Novel Local Sustained Released Varnish for Reducing Oral Bacteria in Dogs

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ABSTRACT

Dental diseases are prevalent in dogs. The process of most oral disorders starts with a high quantity of salivary bacteria in the oral cavity. Reducing the amount of supra-gingival bacteria would be important in prevention of those diseases. Oral hygiene of dogs is not practiced routinely, mainly due to low compliance by both owners and their dogs. A sustained release varnish, applied on the teeth, designed specifically for dogs, in which the drug is released for a prolonged time, would have both clinical and user advantages. This research studies the fundamental properties of potential sustained release varnishes containing antimicrobial agents for treatment of supra-gingival bacteria. The veterinary sustained release varnish (VSRV) contains a polymeric matrix in which the active drug is embedded, and released over a prolonged period of time. The varnish has demonstrated a prolonged anti-bacterial effect in dogs, which lasted up to 10 days depending on the pharmaceutical formulation. The use of VRSV as a prophylactic measure will improve dogs' oral health and thereby dramatically decrease the need for dental therapy which usually requires anesthesia and high expense.

Keywords: Dog, Varnish, Supra-gingival bacteria, Dental disease, Compliance

INTRODUCTION

Oral health problems are among the most prevalent diseases in pets. The prevalence of gingivitis has been estimated to be 19.5% in dogs and 13.1% in cats, while that of dental tartar, 20.5% in dogs and 24.2% in cats (1). Others have reported that by the age of 4 years, about 80% of dogs and cats already suffer from different stages of periodontitis (2-9). Caries are found in dogs (10) but are not as common as other dental disorders (1). In a study reviewing the dental records of 435 dogs, 5.3% were found to have one or more caries lesions including pit and fissure caries, smooth surface caries and root caries (10). Prevention of these dental related diseases is important since their clinical effect may extend beyond the oral cavity and affect other tissues and organs (11, 12).

Supragingival bacteria in dogs were identified by comparative 16S rRNA gene sequencing. More than half of the phenotypes identified were found to be members similar to the indigenous oral microbiota of humans. The most frequently isolated bacteria from saliva were *Actinomyces* (26%), *Porphyromonas* (20%) and *Streptococcus* (18%) (13). The streptococci were highly associated with the dental biofilm and caries, while actinomyces were associated with root caries, and phorphyrimonas are anaerobic bacteria associated with periodontal diseases (14-16). Therefore by reducing

the quantity of supragingival bacteria which are highly associated with dental diseases, could reduce the prevalence of caries, gingivitis, periodontal diseases, halitosis and dental tartar (16-18). Unfortunately, dog owners often neglect dental hygiene and treatment of dental diseases (19-20). The main reason for this is low compliance, as conventional dental drug delivery requires daily application. Dental sustained release delivery systems specifically designed for prevention and treatment of oral diseases in pets are scarce (21-22). A product aimed to prevent plaque forming bacteria is an OraVet™ barrier sealant system (Merial. Oravet.au.merial.com). Clearly, a sustained release application, which would prolong the duration of the drug in the oral cavity, and therefore reduces the number of applications, would be beneficial to the oral health of these animals.

Sustained release devices (SRDs) are novel drug delivery systems used in humans (23). The use of this type of pharmaceutical application in animals has numerous pharmacological and clinical advantages. The main advantage of these drug delivery devices is that they prolong the duration of the drug in the oral cavity, therefore minimizing the frequency of application while maximizing its clinical efficacy. Elimination of a drug from the oral cavity is rapid due to salivary flow, food and water consumption. Increasing the amount and duration of drug in the oral cavity is one of the pivotal pharmacological factors associated with the prevention and therapy of oral diseases (23-25).

In most cases, the prevalence of oral diseases can be minimized if appropriate oral health measures are applied. It is, therefore, important to establish alternative and simple means to prevent these diseases in animals. The aim of this study was to develop a pharmaceutical dental sustained release varnish designed specially for use in dogs.

MATERIALS AND METHODS

Pharmaceutical formulation

Pharmaceutical formulations were prepared similarly to those described by Steinberg *et al.* 2006 (24). The basic veterinary sustained release varnish (VSRV) formulation used for this study consisted of: ethyl cellulose as polymeric matrix (N-100 type, Hercules, Wilmington, USA), PEG (polyethylene glycol) 400 (Merck, Germany), klucel HF (hydroxypropyl cellulose) (Hercules, Wilmington, USA) as pharmaceutical additive, and flavoring agents (bacon flavor (0.2% w/s), lard flavor (0.05-0.3% w/s), type tuna-L flavor (0.05-0.3% w/s) (Bigarol®, Symrise, Eltville, Germany)) mixed with the active agents: CHX (chlorhexidine) (Sigma-Aldrich, St. Louis, USA) at 2%, 4% and 5% or triclosan (Taro, Haifa, Israel) at 5%. For the cetylpyridinium CPC (chloride monohydrate) (Sigma-Aldrich, St. Louis, USA) at 5% formulation the ethylcellulose was replaced with eudragit (Rohm Pharma, Darmstadt, Germany). The varnish was kept in air tight containers at room temperature.

Agar bioassays

The veterinary sustained release varnish (VSRV) was dried into a film and cut into disks of 1 cm in diameter. The disks were placed on Mueller-Hinton agar plates pre-cultured with clinically isolated *Streptococcus mutans* from dogs. The pre-seeded agar plates were incubated for 24 hours in 5% CO_2 at 37°C. The disks were removed from the agar plate daily, placed on newly pre-seeded plates and incubated again under the same conditions. The diameters of the bacterial inhibition zone around the disks were measured and recorded as a mean of two readings. Three triplicates of each formulation were used. A control group of placebo VSRV was used as a reference. The placebo VSRV comprised of the base of the product, as mentioned in pharmaceutical formulation without any active agent.

Clinical applications

Dog population

Dachshund breed dogs raised at a dog farm "Bet Erez" located in the Tel Aviv area, Israel, were chosen for the clinical trial. All dogs were in general good health, ate commercial dry food, and had not received any medication or any dental care. Each test was performed on 10 intact dogs (2-4 males and 6-8 females, depending on the trial), age between 2 and 6 years. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University of Jerusalem.

Application of the veterinary sustained release varnish (VSRV)

Individual bottles containing the different VSRV formulations were prepared for each dog. Each formulation was tested in a blinded manner; the VSRVs were coded and the

Figure 1. Application of the VSRV to the teeth surfaces (incisors, canines and pre-molars) and the gingivae on the buccal site, using a synthetic hair varnish brush as two thick layers. The active ingredients in the varnishes were, separately, CHX, CPC and triclosan.

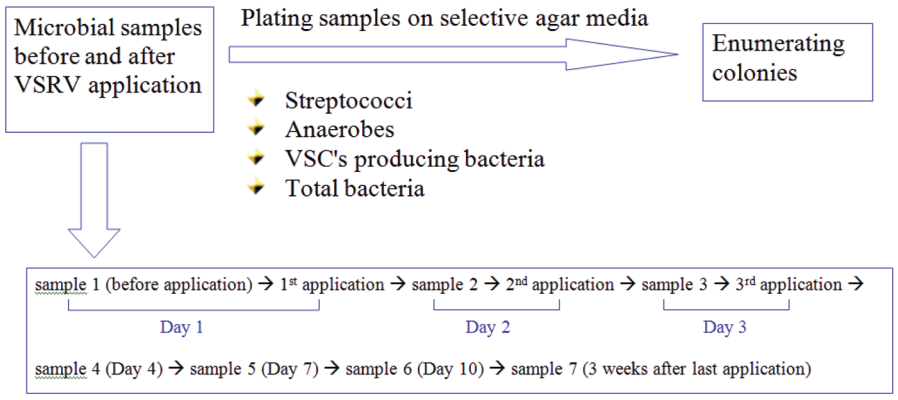

Figure 2. Schematic diagram illustrating the *in-vivo* application.

codes were revealed only after results were obtained. Dogs were identified by pet microchip scanner (Trovan®, United Kingdom). The VSRV was applied with a small brush immersed in the varnish. Dogs were not anaesthetised for application of the varnish and none of the dogs were hospitalized before, during and after the procedure. No special preparations were required excluding feeding 24 hr prior to the collection of samples and application of the varnish. Two coats of the varnish were applied onto the dogs' incisors and premolar teeth in the upper and lower jaw on the buccal surface (Figure 1). All VSRVs were applied by one individual proficient in the technique.

Oral microbial samples were taken from the tongue before VSRV application during the first 3 days and after application on days 4, 7, 10 and 21 days following (Figure 2). The samples were taken using a sterile swab. Application and sampling of subjects were carried out at the same time of day (8am-10am), before feeding. Dogs were kept away from food 24 hours prior to sampling; water was provided *ad libitum*. The collected bacteria were plated on agar media. For enumeration of streptococci the samples were plated on selective *mitis salivarius* agar and incubated in 5% CO_2 at 37°C for 48 hours (26). Anaerobic bacteria were cultured on BHI (Brain Heart Infusion Agar) in anaerobic gas jars (Mitsubishi®, New York, USA) for 48 hours at 37°C. Aerobic facultative bacterial counts were enumerated in BHI grown in 5% CO_2, 37°C for 48 hours. Each sample was plated in quadruplicate. Enumeration of bacteria was conducted by ranking the amount of bacteria from 0-7 using semi-quantitative commercial charts (27).

Statistical analysis

In order to assess the change of the bacterial counts between two time points the non-parametric Wilcoxon signed rank test was applied. P value of 5% or less was considered statistically significant.

RESULTS

In vitro bioassays

All SRV samples demonstrated a prolonged antimicrobial effect which lasted for at least 45 days (Figure 3). Both CHX formulations had an initial longer effect compared to the other two formulations of CPC and triclosan. However, the SRV of triclosan showed an enhanced effect over the test period.

In vivo tests

VSRV containing CHX of 2% had almost no antimicrobial effect in the oral cavity (Figure 4). The VSRV containing 4% CHX had an antimicrobial effect which lasted only

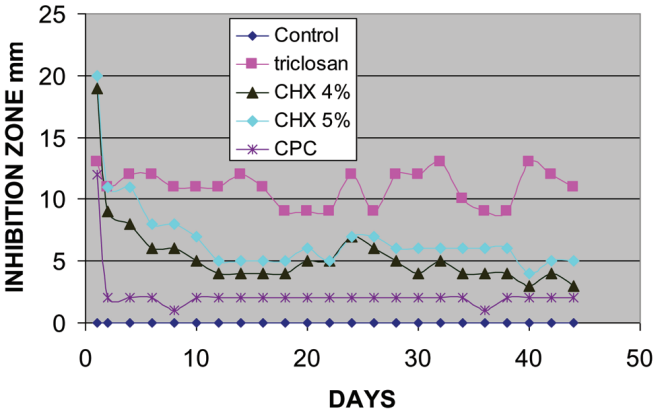

Figure 3. In vitro Bioassay of CHX 4%, CHX 5%, triclosan and CPC formulations.

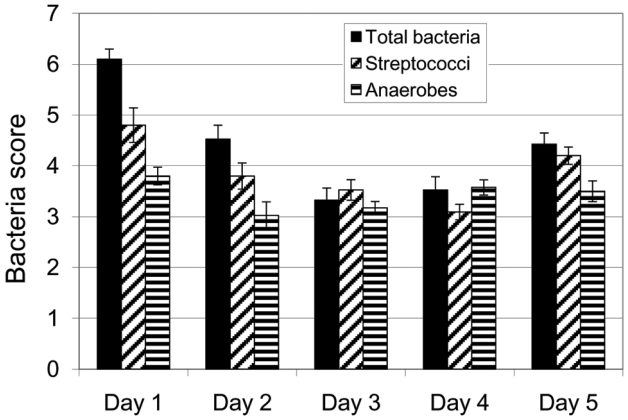

Figure 5. Mean score oral cavity bacteria before and after VSRV application with 4% CHX (w/v).

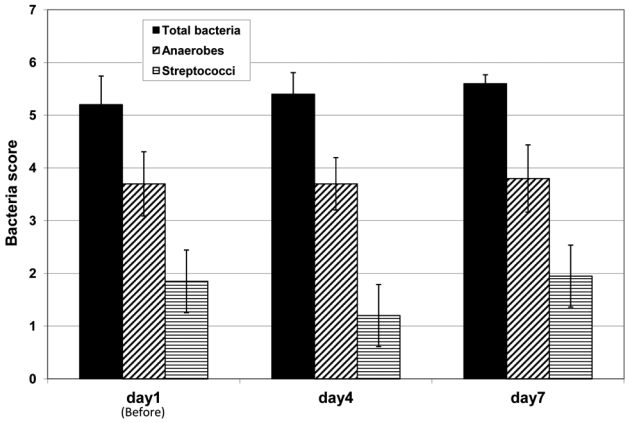

Figure 4. Mean score oral cavity bacteria before and after VSRV application with 2% CHX (w/v).

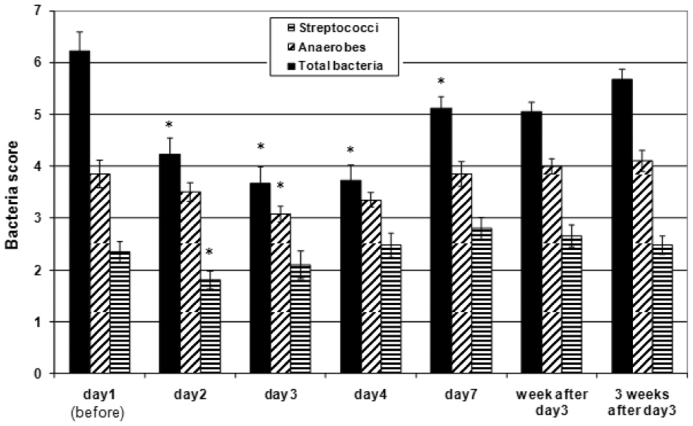

Figure 6. Mean score oral cavity bacteria before and after VSRV application with 5% CHX (w/v). * = P < 0.05 from day 1 (Wilcoxon signed rank test).

4-5 days (Figure 5). The best results were achieved by using VSRV containing 5% w/w CHX (Figure 6). Application as above (day 1, 2 and 3) showed a reduction in tested bacteria and a sharp statistically significant (P<0.05) decrease which lasted for 7 days after which repopulation occurred. Three weeks after application the levels of bacteria were similar to those on day 1.

Substituting the active ingredient by 5% triclosan also demonstrated a prolonged antimicrobial effect, mainly for the total amount of bacteria, which was statistically significant (P<0.05) for 10 days after the application. Repopulation of streptococci was recorded after about three weeks (Figure 7).

Next, we replaced the active ingredient of the formulation with 5% CPC (Figure 8). The microbial results showed a statistically significant (P<0.05) effect in reduction of total bacterial counts for 8 days, after which a gradual increase of bacterial counts was recorded until about three weeks after the application.

No side effects were seen for any of the formulations used.

DISCUSSION

Microbial oral research in veterinary medicine has clearly indicated a strong relationship between oral bacteria and oral disorders (8, 28). The prevalence of oral diseases in dogs increases up to more than 70% by 5 years of age and varies between 5%-75% (4, 6, 8, 9). As in humans, gingivitis, periodontal diseases and dental caries are highly associated with presence of supragingival bacteria in the oral cavity. Clearly,

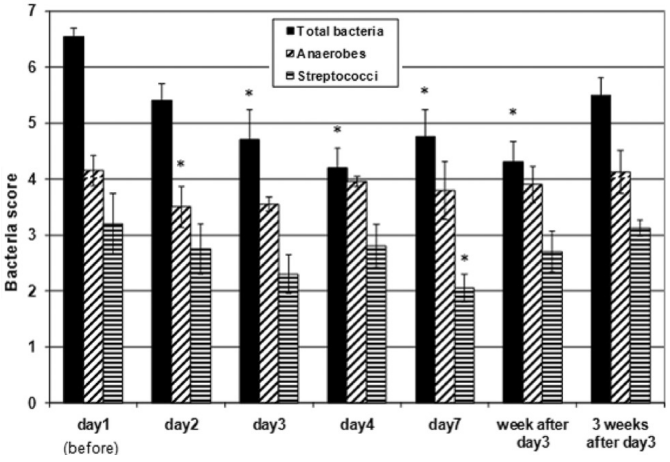

Figure 7. Mean score oral cavity bacteria before and after VSRV application with 5% triclosan (w/v). * = P < 0.05 from day 1 (Wilcoxon signed rank test).

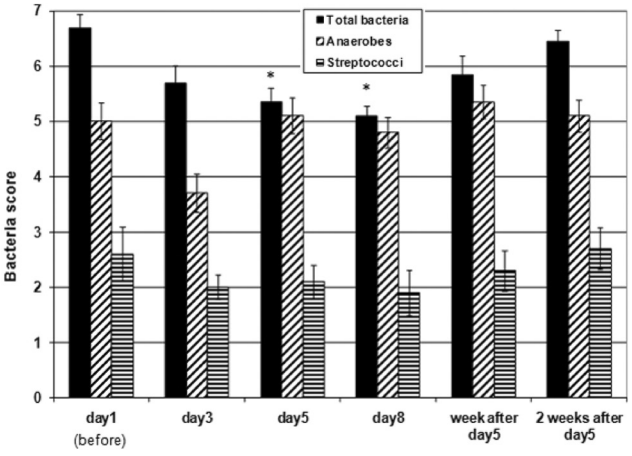

Figure 8. Mean score oral cavity bacteria before and after VSRV application with 5% CPC (w/v). * = P < 0.05 from day 1 (Wilcoxon signed rank test).

elimination of these bacteria will result in reduction of those dental disorders which affect the well-being of dogs.

Oral disorders are highly prevalent in dogs and affect their quality of life (1, 16). Unlike humans, most dental procedures in dogs require full anesthesia (16) In addition, these diseases are more critical in elderly dogs as they might suffer from underlying diseases such as renal and hepatic diseases which rank them at higher risk for anesthesia. In addition to health concerns, the cost of such procedures may prevent dog owners from providing dental treatment for their dogs, which of course compromises the dog's general health and quality of life.

The main disadvantage of the existing veterinary dental drug delivery systems is compliance (29). The compliance of owners to carry out daily tooth brushing for their dogs is very low – resulting in poor dental hygiene. The advantage of the VSRV is that it provides sustained release of the drug in the oral cavity, thus reducing the frequency of the application. Furthermore, SRD's (sustained release delivery systems) allows for an increase of the drug concentration, compare to non-SRD's antimicrobial products, with the added advantage of minimal side effects (23).

In this study the antimicrobial effect of three different active agents incorporated into VSRV was tested. CHX, CPC and triclosan are well known antimicrobial drugs, abundant in oral hygiene products for human use. They all demonstrated good antimicrobial effects and have been shown to reduce the prevalence of oral bacteria in human studies (23, 30). In addition to their different antimicrobial efficacy and mode of action, their physico-chemical properties differ. CHX is a quaternary amine charged positively at two domains (31). CPC is a cationic quaternary ammonium positively charged at one domain (16), while triclosan is a chlorinated aromatic compound which has functional groups representative of both ethers and phenols (16). Our agar bioassay showed that incorporation of these agents into the matrix of the device did not influence their antimicrobial effect.

The clinical results show that the VSRV can suppress oral bacteria up to 10 days after application, depending on the drug. Advantageously, the longer the persistence of the drug in the oral cavity the more favorable the clinical results. VSRV may be used as a treatment for dental diseases as well as a prophylactically. In this study a dose-response effect was observed. Applying CHX varnish of 2% had almost no microbial effect in the oral cavity (Figure 4); however, 4% and 5% CHX in a VSRV resulted in a significant decrease in oral bacteria. Changing the active ingredient to CPC demonstrated a reduction in bacteria, but its effect was less than the effect of CHX application. Triclosan as an active ingredient in the VSRV also showed microbial reduction which lasted 10 days. Although this study was performed in Dachshund dogs it can be considered relevant to all other dog breeds.

The prolonged effect of the drugs observed in this study was confirmed by the reduction of total bacterial counts. One of the main pharmaceutical goals in preventing oral disorders is decreasing the total supragingival bacterial counts, as they

directly reflect the biomass which is highly associated with periodontal diseases, gingivitis and dental caries (5, 7, 32-35). Obviously, reducing specific perio-pathogenic or cariogenic bacteria is also essential and should be considered in the development of future VSRVs.

Spurred by the debate regarding which drug or drug delivery system is optimal in the treatment of dental diseases in dogs, the search continues for novel pharmaceutical dosage forms to prevent and treat dental diseases in canines. As teeth are important structures in animals, much beyond just chewing food, it is of the utmost importance to maintain and restore full dentition. Scarce information is available on dental sustained release delivery systems specifically designed for prevention and treatment of oral diseases in dogs (21-22) as in other species as well (36). It is proposed that the VSRV dosage delivery system may be applied to animals, such as dogs and cats, and thereby reduce the low compliance associated with conventional dental treatments.

Clearly, a VSRV extending the duration of the drug in the oral cavity will result in better clinical effects. Our study describes the concept of a local sustained delivery system for oral usage in dogs which may be further developed and used as a means to improve dogs', and potentially other animals' oral hygiene resulting in a healthier and better quality of life.

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Conflict of interest: There was no conflict of interest in this study.

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