Interference of antibiotics in the growth curves of oral streptococci

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Abstract

The growth curve interference (GCI) induced by different antibiotics was studied using reference strains of oral streptococci. This parameter may prove to be useful in preventing subacute endocarditis of odontological origin in high-risk patients. The growth curves using different concentrations of antibiotics and with an initial optical density (OD) of 0.05 ± 0.01 were observed until an OD of 0.9–1.0 was reached. The GCI was defined as the lowest concentration of antibiotic that modified the growth curve with respect to a control without antibiotic. The GCI values were then compared with the minimum inhibitory concentration (MIC) values. In all cases, interference in the growth curves was at least one concentration lower than the MIC in a time ranging from 4–6 h.

Keywords: Antibiotic susceptibility; MIC; Oral streptococci

1. Introduction

The viridans group streptococci comprise a heterogeneous group of microorganisms that are an important part of the normal flora of the oral cavity, and for this reason are also known as oral streptococci. They may also be isolated from areas such as the pharynx, skin, vagina or intestine, where they are not usually considered pathogenic. However, viridans group streptococci may be involved in sepsis and pneumonia in subjects who are immunodepressed [1–3], in sepsis and meningitis in neonates [4] and, particularly, in subacute endocarditis, often associated with bacteraemia after dental procedures [5]. They have also been isolated from invasive pyogenic infections in immunocompetent hosts [6–8].

These microorganisms were traditionally considered to be uniformly sensitive to a wide range of antibiotics [9]. Recent studies have come to refute this assumption, with reports of strains with a high resistance to aminoglycosides [10,11] and of strains tolerant with respect to penicillin [12–14] and resistant to macrolides [14,15] and other antibiotics [16].

Not only does resistance create problems in treating the infections produced by these microorganisms, it is also important because resistance can be transmitted to other bacteria, for example to Streptococcus pneumoniae in the case of penicillin [13,14,16]. Increased bacterial resistance has been related to the use of antibiotics in dental treatment [17,18]. Other studies identify an in vitro relationship between the production of slime, certain biotypes and the efficacy of antibiotics [10,19].

The objective of the present study was to evaluate the interference in the growth curves of oral streptococci produced by certain antibiotics and to relate this interference to their minimum inhibitory concentration (MIC) value.

2. Materials and methods

2.1. Assayed strains

The following streptococcal reference strains were used: Streptococcus mutans Ingbritt, Streptococcus sanguis DSMZ...
Table 1

Minimum inhibitory concentrations (MICs) of the antibiotics tested against the streptococcal bacterial strains

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (mg/L)</th>
<th>S. mutans Ingbritt</th>
<th>S. sanguis DSMZ 6777</th>
<th>S. gordonii NCTC 3165</th>
<th>S. oralis NCTC 11427</th>
<th>S. salivarius HHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0.07</td>
<td>0.07</td>
<td>0.015</td>
<td>0.07</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.03</td>
<td>0.12</td>
<td>0.12</td>
<td>0.07</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>0.015</td>
<td>0.07</td>
<td>0.07</td>
<td>0.12</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.0003</td>
<td>0.015</td>
<td>0.015</td>
<td>0.003</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.03</td>
<td>0.015</td>
<td>0.25</td>
<td>0.03</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Spiramycin</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Josamycin</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
<td>0.7</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>0.25</td>
<td>0.07</td>
<td>0.07</td>
<td>0.12</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.015</td>
<td>0.03</td>
<td>0.015</td>
<td>0.07</td>
<td>0.015</td>
<td></td>
</tr>
</tbody>
</table>

6777, Streptococcus gordonii NCTC 3165, Streptococcus oralis NCTC 11427 and Streptococcus salivarius HHT.

2.2. Determination of the MIC

Antibiotics were tested at concentrations ranging from 0.00007 mg/L to 16 mg/L. The MIC was determined using an agar dilution method, a Steer’s replicator and Wilkins Chalgrens agar (Difco Laboratories, Madrid, Spain). An inoculum of \(10^5 – 10^6\) colony-forming units/mL was used. Plates were incubated at 36 ± 1°C in an anaerobic atmosphere containing 85% N₂, 10% H₂ and 5% CO₂ and readings were taken after 48h [9]. The MIC was defined as the lowest concentration of antibiotic that visibly inhibited growth of the microorganisms.

2.3. Growth curve interference (GCI)

The tested strains, taken from a 24h culture in Wilkins Chalgrens broth (Difco Laboratories), were inoculated in 9 mL of the same medium until reaching an optical density (OD) of 0.05 ± 0.01 at a wavelength of 600 nm in a spectrophotometer (Zuzi series 4200; Zuzi, Beijing, China). For each strain, a series of tubes was prepared with the aforementioned OD value and the nine studied antibiotics were added in a scaled series of seven concentrations greater than, equal to and below the MIC, giving a total of 315 experiments. As the growth control for each assay, a tube with the same medium and inoculum but with no antibiotic was used. The tubes were incubated in a bain-marie at 36 ± 1°C. Spectrophotometry readings of the OD were taken every hour. The experiment was considered complete when an OD of 0.9–1.0 was reached with the lowest concentration of antibiotic that modified the growth curve with respect to the control. This value is referred to as the GCI.

The following antibiotics were used: penicillin, amoxicillin, cefuroxime, erythromycin, spiramycin, roxithromycin and clindamycin (Sigma Chemical Co., St Louis, MO), imipenem (Merck Sharp Dohme, Madrid, Spain) and josamycin (Ferrer Grupo, Barcelona, Spain).

3. Results

The MICs of the antibiotics tested are given in Table 1. Table 2 shows the GCI of the 45 tests in which the OD of 0.9–1.0 was reached with the minimum concentration of antibiotic. In 25 cases (55.6%) this OD was obtained with the concentration immediately below the MIC (Fig. 1). This
Table 2
Concentration of antimicrobial and time corresponding to optical density values of 0.9–1 for the streptococcal bacterial strains tested

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>S. mutans Ingbritt</th>
<th>S. sanguis DSMZ 6777</th>
<th>S. gordonii NCTC 3165</th>
<th>S. oralis NCTC 11427</th>
<th>S. salivarius HHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0.03 (6)</td>
<td>0.015 (4)</td>
<td>0.007 (6)</td>
<td>0.007 (4)</td>
<td>0.007 (6)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.015 (6)</td>
<td>0.07 (5)</td>
<td>0.07 (6)</td>
<td>0.03 (4)</td>
<td>0.007 (6)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>0.007 (5)</td>
<td>0.03 (5)</td>
<td>0.03 (6)</td>
<td>0.07 (6)</td>
<td>0.007 (6)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.0003 (5)</td>
<td>0.003 (6)</td>
<td>0.007 (6)</td>
<td>0.0003 (4)</td>
<td>0.015 (6)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.0015 (6)</td>
<td>0.007 (6)</td>
<td>0.12 (6)</td>
<td>0.007 (5)</td>
<td>0.015 (6)</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>0.07 (5)</td>
<td>0.25 (6)</td>
<td>0.12 (6)</td>
<td>0.25 (4)</td>
<td>0.07 (5)</td>
</tr>
<tr>
<td>Josamycin</td>
<td>0.07 (6)</td>
<td>0.07 (6)</td>
<td>0.07 (5)</td>
<td>0.07 (6)</td>
<td>0.007 (6)</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>0.015 (4)</td>
<td>0.03 (4)</td>
<td>0.015 (4)</td>
<td>0.015 (4)</td>
<td>0.015 (5)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.0007 (5)</td>
<td>0.007 (5)</td>
<td>0.007 (5)</td>
<td>0.03 (6)</td>
<td>0.007 (6)</td>
</tr>
</tbody>
</table>

MIC, minimum inhibitory concentration.

Finding was consistent for cefuroxime with all the strains, and it was nearly always the case for amoxicillin (with one exception for S. salivarius HHT). In nine cases (20%) the OD of 0.9–1.0 was obtained with concentrations two under the MIC value (Fig. 2), and in 11 instances (24.4%) the concentrations were clearly lower than the MIC (Fig. 3). This clear difference between the MIC and the GCI was not observed at all for S. sanguis or S. gordonii, nor was it associated in any case with amoxicillin or cefuroxime. It was more frequent for S. mutans Ingbritt and S. oralis NCTC 11427, and with the antibiotics imipenem, spiramycin, josamycin and roxithromycin. The OD 0.9–1.0 values were reached in varying periods of time. In seven cases (15.6%) it took 4 h. It is noteworthy that S. mutans Ingbritt, S. gordonii NCTC 3165 and S. salivarius HHT did not reach the established OD in 4 h. It took 5 h in 14 cases (31.1%) and 6 h in the remainder of the experiments (53.3%).

4. Discussion

Viridans group streptococci, also known as oral streptococci, are increasingly involved in human infections, most notably perhaps in subacute endocarditis. These infections often take place after some sort of dental procedure. For this reason, preventive clinical trials have been developed using antibiotics, especially for high-risk patients. Depending on the patient, such measures may involve amoxicillin, clindamycin or erythromycin given orally, or parenteral administration of ampicillin and vancomycin [20]. Preventive interventions are also used when there is a risk of bacteraemia of oncohaematological origin [21]. As far as the treatment of viridans streptococcal endocarditis is concerned, combinations of antibiotics such as penicillin and aminoglycosides or vancomycin with aminoglycosides may be used, with the duration of therapy depending on the patient’s response. Alternative therapeutic or prophylactic regimens have been proposed by various authors [22,23].

The different patterns of susceptibility of different species to a number of antibiotics and the risk of transmission of such resistance [11,16] make it necessary to increase our knowledge of the activity of antibiotics on oral streptococci.

A number of authors have described parameters that provide a dynamic view of antibiotic activity in vitro. Such is the
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