Influence of Alcohol Containing Cough Syrup on Oral Streptococci Biofilm Formation

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Influence of Alcohol Containing Cough Syrup on Oral Streptococci Biofilm Formation

Abstract:

Purpose: The purpose of our study was to investigate the effect of low concentrations of alcohol and alcohol containing cough syrup on streptococcal biofilm formation.

Methods: Seven-day biofilms were prepared from $1 \times 10^7$ cells of *S. mutans* and *S. sanguinis* in a 48-well culture plate, and on hydroxyapatite (HA) discs. The biofilm cells were then incubated with alcohol or cough syrups (CS) for six hours. Viable bacteria were determined using the MTT viability assay.

Results: Alcohol at concentrations up to 10% and cough syrup with alcohol showed an increase of 60-72% viable bacteria in the biofilm, while non-alcoholic cough syrup had no such effect. However, supplementation of the same syrup with ethanol enhanced the formation of biofilm on HA and culture dish surfaces to a degree similar to alcohol. Treatment of pre-formed biofilm with ethanol (< 10%) or CS failed to disrupt the biofilm.

Conclusions: Our results demonstrated that low concentration of alcohol or CS containing alcohol favors the formation of streptococcal biofilm which may play a role in dental caries. The findings of the study should be taken into consideration by pediatricians before administering the CS especially to patients who are at high caries risk.

Introduction

Alcohols such as ethanol and isopropanol are widely used as disinfectants in various home and medical settings. It is generally thought that alcohols act by disrupting membranes and denaturing proteins in bacteria. Alcohol also has numerous applications as stabilizers and solvents in liquid medications. A large variety of therapeutics, such as cough suppressants, expectorants, and oral tranquilizer suspensions, contain ethanol at various concentrations.\(^1\) Previous research by Knobloch *et. al.* in 2002 demonstrates that low levels of alcohol supplementation in media actually increases *Staphylococcus epidermis* biofilm production.\(^2\) Kantorski *et. al.* in 2007 also show that diets with alcohol solutions are found to facilitate colonization of *Streptococcus mutans* and increase the occurrence of smooth surface dental caries in rat molars when compared to a control diet.\(^3\) Recently, Redelman *et. al.* in 2012 report that treating preformed biofilms of *Staphylococcus aureus* with ethanol also enhances biofilm levels and many of the bacteria within these biofilms are found to be alive and metabolically active.\(^4\)
Streptococcus mutans is known to be a highly virulent bacteria in the human dental caries process. One of the important virulent properties of these organisms is their ability to form biofilms known as dental plaque on tooth surfaces. Streptococcus Sanguis is known to be one of the first colonizers of the tooth surface. Streptococcus sanguis plays a key role in the biofilm formation found on human teeth. It is well known that Streptococcus sanguis forms extracellular polysaccharides that contribute to the overall dental plaque and facilitate adhesion of Streptococcus mutans and other bacteria to the surfaces of oral cavity.

Many children consume over the counter cough syrups regularly, and many of these cough syrups contain alcohol as an ingredient. When administered to the pediatric patient, this low concentration of alcohol could affect the colonization and enhance biofilm formation in the oral cavity. This could be of concern to pediatric dental patients especially to those that are at a high risk for dental caries.

Refer to Table 1

Our study was designed to test the effect of alcohol and alcohol containing cough syrup on the formation of mixed biofilms of Streptococcus mutans and Streptococcus sanguis. We also tested the effects of these compounds on preformed mixed biofilms of the same bacteria.

Materials and Methods:

Cough syrups tested:

Walgreen’s brand nighttime cough relief with 10% alcohol (Walgreen Co., Deerfield, IL) was used in the study. Walgreen’s brand daytime cough relief (Walgreen Co, Deerfield, IL) with the same active ingredients except alcohol was used as a control.

Bacteria:

ATCC strains of S. mutans (700610) and S. sanguis (51656) were grown in Trypticase soy broth (TSB) for 24 hours at 37C. Bacteria were harvested by centrifugation and re-suspended to a concentration of 1x10^7 cells/ml.

Biofilm formation:

Using a 48 well culture dish, a 7-day biofilm was grown by placing 1.0 ml of the bacteria (1x10^7 cells) and incubating at 37C for 7 days. An aliquot (0.2 ml) of media from each well was discarded and the same amount of fresh TSB supplemented with 10% dextrose was added back to each well every 2 days. In a similar fashion biofilm was also prepared on hydroxyapatite.
(HA) discs. HA discs (Hi Med Labs) were sterilized by autoclaving and then incubated with Clarified human saliva (2.0 ml/disc) for 24 hours. Discs were rinsed with saline, and then 1.0 ml of bacterial suspension (1x10^7 cells) was added and incubated for 7 days as described above. Bacteria were collected from plastic wells and HA discs, centrifuged and suspended to a concentration of 1x10^7 cells/ml and employed in the study.

**Treatment of planktonic and biofilm bacteria with alcohol containing cough syrup:**

One ml of freshly cultured and biofilm bacteria (1x10^7 cells/ml) were placed in a 48 well culture dish, to which 0.2 ml of the cough syrup or varying concentrations of lab grade ethanol was added to each well and incubated for six hours, and the bacterial viability was determined (see below) by MTT assay.

**Bacterial viability:**

Viable bacteria following the treatment with alcohol or cough syrups were determined by using the MTT assay using the kit obtained from (Roche labs, Indianapolis, IN). Cells were incubated with MTT labeling agent for 4 hours and then with solubilizing agent, provided in the kit by the manufacturer, over night. The resulting optical density was measured at 550 nm using a spectrophotometer.

**Statistical analysis:**

All experiments were conducted with triplicate samples, and each experiment was repeated three times. Data was analyzed for statistical significance by ANOVA followed by Scheffe’s f-test, with a p-value less than 0.05 considered significant.

**Results**

**Effect of cough syrup and low concentrations of alcohol on S. mutans and S. sanguinis biofilm formation:**

S. mutans a cariogenic organism and S. sanguinis, an early colonizer of the oral cavity were reported to be associated with dental plaque. We tested the effect of varying concentrations of alcohol-containing cough syrup on formation of biofilm in a 48-well culture dish. The results (Fig 1) show that low concentrations of ethanol as well as the cough syrup enhanced the biofilm formation. 60% increase in biofilm formation was seen in the presence of alcohol-containing Cough syrup (CSA). CSA at lower concentration (10-25%) had only 15-36% increase in biofilm over the buffer control. The non-alcohol cough syrup (CS2) had no effect on the biofilm formation by these bacteria. However, addition of lab grade ethanol resulted in an increase in biofilm comparable to that of ethanol (Fig 1). The results suggest that alcohol is the influencing factor in enhanced biofilm formation.

**Figure 1:**
Effect of cough syrup and low concentrations of alcohol on S. mutans and S. sanguinis biofilm formation on hydroxyapatite discs:

The effect of low concentrations of alcohol and cough syrups with alcohol (CSA) and without (CS2) were tested on biofilm formation on saliva-coated HA discs. Both alcohol and CSA had a significant impact upon biofilm formation. Alcohol had a dose-dependent increase of biofilm mass on HA discs. The presence of 1.25% alcohol had about a 15% increase, but at 7.5% an increase of 69% was seen of the control (Fig 2). In a similar fashion, CSA also enhances biofilm mass, up to a 70% increase was observed. The CS2 had no effect, but upon addition of 7.5% ethanol the biofilm mass was increased by 61%. Once again the data (Fig 2) suggest that low concentrations of alcohol appear to favor biofilm formation.

Figure 2:

Effect of cough syrups on pre-formed biofilm on HA discs:

We also tested the effect of alcohol and cough syrups on pre-formed biofilm on HA discs. Similar to previous results, both alcohol and CSA enhanced the bacterial growth of the biofilm cells. About 60-73% increase in biofilm cells over the control was observed when treated with CSA (Fig 3). The CS2 (non-alcohol containing) cough syrup failed to alter the biofilm cells. The addition of alcohol (2.5% to 10%) significantly enhanced the growth of biofilm cells. An increase of 40-64% was seen when CS2 was supplemented with alcohol (2.5% - 10%). The results (Fig 3) suggest that low concentrations of alcohol appear to promote growth of oral streptococci.

Figure 3:

Discussion

Existing studies show that treating both preformed biofilms and developing biofilms of Staphylococcus strains with low levels of alcohol enhances viable bacterial number and biofilm production. Previous research by Knobloch et al. shows that treatment with alcohol during initial biofilm formation increases Staphylococcus epidermidis biofilm production. This increase is attributed to altering gene expression in particular the ica gene for polysaccharide adhesion production. Redelman et al. demonstrate that treating preformed biofilms of Staphylococcus aureus with alcohol also has a positive effect and enhances the number of viable cells. Their research also suggests a variance in gene expression as a strong factor in the increase in bacterial biofilm. Our study was designed to expand on this knowledge and determine if alcohol exposure had the same effects on biofilms of oral Streptococcal strains.

Our results demonstrated that low concentrations of alcohol had a significant effect on the formation of our mixed oral streptococci biofilm. Treating preformed biofilms of the same mixed bacteria with the same reagents also had a significant enhancement on the number of viable cells in the biofilm. We chose saliva coated hydroxyapatite discs to replicate the human
tooth as a surface for the biofilm to form. The increase in the number of cells was seen in the biofilms grown both in microtier plates and on the hydroxyapatite discs.

The positive influence of the alcohol was also noted in each experiment whether the biofilm was treated with alcohol alone or with cough syrup that contained alcohol. Cough syrup with the same active ingredients but free from alcohol was also tested as a control. It had no significant effect in any study design, however, we were able to influence the formation of our biofilm and increase the number of viable bacteria in preformed biofilms by adding alcohol to the non-alcohol containing cough syrup.

One possibility of the results that we found could be that the streptococci are able to cleave the hydroxyl group from the alcohol when it is in low concentrations converting it to a carbon source that is then used as food for the biofilm. Higher concentrations of alcohol are able to have the opposite effect and decrease viable bacteria. The concentrations of alcohol found in numerous over the counter medications would fall in the range that was shown to increase bacteria in our study.

We were able to enhance biofilms of known oral streptococci by exposing them to alcohol containing cough syrups. Our results shed light on a consideration that should be made when advising pediatric patients. The caregivers to pediatric dental patients should be informed of the possibility that medications that contain alcohol could be increasing the cariogenic bacterial load in their patients. This concept should be incorporated into the general home care instructions given to these patients. This is of special importance to the patients that are considered at a high risk for dental caries. Dental providers should inform parents and patients that take these medications of the importance to brush and clean the oral cavity and tooth surfaces after each exposure to the medication being taken. This information should also be shared with pediatricians that recommend or prescribe these alcohol containing medications to their patients.

Conclusions:

1. The presence of low levels of alcohol enhanced the formation of oral streptococci biofilm.
2. Exposure of low levels of alcohol to preexisting oral streptococci biofilm increased the number of viable bacteria.
3. Prescribers of medicines that contain low levels of alcohol should be aware of the effect of alcohol on cariogenic bacterial load and advise the patients accordingly.
Acknowledgements

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References


Table 1:

Common Medications That Contain Alcohol

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<tr>
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<tbody>
<tr>
<td>Liquid Theraflu</td>
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</tr>
<tr>
<td>Tylenol Liquid</td>
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<td>Benadryl Elixir</td>
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<td>Benadryl Decongestant</td>
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<td>Contact Severe Cold</td>
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<tr>
<td>Dimetapp</td>
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<tr>
<td>Formula 44 Cough</td>
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<tr>
<td>Novahistine Cough</td>
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<td>Pediquil</td>
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</tr>
<tr>
<td>Robitussin</td>
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<tr>
<td>Sudafed Cough Syrup</td>
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<tr>
<td>Vicks Cough</td>
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Influence of alcohol and cough syrups on growth of biofilm in plastic wells

Biofilm formed in the presence of:

194x148mm (150 x 150 DPI)
Biofilm grown on HA discs in the presence of.

201x152mm (150 x 150 DPI)
Preformed biofilm on HA discs incubated with...