Local anaesthetic inhibition of lidocaine on the growth of streptococcus mutans in vitro

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Abstract

Objective: This study aims to determine the effectiveness of lidocaine of local anaesthetic agent in inhibiting the growth of streptococcus mutans bacteria.

Methods: This type of research is a laboratory experimental with a post test only control group design. Microorganism used was streptococcus mutans and cultured at the Microbiology Laboratory of the Pharmacy, Universitas Sumatera Utara. The local anaesthetic tested was a solution of lidocaine diluted with NaCl 0.9% to 0.5%, 1%, and 2%. The antibacterial activity test was carried out using diffusion method by dripping 25 µl of lidocaine (0.5%, 1%, 2%), positive control (amoxicillin), and negative control (NaCl 0.9%) on disc paper, then placed on nutrient agar, and incubated. After 24 hours, the inhibition zone formed around the disc paper was observed.

Results: Lidocaine showed antibacterial activity with the inhibition zone of 0.5%, 1%, 2% concentration was 6.98 mm, 7.66 mm, 8.36 mm. Then the data was analyzed using the One-way ANOVA test. From these results, there was a significant difference in the mean of the inhibitin zone (p < 0.05) between 0.5%, 1%, and 2% lidocaine, positive control, and negative control.

Conclusion: Lidocaine has antibacterial activity against Streptococcus mutans bacteria with an effective concentration of 2%.

Keywords: Antibacterial, Lidocaine, Local anaesthetics, Streptococcus mutans

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Introduction

The human oral cavity has the most complex microbial flora in the body. Generally, these microorganisms are non-pathogenic opportunistic commensals to maintain oral health condition and defend against pathogenic microorganisms. However, an imbalance of the oral environment can cause microorganism homeostatic to be disrupted and cause potentially pathogenic microorganisms.1

Streptococcus mutans are one of the normal flora found in the oral cavity, especially in plaque. This bacterium is known as the main etiology of dental caries by producing large amounts of organic acids.2 In severe cases, the infection produced by S. mutans can spread to the pulp tissue to the periapical tissue, causing odontogenic infections. Antibiotics are one of the measures needed in dealing with infections, but their effectiveness is still in doubt because it can cause bacterial resistance and allergic reactions.2

Local anaesthetics are drugs used in invasive procedures in addition to antibiotics. It is needed to relieve temporary pain sensations during dental procedures.1 Lidocaine is a local anaesthetic agent for the amide group and is one of the most commonly used local anaesthetics in Indonesia.1 Lidocaine has a fast and stable onset of action, and low levels of toxicity and allergenicity.2 As an anaesthetic agents, lidocaine is known to have analgesic, antiarrhythmic, and antiinflammation effects.2 Clinically, it has been known in dentistry that local anaesthetic agents rarely cause local infection in the injection site without administering disinfection. Several studies have shown that some local anaesthetics can inhibit various bacteria and fungi.2,3

Research conducted by Pelz et al.3 reported that lidocaine had inhibitory action on nearly 52 tested bacterial strains, except for pseudomonas aureginosa, enterococcus faecalis, dan candida albicans.6 Kesici et al.7 reported that lidocaine can inhibit the growth of Staphylococcus aureus dan Escherichia coli, except Pseudomonas aureginosa. When lidocaine is combined with adrenaline there is an increased antibacterial effect, then its against Pseudomonas aureginosa. It was also found that the anaesthetic agent did not have a bactericidal effect.7 A wider application of local anaesthetics could be suggested in the treatment of surgical wound infections.3

Based on the description above, the authors are interested in conducting research on the inhibition of local anesthetic lidocaine with various concentrations of 0.5%, 1%, and 2% against Streptococcus mutans.

Material and Methods

This type of research is a laboratory experimental study with a post test only control group design. This research was conducted at the Faculty of Pharmacy Microbiology Laboratory, Universitas Sumatera Utara, in February 2020. The samples used were streptococcus mutans obtained from the Faculty of Pharmacy Laboratory, Universitas Sumatera Utara, in February 2020. The samples were plated on nutrient agar, and incubated. After 24 hours, the inhibition zone formed around the disc paper was observed.
Sumatera Utara. The local anaesthetic tested was a commercially available solutions of lidocaine. It was diluted with 0.9% NaCl to get a concentration variation of 0.5%, 1%, and 2%.

The process of nutrient agar (NA) media is carried out by inserting 28 g of NA into the erlenmeyer, adding up to 1000 ml of aquadest and covering it with cotton. This medium was heated until completely dissolved, then sterilized in an autoclave at a temperature of 121°C for 15 minutes. Preparation of bacterial rejuvenation media using NA sterile as much as 3 ml, then put it in a test tube and let it stand at room temperature at an angle of 45°C. Furthermore, the media is stored in a refrigerator at 5°C.

After that, making a culture stock of S. mutans by taking one loop of bacteria from the pure culture using a sterile loop needle, then scratched it on the surface of the media to make it tilted, covered with cotton, and incubated at 37°C for 24 hours. Preparation of bacterial suspensions was carried out by taking bacteria that have grown on the media to tilt using a sterile loop needle, then suspending them in a test tube containing 10 ml of NA to make it sterile. The turbidity of the solution was measured using a spectrophotometer to obtain a transmittance of 25% equivalent to 10⁶ CFU (colony forming units).

The method used to test the antibacterial activity of lidocaine against S. mutans is the kirby-bauer disc diffusion method. The antibacterial activity test was carried out by providing sterile petri dishes, then adding 0.1 ml of S. mutans suspension, adding 15 ml of NA at a temperature of 45-50°C, then homogenizing and leaving until the media solidified. Next, provide five sterile disc papers containing the test materials, 0.5%, 1%, and 2% lidocaine, positive control (amoxicillin), and negative control (NaCl 0.9%), as much as 25 µl. Then, the paper discs were placed on the solidified media, let stand for 15 minutes, then incubated in an incubator at 37°C for 24 hours. Measurement of the diameter of the inhibition zone formed around the disc paper was carried out using a caliper after 24 hours of incubation.

The data obtained are the results of laboratory observations which analyzed parametrically with the One-way ANOVA test. If the test results show significant results, then proceed with the post-hoc Least Significant Difference (LSD) test.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Group</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>D* (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Streptococcus</strong></td>
<td>0.5%</td>
<td>6.45</td>
<td>6.95</td>
<td>7.05</td>
<td>7.30</td>
<td>7.15</td>
<td>6.98</td>
</tr>
<tr>
<td>mutans</td>
<td>1%</td>
<td>6.80</td>
<td>8.00</td>
<td>7.95</td>
<td>8.05</td>
<td>7.50</td>
<td>7.66</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>7.45</td>
<td>8.80</td>
<td>8.30</td>
<td>8.85</td>
<td>8.40</td>
<td>8.36</td>
</tr>
<tr>
<td></td>
<td>K+ **</td>
<td>19.90</td>
<td>20.00</td>
<td>20.10</td>
<td>20.70</td>
<td>21.20</td>
<td>20.38</td>
</tr>
<tr>
<td></td>
<td>K - ***</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 2. Shapiro-Wilk test

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>5</td>
<td>0.408</td>
</tr>
<tr>
<td>1%</td>
<td>5</td>
<td>0.098</td>
</tr>
<tr>
<td>2%</td>
<td>5</td>
<td>0.275</td>
</tr>
<tr>
<td>K+</td>
<td>5</td>
<td>0.262</td>
</tr>
<tr>
<td>K-</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

The data obtained are the results of laboratory observations which analyzed parametrically with the One-way ANOVA test. If the test results show significant results, then proceed with the post-hoc Least Significant Difference (LSD) test.

Results

The results of observing the diameter of the inhibition zone of each groups on the growth of Streptococcus mutans after incubation for 24 hours can be seen in table 1.

Based on table 1, it can be seen that there are inhibition zones in lidocaine with various concentrations and positive control, except negative control. Then, the data were tested for data normality using the Shapiro-Wilk to see whether the research was normally distributed or not. Table 2 shows that the research data are normally distributed with p > 0.05.

After performing the data normality test, the homogeneity test continued using the Levene test. Based on the tests that have been carried out, it
**Table 3. One-way ANOVA test**

<table>
<thead>
<tr>
<th>Combination</th>
<th>0.5%</th>
<th>1%</th>
<th>2%</th>
<th>K+</th>
<th>K-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>1081.326</td>
<td>4</td>
<td>270.331</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within groups</td>
<td>4.030</td>
<td>20</td>
<td>0.201</td>
<td>1341.595</td>
<td>0.000*</td>
</tr>
<tr>
<td>Total</td>
<td>1085.356</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Information: Ss = sum of squares, df = degrees of freedom, MS = mean of squares, F = F counts.
*There is a significant difference (p < 0.05)

**Table 4. LSD test**

<table>
<thead>
<tr>
<th>Combination</th>
<th>0.5%</th>
<th>1%</th>
<th>2%</th>
<th>K+</th>
<th>K-</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>-</td>
<td>0.027*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>1%</td>
<td>-</td>
<td>0.023*</td>
<td>0.000*</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>-</td>
<td>0.000*</td>
<td>0.000*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K+</td>
<td>-</td>
<td>0.000*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*There is a significant difference (p < 0.05)

shows the research data is homogeneous with a significance of 0.563 (p > 0.05). Furthermore, the data were tested in a parametric manner using the One-way ANOVA test to determine the difference in antibacterial activity in all groups against the growth of S. mutans.

Table 3 shows that the significance value of the data that have been tested by One-way Anova is 0.000 (p < 0.05), which means that there are significant differences between all groups on the growth of S. mutans, further analysis were carried out using the post test hoc Least Significant Difference (LSD). Based on the results of the LSD test in Table 4, it shows that each groups when compared to one another have a significant difference (p < 0.05).

**Discussion**

The method used to evaluate the antibacterial activity of lidocaine against streptococcus mutans is the kirby-bauer disc diffusion method or diffusion method. The results showed the formation of an inhibition zone around the disc paper after 24 hours of incubation in all lidocaine groups and positive control, except negative control. Lidocaine at concentration of 0.5% had an inhibition zone of 6.98 mm, a concentration of 1% had an inhibition zone greater than 0.5% concentration which was 7.66 mm, and a concentration of 2% had the largest inhibition zone of 8.36 mm. From these results, it can be seen that each increase in the concentration of lidocaine results in a wider diameter of the inhibition zone. While, positive control had an inhibition zone of 20.38 mm.

According to Davis and Stout, the bacterial inhibitory response can be categorized as weak if the inhibition zone is 5 mm or less, moderate is 5 to 10 mm, strong is 10 to 20 mm, and very strong is 20 mm or more.9 In this study, lidocaine concentrations of 0.5%, 1%, and 2% had a moderate inhibitory response because the resulting zone of inhibition was between 5 to 10 mm, while positive control had a moderate inhibitory response, its more than 20 mm. The inhibition zone produced by lidocaine is less effective than positive control in inhibiting the growth of S. mutans. It may be caused by several factors such as; organism sensitivity, culture medium, incubation conditions, and agar diffusion rate. The rate of agar diffusion is influenced by the concentration of microorganisms, composition of the media, incubation temperature, and incubation time.9

Previous research by Kesici et al.7 which uses bacteria cause nosocomial infections, showed that lidocaine can inhibit Staphylococcus aureus and escherichia coli. Based on the results, it was found that lidocaine inhibited the growth of Staphylococcus aureus with an inhibition zone of 12.00 mm and Escherichia coli with 15.60 mm.2 The resistance was quite small at 8.36 mm. This difference can be caused by the type of bacteria used. Kesici, et al. using aerobic bacteria, so it is likely that lidocaine is more effective against aerobic bacteria than facultative anaerobic bacteria.

Several hypotheses have been proposed to explain the antibacterial mechanism of local anaesthetics. Some researchers reported that bacterial growth can be inhibited by local anaesthetic agents caused by damage to the bacterial cell wall or cytoplasmic membrane, leakage of intracellular components, inhibition of dehydrogenase activity, and increased cell wall permeability. The result of penetration of local anesthetics from the bacterial membrane comes from the electron binding of the anaesthetic molecule to the polar bond, it is related to the hydrophobic nature of the anaesthetic on the surface of cell membrane. Local anaesthetics can inhibit the membrane activity of cell respiration and change their permeability and solubility, thus causing leakage of components from the cytoplasm characterized by the release of metal ions which play an important role in cell metabolism.10,11 In addition, local anesthetics can also inhibit membrane protein synthesis by causing increase in lipid molecules, resulting in changes in membrane fluidity in protein selective processes. Protein synthesis is known to be slightly more sensitive to inhibition.
than the production of DNA or RNA.\textsuperscript{12} It makes lidocaine selectively inhibit DNA, RNA, or protein synthesis through the cell wall or cytoplasmic membrane, causing damage and causing bacteria to become inactive and lysis.\textsuperscript{12}

After 24 hours of incubation there was a significant difference between the respective concentrations of 0.5%, 1%, and 2% when compared to one another. This indicates that the antibacterial activity of lidocaine will be more effective as the concentration increases.

Various studies have reported a lot of lidocaine activity in vitro against various pathogenic bacteria, therefore researchers suggest that further testing of the antibacterial activity of lidocaine against the growth of streptococcus mutans bacteria in vivo is needed to determine its clinical effectiveness and it can be used in dentistry.

\textbf{Conclusion}
This study proved that the local anaesthetic agent lidocaine has antibacterial activity in inhibiting the growth of Streptococcus mutans in vitro with 2\% lidocaine being the most effective concentration. According to Davis and Stout's classifications the inhibitory response to lidocaine has moderate potential.

\textbf{Acknowledgment}
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\textbf{Conflict of Interest}
The authors report no conflict of interest.

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