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Short communication

Antibacterial effect of parabens against planktonic and biofilm *Streptococcus sobrinus*

Steinberg Doron [a,*], Michael Friedman [b], Maher Falach [a,b], Ester Sadovnic [c], Hirschfeld Zvia [c]

[a] *Department of Oral Biology, Faculty of Dental Medicine, Hebrew University-Hadassah, P.O. Box 12272, Jerusalem 91120, Israel*
[b] *School of Pharmacy, The Hebrew University, Jerusalem, Israel*
[c] *Department of Restorative Dentistry, Faculty of Dental Medicine, Hebrew University-Hadassah, Jerusalem, Israel*

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Abstract

Tooth decay is an infectious disease caused by bacteria immobilized on the tooth surfaces. Eradication of these bacteria, for example *Streptococcus sobrinus* (*S. sobrinus*), from the oral cavity is essential in the prevention and treatment of tooth decay. We have tested the antimicrobial effect of several paraben derivatives such as methyl (MP), ethyl (EP), propyl (PP) and butyl (BP) against immobilized and planktonic *S. sobrinus*. The antibacterial effect was as follows: MP > EP > PP = BP on immobilized bacteria and MP > EP = PP > BP on planktonic bacteria. An antibacterial synergistic effect was found between several combinations of parabens on immobilized and planktonic *S. sobrinus*. Our results indicate that parabens are potential antibacterial agents against immobilized or planktonic bacteria found in the oral cavity.

Keywords: Paraben; Biofilm; Dental plaque; *Streptococcus sobrinus*

1. Introduction

Tooth decay (caries) is a worldwide oral disease affecting all ages, ethnic groups and genders. Bacterial accumulation on the surface of the tooth (dental plaque biofilm) is the main precursor of caries. Properties of bacteria in the biofilm are unique and may differ from planktonic bacteria. It is conceivable that due to these differences, the effect of antibacterial agents may differ between immobilized bacteria in the biofilm and bacteria in suspension [1,2].

Streptococcus sobrinus (*S. sobrinus*) is one of the most cariogenic bacteria of mutans streptococci [3,4] and elimination of cariogenic bacteria such as *S. sobrinus* is a fundamental step in preventing and treating dental caries. Several antibacterial drugs are being used for prevention or treatment of tooth decay [5,6].

Parabens (hydroxybenzoates), are one of the most common preservative agents in the food and pharmaceutical industries. Parabens possess minimal side effects [7]; thus, they can act as potential drugs for use in the dental field and lately attention has been drawn to their use as antibacterial agents in the dental field. For example, it was shown that parabens could affect glycolysis of *Streptococcus mutans* by irreversibly inhibiting the phosphotransferase system (PTS) [8]. Furthermore, parabens were found to be potent inhibitors of arginolysis in several oral streptococci [9]. Sissons et al. [10] have shown that methyl paraben is effective against immobilized dental plaque bacteria in a biofilm model. Steinberg et al. [11] reported that parabens had an antibacterial effect when used in mouthwashes or when incorporated into slow release devices in human volunteers.

The purpose of this investigation was to assess the antimicrobial activity of several derivatives of parabens and a possible antibacterial combination against immobilized and planktonic *S. sobrinus*, as a step in optimiz-

* Corresponding author. Tel.: +972-2-6757633; fax: 972-2-6439219.
E-mail address: dorons@cc.huji.ac.il (S. Doron).

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ing the concentration of parabens as antibacterial agents in the oral cavity.

2. Materials and methods

2.1. Active agents

Four derivatives of parabens were used in this study: methyl paraben (MP), ethyl paraben (EP), propyl paraben (PB) and butyl paraben (BP) (Sigma, St. Louis, MO, USA).

2.2. Immobilized biofilm bacteria

The microorganism used in this study was *S. sobrinus* 6715. The in vitro model used for testing the effect of parabens on dental plaque was similar to a model previously described by Schilling et al. [12], Steinberg et al. [13] and Steinberg and Rothman [14].

2.2.1. Bacteria preparation

S. sobrinus were grown at 37 °C under aerobic conditions supplemented with 5% CO_2. Following 18 h incubation, the bacterial suspension was centrifuged for 10 min at $3000 \times g$. The supernatant fluid was then discarded and the bacterial pellet was resuspended in buffered KCl (pH 6.5, 55 mM). This washing procedure was repeated three times. The optical density of the suspension was adjusted to 1.5 at 540 nm with the buffered KCl.

2.2.2. Biofilm formation on hydroxyapatite

Hydroxyapatite (HA) beads were prepared as follows. Forty milligrams of HA beads (Type 1 Bio-Rad Hercules, USA) were washed three times with buffered KCl. Next, the washed beads were covered with 1 ml of the above prepared suspension of *S. sobrinus* 6715. After incubation for 2 h at 37 °C, the beads were washed three times with buffered KCl to remove loose and unbound bacteria.

2.2.3. Effect of parabens on biofilm bacteria

The immobilized bacteria on HA, prepared above, were exposed to different concentrations of parabens, either separately or in combinations of two types of parabens, and incubated for 18 h at 37 °C. The paraben solution was then discarded and the beads were washed three times with buffered KCl. The viability of the surface-bound bacteria was assessed as follows: the HA beads were subjected to sonication by a probe for three intervals of 1 min each in an ice bath, after which aliquots of bacteria from the supernatant fluid were serially diluted in PBS. The viability of bacteria was determined by plating 0.05 ml of each bacterial dilution on mitis salivarius agar supplemented with bacitracin [15], a selective agar medium for mutans streptococci. Following 72 h of incubation, bacterial growth on the agar plates was recorded using a colony counter (New Brunswick Scientific, New Brunswick, USA). Viable bacteria were recorded by calculating the number of colony forming units (CFU) and the dilution factor. Bacterial growth in biofilm, not exposed to parabens, was used to determine the maximal growth levels of *S. sobrinus*. The results are presented as percentage bacterial viability calculated from the maximal viability counts. Each experimental set was repeated three times.

2.3. Planktonic bacteria

The antibacterial activity of the four types of parabens was examined for each derivative. Briefly, 0.1 ml of an overnight culture of *S. sobrinus*, grown as described above, was added to 5.5 ml of TSB supplemented with 0.5 ml of parabens at different concentrations. The test tubes were incubated at 37 °C in an atmosphere enriched with 5% CO_2. After 18 h of incubation, the bacterial suspensions were serially diluted and each dilution was plated on four plates of selective agar media for mutans streptococci [15]. Viable bacterial counts were performed as described above for the biofilm bacteria. Each experiment was repeated three times.

2.4. Combination effect

After establishing the MIC for each of the derivatives of the parabens separately, the potential combination effect between MP, EP, PP and BP derivatives of parabens compounds in solution and in biofilm was investigated.

3. Results

Our results demonstrate a dose-dependent antibacterial effect of parabens against *S. sobrinus*. The antibacterial effects for the paraben derivatives tested (methyl, ethyl, propyl, butyl) against *S. sobrinus* immobilized in biofilm were between 0.5 and 0.062%. (MP > EP > PP = BP) (Table 1). Similar trends in antibacterial values were also obtained with planktonic bacteria (MP > EP = PP > BP).

The effects of combinations of two different types of parabens were tested on immobilized bacteria and on planktonic bacteria.

Bacterial growth on biofilm was affected by different combinations of pairs of different parabens (Fig. 1). No bacterial growth was recorded in the biofilm when either EP or PP or BP was introduced at a concentration of 0.03% together with MP at concentrations

Table 1
Antibacterial values (w/v%) of parabens against *S. sobrinus*

	MP	EP	PP	BP
Immobilized bacteria	0.5–0.25	0.25–0.125	0.125–0.062	0.125–0.062
Planktonic bacteria	0.5–0.25	0.25–0.125	0.25–0.125	0.125–.062

Range of minimal inhibitory concentrations of methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), butyl paraben (BP) against HA-immobilized and planktonic *S. sobrinus*.

higher than 0.03%. Combinations of parabens showed different antibacterial patterns of inhibition of planktonic bacteria compared with the immobilized bacteria (Fig. 2). The occurrence of full inhibitory effect for planktonic bacteria required higher concentrations of parabens compared with immobilized bacteria. A stronger antibacterial effect occurred with the combinations of parabens on immobilized bacteria than on planktonic bacteria.

4. Discussion

The debate in the dental field regarding the eradication of cariogenic bacteria has not ceased. New drugs and drug applications are constantly being tested. The use of combinations of parabens has been shown to have a synergistic effect on planktonic bacteria [16,17] although a complete antibacterial effect is not always

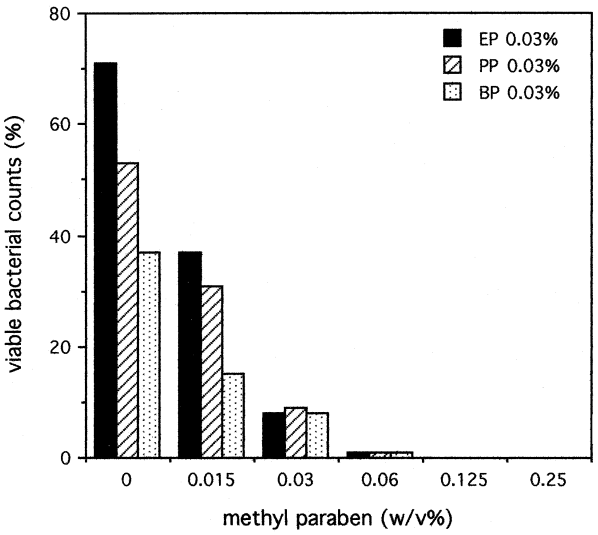

Fig. 1. The antibacterial effect of a combination of methyl paraben (MP) with ethyl paraben (EP), propyl paraben (PP), butyl paraben (BP) against immobilized *S. sobrinus*.

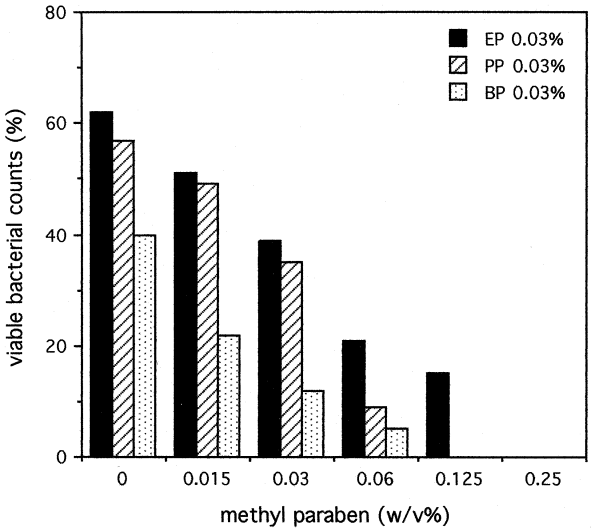

Fig. 2. The antibacterial effect of a combination of methyl paraben (MP) with ethyl paraben (EP), propyl paraben (PB), butyl paraben (BP) against planktonic *S. sobrinus*.

achieved. The exact antibacterial activity of parabens is not fully understood, but appears to be via alteration of cell membrane properties [18]. Changes in the integrity of the membrane in the presence of parabens, allow intercellular solutes to leak from the cells [19]. Ma and Marquis [8] have shown that the level of effectiveness of parabens in affecting a drop in pH values due to bacterial fermentation in an excess of glucose was BP > PP > EP > MP. According to our results, the antibacterial values of these parabens on planktonic *S. sobrinus* were also in this order. Ma and Marquis [8] have further shown that BP can irreversibly inhibit F-ATPase of *S. mutans*. Our study and Ma and Marquis' [8] study indicate that BP has the greatest potential as an antibacterial and anticaries agent compared with other parabens tested. BP was also found to be superior to MP, EP and PP in solution in biofilm for killing bacteria. Sissons et al. [10] have tested the duration of the effect of MP on immobilized bacteria. They have found that MP inhibited the growth of plaque bacteria for three days but after this period it had no effect.

Surprisingly, most of the assays determining the antibacterial effects of agents against oral bacteria were performed in suspension, where it is clear that the most important ecological niche of the oral bacteria is the dental plaque biofilm. It is conceivable that bacteria immobilized in the dental plaque may have a susceptibility to antibacterial agents which is different from the same bacteria in suspension [1,14,20,21]. The difference is probably due to environmental and physiological differences between planktonic phase and biofilm [22,23].

We have tested the effect of several paraben derivatives in a planktonic as well as in the immobilized phase and found several differences between the activity of parabens on planktonic and immobilized *S. sobrinus*. The killing effects of parabens on planktonic bacteria or immobilized bacteria were similar. However, different antibacterial effects of combinations of parabens were found when testing planktonic bacteria and immobilized bacteria. The effect of the combination of parabens on immobilized bacteria was more effective than that combination in solution. This enhanced antibacterial effect on the surface may be due to the adsorption properties of the parabens allowing them to reach higher local concentrations on the surface compared with solution, which results in greater antibacterial efficacy. It is possible that the presence of extracellular polysaccharides synthesized in situ by oral bacteria will decrease the susceptibility of such bacteria to parabens, especially in biofilms [24]. Such conditions would require an increase in parabens concentrations to produce the same effect.

Parabens are antibacterial agents that have received little attention in the dental field. This study on *S. sobrinus* bacteria, along with other studies on oral bacteria, may lead to further tests on the potential effect of parabens in this area.

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