Short communication

Influence of natural coffee compounds, coffee extracts and increased levels of caffeine on the inhibition of Streptococcus mutans

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ABSTRACT

The inhibition of Streptococcus mutans by Coffea arabica extracts incorporated or not with natural coffee compounds was investigated by the disk diffusion method. Additionally, the turbidimetric test was used to verify the influence of caffeine concentration on the inhibition of S. mutans. Extracts of different samples of Arabica coffee showed antibacterial activity against S. mutans. The inhibitory effect was not affected by the brewing method (filtered or espresso) or by the different Arabica coffee samples. Plain caffeine, trigonelline, caffeic acid, protocatechuic acid and chlorogenic acid at 2.0 mg/mL provided similar antimicrobial effect against S. mutans. However, there was an increase in the antibacterial activity when these compounds were added to the coffee extract, except for chlorogenic acid which did not affect the inhibitory effect. Caffeine at concentrations found in Arabica beverages inhibited S. mutans temporarily, whereas higher caffeine concentrations provided a stronger and longer lasting inhibition.

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1. Introduction

Dental caries, one of the most prevalent and costly infectious disease affecting mankind, is the consequence of the interaction among oral microflora, diet, deitition and oral environment. Streptococcus mutans is regarded as the main microbial agent, although additional acidogenic microorganisms may be involved. The ability to metabolize carbohydrates, to adhere to and to form biofilm on tooth surfaces is associated with the cariogenicity of this pathogen (Antonio, Farah, Santos, & Maia, 2011; Ferrazzano et al., 2011).

Many studies on caries-related microorganisms indicated that some natural products can affect survival and virulence of S. mutans. The inhibitory effects of cranberry, propolis, coffee, wine, cocoa, tea, and some dairy products on S. mutans, in vitro and in experimental animal studies, have been reported (Almeida, Silveira, Nunan, Santos, & Gloria, 2004; Antonio et al., 2010; Yamanaka-Okada et al., 2008). The high contents of calcium and phosphorus are responsible for the cariostatic mechanism of yogurt and cheese (Antonio et al., 2011). For the other products, the literature supports the anti-bacterial role of polyphenols suggesting a direct effect against S. mutans, an interaction with microbial membrane proteins inhibiting adherence of bacterial cells to the tooth surface, an inhibition of glucosyl transferase and amy-lase, a reduction of the contents of food derived acids that can damage tooth enamel, and an inhibition of tooth demineralization by interactions with the organic matrixes (Antonio et al., 2011; Ferrazzano et al., 2011; Smullen, Koutsou, Foster, Zumbé, & Storey, 2007).

In addition to polyphenols, other natural substances such as trigonelline, caffeine and α-dicarbonyl compounds are found in coffee and show antibacterial activity against S. mutans. Results involving caffeine are controversial. Daglia et al. (2007), Cogo et al. (2008) and Antonio et al. (2010) did not find antibacterial effect of plain caffeine in susceptibility tests. However, Daglia et al. (2007) found caffeine to possess inhibitory activity from 5.0 to 12.5 mg/mL. They also found a synergistic effect of caffeine with α-dicarbonyl compounds in coffee. Moreover, Antonio et al. (2010) observed that decaffeinated extracts showed lower antibacterial activity against S. mutans compared to the respective non decaffeinated extracts. Therefore, studies clarifying the role of caffeine and other natural coffee compounds on the antibacterial activity of coffee are still needed.

It is well known that the presence of bioactive compounds in coffee, including those exerting antimicrobial activity, is affected by species, agricultural practices, processing and storage conditions (Antonio et al., 2011; Esquivel & Jiménez, 2012; Moretti, Mattos, Calbo, & Sargent, 2010). Therefore, knowledge on the concentrations of compounds needed to obtain the desired biological effects will be essential for selection of species currently available in germplasm banks or for designing genetic improvement to produce coffee with the desired functional properties.

In the present study, the inhibitory effect of aqueous extracts of four samples of Arabica coffee on S. mutans was investigated. The inhibitory
effect of coffee extracts, incorporated or not with some natural coffee compounds, against S. mutans was also tested. Additionally, the influence of caffeine concentrations on the inhibition of S. mutans was investigated.

2. Materials and methods

2.1. Material

Caffeic, protocatechuic and chlorogenic (5-caffeoylquinic acid) acids and trigonelline were from Sigma-Aldrich and caffeine was from Reagen.

Four commercial roasted Coffea arabica samples (A–D) were used. The beans were ground and sieved (20 mesh). Aqueous extracts of each sample were prepared by adding 8.0 g of ground coffee to 40 mL of boiling water. The mixture was kept in boiling water for 3 min and filtered through filter paper. Espresso coffee was prepared with 8.0 g of ground coffee and 40 mL of water in an espresso machine. The extracts incorporated with natural coffee chemical compounds were prepared by adding 2.0 mg/mL of trigonelline, caffeine, chlorogenic, caffeic and protocatechuic acids to extract sample B.

S. mutans (American Type Culture Collection: ATCC 25175) were grown under microaerophilic conditions at 36.5 ± 1.0 °C for 48 h on Brain Heart Infusion broth (BHI, Dialab Diagnósticos, MG, Brazil) and stored at 4 °C.

2.2. In-vitro antibacterial activity of plain coffee chemical compounds and coffee extracts against S. mutans

The agar diffusion method (NCCLS, 1993) was used. The inoculums were standardized by transferring colonies from the nutrient agar to sterile saline up to 10^8 cfu/mL (50% transmittance at 580 nm) and 200 μL of the suspension was homogeneously placed on the surface of 50 mL Mueller-Hinton agar in 150 mm Petri dish. Sterile disks (6.0 mm diameter) were impregnated with 20 μL coffee extracts and/or chemical compound solutions and placed on the surface of the agar containing S. mutans. Additionally, each plate carried control disks: solvent control (20 μL sterile distilled water) and positive control (30 μg amoxicillin/clavulanic acid, Cecon, SP, Brazil). The plates were incubated at 36.5 ± 1.0 °C for 48 h under microaerophilic conditions. The diameters of the inhibition zones were measured with a caliper.

2.3. Influence of caffeine concentration on the in vitro antibacterial activity against S. mutans

The turbidimetric method was used to determine the influence of caffeine concentration on the growth inhibition of S. mutans (USP, 2006). Caffeine solutions (1 mL) and 100 μL of the inoculums (10^6 cfu/mL) were added to test tubes containing 9 mL of BHI. The tubes were incubated at 36.5 ± 1.0 °C under microaerophilic conditions. Absorbances (530 nm) were recorded at 0, 2, 4, 6, 8, and 24 h. The percent inhibition [I (%)] was calculated as 100(A0 – Ai)/A0 (Ai = absorbances for control and samples, respectively).

2.4. Statistical analysis

The experiments were performed in triplicate. Results were submitted to analysis of variance and the means were compared by the Tukey’s or the Bonferroni’s test (5% significance).

3. Results and discussion

3.1. Influence of extraction/brewing processes and coffee samples on the antibacterial activity against S. mutans

All extracts showed in vitro antibacterial activity against S. mutans (Fig. 1). No significant difference was observed on the diameters of the inhibition zones among coffee samples and between filtered or espresso extracts.

Similar results regarding the brewing processes were reported by Daglia, Cuzzoni, and Dacarro (1994) and Furuhata, Dogazaki, Hara, and Furuyama (2002). According to Antonio et al. (2011), the concentration of lipophilic compounds in espresso is higher compared to filtered coffee extracts. The lack of difference between the antibacterial effects for the espresso and filtered coffee extracts suggests that the antimicrobial activity might be associated to water soluble compounds, which were similar in the extracts.

Even though there were significant differences on the physicochemical characteristics of the different samples of the Arabica coffee included in this study (same samples as those described by Almeida, Farah, Silva, Nunan, & Gloria, 2006), such differences were not sufficient to affect the antibacterial effect on S. mutans.

3.2. Influence of selected coffee compounds in water and in coffee extract on the antibacterial activity against S. mutans

All tested coffee chemical compounds at 2.0 mg/mL produced similar inhibitory effect against S. mutans (Table 1). When these compounds, at the same concentrations, were added to coffee extract B, there was a significant increase in the antimicrobial activity, except for chlorogenic acid.

Trigonelline, caffeine, caffeic acid and protocatechuic acid incorporated to the coffee extract caused a significant increase on the antimicrobial effect compared to the original extract. The increase in the concentration of trigonelline from 0.82 to 2.82 mg/mL caused a 14% increase of the microbial effect compared to the original extract.

Table 1

<table>
<thead>
<tr>
<th>Chemical compound</th>
<th>Inhibition zones (mm)/coffee chemical compound in</th>
<th>Aqueous solutions</th>
<th>Coffee extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid (5-CQA)</td>
<td>7.6 ± 0.38*</td>
<td>8.3 ± 0.66*</td>
<td></td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>7.3 ± 0.29*</td>
<td>8.7 ± 0.29*</td>
<td></td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>7.5 ± 0.50*</td>
<td>8.5 ± 0.50*</td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>7.5 ± 0.50*</td>
<td>8.3 ± 0.58*</td>
<td></td>
</tr>
<tr>
<td>Trigonelline</td>
<td>7.3 ± 0.29*</td>
<td>8.3 ± 0.58*</td>
<td></td>
</tr>
</tbody>
</table>

Means (± standard deviations) with different letters in the same line (ab) are significantly different (Tukey’s test, p ≤ 0.05). No significant difference was observed among chemical compounds (same column). Diameter of inhibition zones: plain coffee extract = 6.9 mm; and amoxicillin/clavulanic acid = 23.0 mm.
increase in the diameter of the inhibition zone; the increase in caffeine from 2.1 to 4.1 mg/mL produced an 11% increase; whereas the incorporation of caffeic and protocatechuic acids (not detected in the coffee extracts) caused an increase of 18% and 13%, respectively, in the diameter of the inhibition zone.

3.3. Influence of caffeine concentration on the antibacterial activity against S. mutans

The concentration of caffeine affected significantly the antimicrobial activity against S. mutans (Table 2). When using 8.0 mg/mL of caffeine, higher inhibitions (>80%) were observed at 4.0 and 6.0 h. However, there was a significant decrease in the inhibition at 24 h, reaching percentages near the IC50 (inhibition of 50% of the growth). The significant decrease in the inhibition observed for every caffeine concentration with time suggests that caffeine had a bacteriostatic effect on S. mutans. At other caffeine concentrations (0.5 to 4.0 mg/mL), higher inhibitions were observed at 4.0 h.

Based on the evidence that coffee and certain of its components may exert significant anticariogenic effect, incorporation of such products into chewing gums, toothpastes, mouthwashes and dental floss is a real possibility, and such avenues are worth exploring. Furthermore, coffee by products of lower commercial value may be a valuable material for this purpose. Since coffee is relatively safe and its taste and aroma are largely appreciated in all parts of the world, such product would be particularly promising; however further investigations including in vivo studies are still required.

4. Conclusions

The antibacterial effect of Arabica coffee extracts against S. mutans, irrespective of the coffee sample and the brewing method was demonstrated. The addition of natural coffee compounds to coffee extract enhanced the antibacterial activity of coffee extracts. Furthermore, it was observed that caffeine concentration in Arabica coffee could inhibit S. mutans temporarily and a stronger and longer lasting effect could be achieved with higher caffeine concentrations. The results obtained make coffee extracts potential inhibitors of dental caries. It is anticipated that in the future, by determining the concentration of effective compounds, the antimicrobial activity of a certain type of coffee will possibly be predicted.

Acknowledgments

The authors acknowledge CNPq, CAPES and FAPEMIG for the scholarship and financial support.

References


<table>
<thead>
<tr>
<th>Contact time (h)</th>
<th>Percent inhibition of S. mutans growth/caffeine concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>13.8±1.9&lt;sup&gt;a&lt;/sup&gt; 48.5±2.2&lt;sup&gt;a&lt;/sup&gt; 48.7±1.2&lt;sup&gt;a&lt;/sup&gt; 75.0±1.0&lt;sup&gt;a&lt;/sup&gt; 81.7±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.0</td>
<td>9.0±1.8&lt;sup&gt;a&lt;/sup&gt; 41.8±2.8&lt;sup&gt;a&lt;/sup&gt; 42.0±1.6&lt;sup&gt;a&lt;/sup&gt; 68.5±2.5&lt;sup&gt;a&lt;/sup&gt; 89.4±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.0</td>
<td>4.3±0.3&lt;sup&gt;a&lt;/sup&gt; 17.9±1.0&lt;sup&gt;a&lt;/sup&gt; 18.1±1.1&lt;sup&gt;a&lt;/sup&gt; 42.0±2.8&lt;sup&gt;a&lt;/sup&gt; 77.3±1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.0</td>
<td>3.9±0.1&lt;sup&gt;a&lt;/sup&gt; 6.6±0.6&lt;sup&gt;a&lt;/sup&gt; 6.6±0.6&lt;sup&gt;a&lt;/sup&gt; 44.8±4.5&lt;sup&gt;a&lt;/sup&gt; 47.8±2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.0</td>
<td>9.0±1.8&lt;sup&gt;b&lt;/sup&gt; 41.8±2.8&lt;sup&gt;b&lt;/sup&gt; 42.0±1.6&lt;sup&gt;b&lt;/sup&gt; 68.5±2.5&lt;sup&gt;b&lt;/sup&gt; 89.4±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>8.0</td>
<td>4.3±0.3&lt;sup&gt;b&lt;/sup&gt; 17.9±1.0&lt;sup&gt;b&lt;/sup&gt; 18.1±1.1&lt;sup&gt;b&lt;/sup&gt; 42.0±2.8&lt;sup&gt;b&lt;/sup&gt; 77.3±1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>24.0</td>
<td>3.9±0.1&lt;sup&gt;c&lt;/sup&gt; 6.6±0.6&lt;sup&gt;c&lt;/sup&gt; 6.6±0.6&lt;sup&gt;c&lt;/sup&gt; 44.8±4.5&lt;sup&gt;c&lt;/sup&gt; 47.8±2.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means of triplicates (± standard deviations) with different letters in the same line (a,b,c,d) and in the same column (e,f,g) are significantly different (Bonferroni’s test, p≤0.05).