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In vitro activity of macrolides and lincosamides against oral streptococci: a therapeutic alternative in prophylaxis for infective endocarditis

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Susceptibility to macrolides and lincosamides was tested in a total of 446 strains of oral streptococci belonging to eleven species, all isolated from dental plaque and/or saliva in 1991. Minimum inhibitory concentrations (MIC) were determined by the double serial dilutions method in agar. Clindamycin was the most effective antibiotic against all species tested. The results of nearly all parameters (range, mean, MIC_{50}, MIC_{90}) in all species showed erythromycin to be less effective than josamycin, making the latter antibiotic a possible alternative to erythromycin in prophylaxis for infective endocarditis caused by oral streptococci, not only in patients allergic to penicillin, but also in all patients with low-risk lesions, who will be treated with low-risk dental procedures. In these latter patients, antibiotic prophylaxis may be indicated if, after careful evaluation of the individual's situation, no other alternatives are available.

Key words: Oral streptococci; In vitro susceptibility; Endocarditis; Macrolides; Lincosamides

Introduction

Streptococci of the so-called *viridans* group are, together with staphylococci, the bacteria most frequently involved in the etiology of infective endocarditis. Approximately 40% of all subacute forms of the disease are caused by these microorganisms [1–4]. Oral streptococci are the largest subgroup within the *viridans* group. Several studies have identified *Streptococcus sanguis*, *S. mitior* and *S. mutans* as the microorganisms most frequently isolated in subacute infective endocarditis caused by *viridans* group bacteria [5,6]. These organisms usually cause left endocarditis, while right endocarditis is less frequent [4]. Adhesion factors, especially glycocalix molecules [5,7–10], have been implicated in their ability to colonize the valves and the endothelium of great vessels; these factors would also afford the bacteria some protection against opsonization and phagocytosis. However, the existence of endocarditis-causing oral streptococci without glycocalix suggests

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that other pathogenic mechanisms are also involved [11–13].

Because of the seriousness of infective endocarditis, there is widespread agreement that treatment should consist of orally administered bactericidal antibiotics, such as a combination of beta lactams and aminoglucosides. In patients allergic to the former, the antibiotics of choice are vancomycin and teicoplanin. In subjects with high-risk lesions (e.g., valvular prosthesis or certain heart diseases), and in high-risk procedures, the prophylactic measures are similar, although the dosages are different [14–16].

There is disagreement as to the best antibiotic prophylaxis in patients with low-risk lesions to be treated with low-risk procedures. Most regimens are based on experimental models with bactericidal antibiotics alone or in combination [17–18]. Experimental studies with bacteriostatic antibiotics have provided conflicting results [19,20].

The objective of the present study was to analyze the susceptibility of oral streptococci to a group of antibiotics (macrolides and lincosamides) in search of a potential alternative for prophylaxis against infective endocarditis caused by these microorganisms in patients with low-risk lesions, who will be treated with low-risk dental procedures.

Materials and methods

Antimicrobial drugs

Standard powders were obtained from different manufacturers: erythromycin (Antibioticos, Madrid), lincomycin and clindamycin (Upjohn Farmoquímica, Madrid), spiramycin (Rhône-Poulenc, Alcorcon, Madrid), acetyl spiramycin (Hubber, Barcelona), josamycin (Ferrer International, Barcelona), roxitromycin (Russell, Madrid), oleandomycin and azitromyicn (Pfizer, Madrid) and diacetylmidecamycin (Menarini, Barcelona).

Isolates

The microorganisms were isolated from dental plaque or saliva of different patients throughout the year 1991. All strains were fixed by lyophilization and identified according to Hardie's [21] or Loesche's [22] criteria. In all, 60 different strains of *Streptococcus mutans*, 36 of *S. sobrinus*, 10 of *S. cricetus*, 10 of *S. macacae*, 10 of *S. rattus*, 56 of *S. mitior*, 40 of *S. mitis*, 68 of *S. salivarius*, 58 of *S. milleri*, 76 of *S. sanguis* and 22 of *S. oralis* were studied.

Susceptibility testing

The antibiotics were tested at concentrations ranging from 0.0015 to 64 μg/ml. The minimum inhibitory concentration (MIC) was determined using an agar dilution method, Steer's replicator and Wilkins-Chalgren medium (Difco Laboratories, Madrid). As the inoculum we used 10^5 to 10^6 cfu/ml, obtained from a 24-h culture grown in trypticase soy broth medium without dextrose (Scott Laboratories, Madrid). Plates were inoculated at 37°C in an anaerobic atmosphere containing 85% N_2, 10% H_2 and 5% CO_2, and readings were taken after 48 h [23]. The MIC was defined as the lowest concentration of antibiotics that visibly inhibited growth of the microorganisms. Linear extrapolation from values obtained from the next higher and next lower values were used to express the exact values of MIC_{50} and MIC_{90}.

As a control for intraobserver variability, replicate cultures were done of eight strains of each species represented in the present study by 18 or more strains, and two strains of each species represented by four to six strains. The results of these cultures were read by independent observers.

Statistical procedure

Snedecor's F statistic was calculated for the statistical analysis of quantitative variables.

Results

The susceptibilities of oral streptococci to antibiotics are shown in Table 1. The highest mean value was recorded for *S. milleri* susceptibility to oleandomycin (14.24 μg/ml), followed by *S. sobrinus* susceptibility to acetylspiramycin (9.27 μg/ml). The lowest mean values were found for *S. sobrinus* (0.08 μg/ml) and *S. mutans* susceptibility (0.11 μg/ml) to clindamycin. The highest MIC_{90} were those of ace-

TABLE 1

Susceptibility of 446 strains of oral streptococci to eight macrolides, lincomycin and clindamycin

Antimicrobial agents	*S. mutans* (60)[a]	*S. sobrinus* (36)	*S. sanguis* (76)	*S. mitior* (56)	*S. mitis* (40)	*S. salivarius* (68)	*S. milleri* (58)	Miscellaneous[b] (52)
Oleandomycin								
Range	0.25–4	1–8	0.12–32	0.06–32	0.25–32	0.5–32	0.5–32	0.25–8
$\bar{x}$	1.38	4.66	3.28	3.67	7.2	9.75	14.24	1.99
MIC_{50}	0.67	3.01	0.45	0.7	0.9	1.79	1.5	1.2
MIC_{90}	2	6.9	4.77	6.3	21.33	25.2	13.21	3.6
Erythromycin								
Range	0.03–16	0.06–4	0.03–4	0.03–16	0.06–16	0.05–16	0.06–16	0.06–8
$\bar{x}$	1.28	0.52	0.75	2.01	2.57	2.56	4.61	0.73
MIC_{50}	0.08	0.12	0.09	0.06	0.12	0.1	0.15	0.09
MIC_{90}	1	1.1	2.01	4.8	5.66	5.79	13.1	0.87
Spiramycin								
Range	0.25–1	0.5–16	0.25–16	0.25–16	0.25–16	0.25–16	0.25–16	0.25–16
$\bar{x}$	0.58	4.5	1.15	2.4	2.48	5.23	5.95	3.43
MIC_{50}	0.37	0.75	0.36	0.43	0.58	0.68	0.48	1.23
MIC_{90}	0.85	5.29	1.74	4.79	4	12.11	13.42	8.9
Acetylspiramycin								
Range	1–4	0.5–32	0.25–16	0.25–32	1–32	0.25–32	0.25–32	0.5–32
$\bar{x}$	1.6	9.27	1.84	3.72	5	6.76	4.41	8.14
MIC_{50}	0.8	1	0.6	0.7	1.33	0.57	0.48	3.2
MIC_{90}	3	24.8	3.2	6.42	4	22.9	24.26	17.6
Diacetylmidecamycin								
Range	0.03–1	0.25–2	0.03–16	0.03–16	0.12–16	0.03–16	0.03–16	0.03–8
$\bar{x}$	0.39	1.05	1.75	1.81	2.6	2.67	2.83	1.44
MIC_{50}	0.18	0.63	0.10	0.08	0.4	0.5	0.31	0.7
MIC_{90}	0.78	1.7	1.2	4.8	4	3.2	10.2	2.4
Josamycin								
Range	0.03–1	0.25–2	0.12–1	0.12–16	0.12–16	0.06–16	0.12–16	0.12–1
$\bar{x}$	0.3	0.8	0.36	1.65	1.55	1.6	2.62	0.52
MIC_{50}	0.18	0.4	0.18	0.17	0.36	0.31	0.4	0.2
MIC_{90}	0.45	1.55	0.64	2.4	2	2.6	6.8	0.8
Roxitromycin								
Range	0.03–0.5	0.25–2	0.06–16	0.06–16	0.06–16	0.03–16	0.06–16	0.06–4
$\bar{x}$	0.21	0.9	1.54	2.3	2.5	2.37	4.4	0.97
MIC_{50}	0.12	0.44	0.1	0.14	0.18	0.12	0.37	0.42
MIC_{90}	0.4	1.64	3.2	8.54	8	5.8	12.68	1.8
Azitromycin								
Range	0.12–1	0.5–16	0.12–16	0.12–16	0.12–16	0.12–16	0.25–16	0.12–16
$\bar{x}$	0.36	4.16	1.86	2.03	2.7	4.8	5.72	2.5
MIC_{50}	0.18	1.5	0.27	0.20	0.25	0.5	0.63	0.3
MIC_{90}	0.70	8.8	4.4	4.84	8	12.11	13.1	6.4
Lincomycin								
Range	0.06–0.05	0.25–1	0.12–4	0.06–4	0.06–4	0.06–4	0.06–4	0.06–8
$\bar{x}$	0.16	9.62	0.65	0.52	0.63	1.47	1.05	0.64
MIC_{50}	0.09	0.40	0.16	0.23	0.29	0.41	0.53	0.18
MIC_{90}	0.23	0.87	1.6	0.6	1	2.3	2.55	1.2
Clindamycin								
Range	0.015–1	0.015–0.12	0.03–2	0.03–4	0.03–4	0.03–4	0.03–4	0.03–4
$\bar{x}$	0.11	0.08	0.22	0.37	0.55	0.29	0.57	0.79
MIC_{50}	0.035	0.14	0.07	0.05	0.072	0.08	0.08	0.04
MIC_{90}	0.18	0.23	0.4	0.3	1	0.45	1.11	0.19

[a]Numbers in parentheses are the number of strains studied.

[b]The miscellaneous group included 22 strains of *S. oralis*, and 10 each of *S. cricetus*, *S. macacae* and *S. rattus*.

tylspiramycin against *S. salivarius* (22.9 μg/ml) and *S. milleri* (24.26 μg/ml), while the lowest were those of clindamycin against *S. mutans* (0.18 μg/ml) and lincomycin and clindamycin against *S. mutans* and *S. sobrinus* respectively (0.23 μg/ml). The MIC_{90} for all species were lowest for clindamycin, followed by lincomycin and josamycin. The exceptions were *S. sanguis*, in which the MIC_{90} for josamycin (0.64 μg/ml) was lower than that for lincomycin (1.6 μg/ml), *S. sobrinus*, in which the MIC_{90} for erythromycin (1.1 μg/ml) was lower than that for josamycin (1.55 μg/ml), and *S. mutans*, for which the MIC_{90} for roxitromycin (0.4 μg/ml) was slightly lower than that for josamycin (0.45 μg/ml).

The results of statistical analysis showed significant differences between antibiotic activities in different species of oral streptococci, except for spiramycin, diacetylmidecamycin and clindamycin (Table 2). Significant differences in susceptibility were also found between the species, with the exception of *S. mitior* (Table 3).

Discussion

Several reviews have reported the frequencies of transient bacteremias caused by oral streptococci after dental procedures, or after simply chewing gum or paraffin, or brushing one's teeth. However, the risk of developing endocarditis is negligible [24,25] except in particularly susceptible patients, where it is nonetheless very low. Notwithstanding these slight probabilities, prophylactic antibiotic regimens have been developed for patients with low-risk lesions, due to undergo moderate-risk procedures (e.g., thorough check-up and cleaning, scaling and extraction) [14–16]. Such regimens have involved a range of measures, from parenteral oral drug treatment [26] to oral amoxycillin [14–16]. These prophylactic measures have been effective in experimental models [17,27]. Erythromycin and clindamycin have been suggested as alternatives in patients allergic to penicillin [14–16]. As noted above, these measures are based on experimental studies in animal models, and the findings cannot be extrapolated to humans. Moreover, in addition to the almost complete absence of risk in dental patients, there is no clinical evidence of any beneficial effect of prophylactic antibiotic therapy [28]. Because the currently accepted norm seems excessively cautious [28], we sought equally reliable alternatives. If antibiotic prophylaxis for oral streptococcus-caused infective endocarditis is nonetheless being considered, the following facts should be taken into account:

- Some strains of oral streptococci are susceptible to penicillin (MIC $\leq$ 0.1 μg/ml). Against these microorganisms, prophylaxis with the same antibiotics as those to be used for treatment (or with similar antibiotics) seems unadvisable, as this would probably reduce the microorganism's susceptibility [29]. In fact, strains moderately resistant to penicillin have appeared in patients who received oral prophylaxis with this antibiotic [30].
- Penicillin-tolerant strains have been described for all the microorganisms tested in the present study [31] and in 20% of endocarditis patients [32]. These patients would probably require larger doses of antibiotics than are available via oral administration to attain bactericidal concentrations in serum. Such findings, although obtained in animal studies,

TABLE 2

Results of the comparisons of the activities of different antibiotics in oral streptococci

Antibiotic	Significance	Antibiotic	Significance
Oleandomycin	$P < 0.01$	Josamycin	$P < 0.05$
Erythromycin	$P < 0.01$	Roxitromycin	$P < 0.05$
Spiramycin	N.S.	Azitromycin	$P < 0.01$
Acetylspiramycin	$P < 0.01$	Lincomycin	$P < 0.1$
Diacetylmidecamycin	N.S.	Clindamycin	N.S.

TABLE 3

Results of the comparison of susceptibilities of oral streptococci to different antibiotics

Microorganism	Significance	Microorganism	Significance
S. mutans	$P < 0.01$	*S. mitis*	$P < 0.05$
S. sobrinus	$P < 0.01$	*S. salivarius*	$P < 0.01$
S. sanguis	$P < 0.01$	*S. milleri*	$P < 0.01$
S. mitior	N.S.	Miscellaneous	$P < 0.01$

should be taken into account, albeit with due caution [18,33–35].

· Some strains have been identified as nutritional variants; although apparently similar to other strains of a given species, they cause a greater tendency toward recurrence in patients with endocarditis [36–38].

· Nearly all species of oral streptococci produce glucanes and fructanes, which impede the penetration of beta lactams when bacterial vegetations lodge in the cardiac tissues [9,10].

This led us to test the hypothesis that macrolide and lincosamide antibiotics are effective in prophylaxis against infective endocarditis, not only in patients allergic to beta lactams, but in dental patients, after careful consideration of each individual case has ruled out present and future alternatives [28].

As shown by the results of the statistical analysis, not all species of oral streptococci were equally susceptible to macrolides and lincosamides, nor did all antibiotics show the same activity. Clindamycin was clearly the most effective antibiotic in vitro against all species we tested, and therefore represents the best alternative in prophylaxis against infective endocarditis caused by oral streptococci. This conclusion is supported by empirical studies of the prevention of endocarditis [15]. When clindamycin was compared with erythromycin, the result of the present study clearly favored the former. Although none of the species were unequivocally resistant to erythromycin, other macrolides were more active, e.g., roxitromycin against *S. mutans*, and josamycin against nearly all species. Erythromycin was less effective against *S. mutans* in the present study than in a previously published report [29]. This finding, together with the possibility of cross-resistance between erythromycin and other antibiotics (including tylosin, used as an additive in the meat packaging industry), and enhanced resistance caused by previous exposure [39], suggest that this antibiotic may be replaced, in prophylaxis against oral streptococci endocarditis, by macrolides such as josamycin, which involve fewer problems related to cross-resistance.

Oral streptococci are widely believed to be susceptible to antibiotics habitually used in clinical practice. Our findings partially confirm this assumption: inhibition of 90% of the strains of a given species required relatively large amounts of some antibiotics, especially acetylspiramycin and spiramycin when tested against *S. sobrinus*, *S. salivarius* and *S. milleri*. The latter microorganism was the least susceptible to the antibiotics we tested, a logical finding in view of the heterogeneity of this group [40]. The other end of the spectrum was occupied by *S. mutans*, a highly susceptible species. As noted in a previous study [29], some antibiotics showed a notable decline in effectiveness between 1985 and 1989, e.g., erythromycin against *S. mutans*, acetylspiramycin and spiramycin against *S. sobrinus*. In contrast, other antibiotics have become more effective, e.g., erythromycin against *S. sobrinus*, and spiramycin against *S. mutans*. These observations exemplify the variations in susceptibility oral streptococci can display within a single environment.

In conclusion, clindamycin was the most effective of the antibiotics tested in vitro against oral streptococci. Although there is no experimental evidence for the usefulness of clindamycin as an antibiotic prophylactic against infective endocarditis caused by streptococci, in subjects with low-risk lesions subjected to low-risk dental procedures, this agent may be the drug of choice. Josamycin could be used as the antibiotic of second choice, given that our findings showed it to be more effective than erythromycin.

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