clinical or statistical differences among the control, CHX, and boric acid on periodontal pathogen reduction at all three study points. These results are significant for clinicians to be mindful of when prescribing antimicrobial rinses and irrigations following NSPT, because their choice of antimicrobial agents may not be any more effective at decreasing periodontal pathogens than NSPT with saline. Finally, the researchers observed that boric acid was nontoxic to human gingival fibroblasts (HGF) and human periodontal ligament fibers (HPDLF). This was established by conducting a pre-clinical *in vitro* test on the cytotoxicity of boric acid and CHX. The researchers used a real-time cell analyzer to analyze the cell viability of HGF and HPDLF after treatment with various concentrations of boric acid and CHX. The results suggested that up to 0.75% boric acid was non-toxic to the HGF and HPDLF. Conversely, all concentrations of CHX tested, which varied from 0.1% to 0.0015625% CHX solutions showed significant cell mortality in both HGF and HPDLF. This indicates that boric acid should be further studied and considered as a potentially safer and more effective subgingival irrigant than what is widely used today in dentistry. This study was the first to examine the clinical effects of boric acid as a subgingival irrigant post-NPST in humans, however the study failed to evaluate the effects of boric acid during surgical procedures or as a home-care aid after dental therapy. Further research is necessary to verify the results of this study and to evaluate the use of boric acid in other dental scenarios.[10]

CONCLUSION

Research regarding the benefits and limitations of CHX demonstrated an inhibition of biofilm formation at the clinical concentration of 0.12%. In addition, it is indicated that CHX can reduce gingival inflammation when applied topically on a daily basis. However, the usage of CHX during and after invasive procedures should be limited due to its cytotoxic properties. Research indicates that if the periodontal ligament is altered during treatment, regenerative cells such as fibroblasts are inhibited, thus delaying wound healing. Alternative methods, such as boric acid and delmopinol, could be considered as appropriate alternatives for use during and after invasive procedures, such as NSPT. In addition, alternative methods such as essential oils and delmopinol should be considered for reducing biofilm and gingivitis post treatment due to their antimicrobial properties with little to no known detrimental effects on wound healing. The goal as health care professionals is to improve the quality of life for our patients. In order to do so, we practice the principle of nonmaleficence, meaning “do no harm”. It is our responsibility to be aware of current research, maintain a healthy level of skepticism when it comes to traditional methods, and what is revered as the “gold standard”, and practice oral health care based on the most current evidence based research.

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