MEDICAL SCIENCES (2000), 2, 25–31

In-vitro activity of synercid and related drugs against *Streptococcus oralis* isolated from septicaemia and endocarditis cases

* Rafay A M

فاعلية عقار السنرسيد وأمثاله ضد المكورات السبحية الفمية المعزولة من حالات التهاب بطانة القلب والأنتانية (انتان الدم وعفونته)

أ. رافع

الملخص : الهدف: مع زيادة المقاومة للبكيتريا موجبة الصبغة وشدة خطورة التهاب بطانة القلب أصبح من الضرورة النظر الى طرق بديلة للعلاج. **الطريقة:** تمت معملياً دراسة منحنى الإبادة لستة من المكورات السبحية تم عزلها من مرضى التهاب بطانة القلب البكتيري، وأثنين من مرضى تسمم الدم ، واثنين من أفواه أشخاص أصحاء ، ثم مقارنة فاعلية السنرسيد مع البنسلين، الأموكسسلين، التيكوبلانين، الفنكومايسين، الكليندمايسين والإرثرومايسين في المختبر. **النتائج:** كانت الفاعلية التثبيطية الصغرى للسنرسيد ضيقة وتتراوح بين 0.06-0.5 ملغ/لتر وكانت قيمة القراءتين 0.25ملغ/لتر. وقد وجد فرق بسيط بين الفاعلية التثبيطية الصغرى (0.25ملغ/لتر) والفاعلية التثبيطية الكبرى (0.5 ملغ/لتر). وبالرغم من أن الفاعلية الصغرى للسنرسيد ضد المكورات السبحية من نوع أورواليس كانت نسبياً أعلى مقارنة بكل من البنسلينن، كليندمايسين، ارثرومايسين وتيكوبلانين إلا أن مقدرة السنرسيد على إبادة الباكتريا في المختبر كانت أكبر بكثير. وجد أن التركيزات القاتلة للسنرسيد مساوية أو أدنى من 4ملغ/لتر لمعظم المعزولات ماعدا إثنتين واحدة كانت 16ملغ/لتر والأخرى أكبر من 64 ملغ/لتر. **الخلاصة:** أظهر السنرسيد فاعلية إبادة عالية متفوقاً على كل من البنسلين والفانكومايسين، بالنسبة لكل المعزولات العشرة من المكورات السبحية بنسبة 99.9% خلال ستة ساعات من الملامسة.

ABSTRACT: ***Objective –*** The increase in resistance to gram positive organisms and seriousness of infective endocarditis, makes it necessary to look for an alternate treatment. ***Method –*** In-vitro activity of synercid was compared with penicillin, amoxycillin, teicoplanin, vancomycin, clindamycin and erythromycin. ***Result –*** Synercid showed minimum inhibitory concentrations (MIC) within the narrow range of 0.06 – 0.5 mg/l. MIC_{50} and mode values were both 0.25 mg/l. There was just two-fold difference between the MIC_{50} (0.25 mg/l) and the MIC_{90}, (0.5 mg/l). Although the MICs of synercid for *S. oralis* were relatively high compared to penicillin, clindamycin, erythromycin and teicoplanin, the in-vitro bactericidal activity of synercid was much greater. Synercid MBC values were < 4 mg/l for most of the isolates, except for one of 16 mg/l and the other >64 mg/l. Killing curve was performed on six isolates of *S. oralis* from infective endocarditis, two from septicaemia patients and two from the oral flora of normal individuals. ***Conclusion –*** Synercid showed superior bactericidal activity when compared to penicillin and vancomycin against all ten isolates of *S. oralis* tested. Synercid was bactericidal (99.9% kill) against all ten isolates of *S. oralis* within six hours of contact.

KEY WORDS: *streptococcus oralis*, synercid, penicillin, amoxycillin, erythromycin, vancomycin, teicoplanin, clindamycin

Viridans streptococci are among the commonest causes of infective endocarditis, except in intravenous drug abusers where *Staphylococcus epidermidis* is frequently isolated.[1] Bayliss found that streptococci or enterococci were the causative organisms in 63% of infective endocarditis cases and that 48% of these cases were viridans streptococci.[2] These findings were also supported by Young.[3] With newer identification methods viridans streptococci have been further classified to its species level and it has been shown that *S. oralis*, S. *sanguis*, and *S. gordonii* are the most frequently isolated species in patients with infective endocarditis.[4,5] Owing to an increasing resistance to β-lactam antibiotics, the treatment of infective endocarditis has become more complicated.[6] Keeping these in mind, a range of antibiotics was considered, where antibiotic sensitivity, bactericidal kill and killing curve were tested to estimate their potential for the treatment of infective endocarditis caused by *S. oralis*. Synercid (RP59500), like pristinamycin, is a streptogramin antibiotic. It is a semi-synthetic modification of the two major constituents of pristinamycin: pristinamycin IA and pristinamycin IIA. Synercid consists of a quinuclidinylthiomethyl pristinamycin IA derivative and a diethlyaminoethyl-sulphonyl pristinamycin IIA derivative in a ratio of 30:70 weight for weight.[7] Its several novel properties have excited the interest of infectious diseases researchers.[7,8,9] One of these is the activity of synercid against a number of resistant Gram-positive pathogens.[10] Because of its

Department of Microbiology, College of Medicine, Sultan Qaboos University, P.O.Box: 35, Postal Code: 123, Muscat, Sultanate of Oman

bactericidal activity against oral streptococci, its role in the treatment of infective endocarditis is indicated.

METHOD

A total of sixty clinical isolates of *S. oralis* were collected from patients either with endocarditis, neutropenia and from the normal oral flora of healthy individuals. Strains from blood culture were primarily isolated using Bactec NR850 and identified using API 20 Strep (bio Merieux, La Balme les Grottes, France), and were further identified using laboratory-devised method.[11]

DETERMINATION OF MIC

The isolates were tested for their susceptibility to penicillin (Glaxo), amoxycillin (Sigma), erythromycin (Abbot), vancomycin (Sigma), teicoplanin (Merrel Dow), clindamycin (Upjohn) and synercid, a new injectable streptogramin. Isolates grown on columbia agar (CA) were used to inoculate 10 ml of iso-sensitest broth (Oxoid CM473) supplemented with 2% horse serum (Wellcome No. 5) and incubated for 4 hours at 37°C in air. Broth suspensions were adjusted by making a standard dilution in iso-sensitest broth in order to obtain a final inoculum on antibiotic containing agar plates of approximately 10^4 colony-forming units (cfu). Doubling dilutions of the antibiotics were prepared in 0.1M phosphate buffer of pH 7 to provide final concentrations in iso-sensitest agar (Oxoid CM471) over the range 0.003–128 mg/l. A multipoint inoculator (Denley Instruments Ltd) was used to inoculate the isolates, which were then incubated aerobically at 37°C for 18 hours. The MIC was defined as the lowest antibiotic concentration that completely suppressed visible growth (one colony being ignored). Standard strain of *S. oralis* (A6) was inoculated with each batch of susceptibility tests to serve as control (MIC 0.12 mg/l for penicillin).

DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATIONS (MBC)

A total of 15 clinical isolates of *S. oralis* were used. Isolates AR3, AR12, AR13, AR19, AR40 were from patients with endocarditis, 92C17, 93C87, T8-2-12 from patients with neutropenia, 23, 24, A26, N4-1-4 from those with normal oral flora and A6, A10, A 9 from those with septicaemia. MIC/MBC values were determined for synercid, penicillin, vancomycin, clindamycin, erythromycin and teicoplanin against 15 strains of *S. oralis* using a microtitre method, with antibiotic concentrations ranging from 0.003–64 mg/ml. Doubling dilutions of antibiotics were prepared in iso-sensitest broth and these were inoculated with 5 hour broth cultures, diluted to give a final concentration of 10^4 cfu/mL. After 18 hours of incubation at 37°C, MBCs were determined by subculture of all wells with no visible growth. MIC was recorded as the highest antibiotic dilution showing no turbidity. MBCs were determined by transferring 100µl from wells showing no growth to CA plates. The inoculum was allowed to dry before spreading and incubated for 18 hours at 37°C in air. The MBC was taken as the lowest antibiotic concentrations of antimicrobial that reduced the number of viable organisms by 99.9% kill after 18 hours incubation.

TIME KILL CURVES.

The in-vitro bactericidal activities of penicillin, vancomycin and synercid were compared against ten isolates of *S. oralis.* Organisms were grown in brain heart infusion (BHI) for 18 hours at 37°C in air and 100 µl added to 100 ml of freshly prepared pre-warmed BHI. After one hour incubation at 37°C on an aerobic shaker, solutions of antibiotics were added to the culture to provide final concentrations of 4 times the previously determined MICs for *S. oralis* under investigation and an antibiotic-free growth control.

Viable counts were performed at one, two, four, six and twenty-four hours by the Miles and Misra[12] method. The counts were converted to $\log_{10}$ and the mean of the duplicate determination calculated. The counts did not differ by more than 10% and the majority of the counts differed by less than 5%.

RESULTS

Table 1 demonstrates the relative activities of penicillin, amoxycillin, erythromycin, clindamycin, vancomycin, teicoplanin and synercid against 60 isolates of *S. oralis* isolated from infective endocarditis, neutropneic and normal oral flora patients. Table 2 shows the MIC_{50}, MIC_{90} and mode MIC values for each of the antibiotics tested. Synercid was the most active of all the agents tested, except with clindamycin and for some strains with erythromycin. One distinct population of isolates could be distinguished with synercid, all of which were inhibited within a narrow 0.06–0.5 mg/l range. The MIC_{50} and mode values were both at 0.25 mg/l, with two-fold difference between the MIC_{50} and MIC_{90}, at 0.25 mg/l and 0.5 mg/l respectively. Synercid was four fold more active compared with penicillin and amoxycillin. MICs for penicillin and amoxycillin were similar, with a range of 0.015–16.0 mg/l. The mode and MIC_{50} values for penicillin and amoxycillin were 0.03 mg/l and 0.125 mg/l respectively, while MIC_{90} values were 2 and 8 mg/l, respectively. The distribution of isolates according to their susceptibility to erythromycin showed one population with an MIC range of 0.015 – 2 mg/l, in an approximate normal distribution with 6% strains requiring MIC 8 mg/l and 2% requiring 64 mg/l.

TABLE 1.

Percentage of S. oralis (n=60) isolates susceptible to penicillin, amoxycillin, clindamycin, erythromycin, , teicoplanin, vancomycin, Synercid, (MIC, mg/l).

Antibiotics	0.0037	0.0075	0.015	0.03	0.06	0.12	0.25	0.50	1	2	4	8	16	32	64
Penicillin	-	-	3	21	14	20	14	5	11	3	6	3	-	-	-
Amoxycillin	-	-	3	24	14	9	18	3	13	3	-	5	8	-	-
Clindamycin	43	14	13	13	3	-	-	-	-	2	7	5	-	-	-
Erythromycin	-	-	2	24	8	14	14	10	14	6	-	6	-	-	2
Teicoplanin	-	-	-	-	14	40	41	2	2	-	1	-	-	-	-
Vancomycin	-	-	-	-	-	-	2	60	36	2	-	-	-	-	-
Synercid	-	-	-	-	5	25	52	18	-	-	-	-	-	-	-

Two very distinct populations of isolates could be distinguished for clindamycin with no isolates showing intermediate susceptibility. Clindamycin was the most active of the antibiotics tested against these isolates with MIC_{50} value of 0.00375 mg/l, with a sensitive population in MIC range of $\leq$ 0.00375 – 0.06 mg/l. The majority of isolates were clustered within this narrow band at 0.00375 – 0.06 mg/l and 14% isolates showed a higher range of MICs of 2–8 mg/l. The distribution of isolates according to their susceptibility to teicoplanin was uni-modal with MICs range of 0.06–1 mg/l, showing a normal distribution curve, and only 1% strain with an MIC of 4 mg/l fell outside this range. Vancomycin showed narrow MIC range of 0.25–2 mg/l, in an approximate normal distribution curve. For teicoplanin, the values for MIC_{50}, mode and MIC_{90} were generally two fold lower than those for vancomycin. Synercid was four fold more active than vancomycin.

TABLE 2

MIC (mg/l) of the 7 antibiotics tested exhibiting mode, MIC_{50} and MIC_{90} against 60 isolates of S.oralis

Antibiotic	Mode	MIC_{50}	MIC_{90}	% of strains sensitive	% of strains outside normal range
Penicillin	0.03	0.125	2	72	28
Amoxycillin	0.03	0.125	8	84	16
Clindamycin	0.00375	0.0075	4	86	14
Erythromycin	0.03	0.25	2	92	8
Teicoplanin	0.125	0.125	0.25	99	1
Vancomycin	0.5	0.5	0.5	100	0
Syncercid	0.125	0.125	0.125	100	0

Table 3 shows the comparative in-vitro bactericidal activity of synercid, penicillin, vancomycin, clindamycin, erythromycin and teicoplanin against fifteen isolates of *S. oralis*, using a microdilution broth technique. Three strains showed moderate penicillin resistance (>0.25 mg/l), but all fifteen isolates were inhibited by $\leq$ 2 mg/L penicillin. Seven of these were penicillin tolerant (MIC/MBC ratio $\geq$1:8) and required >2 mg/l of penicillin for a 99.9% kill. The MIC's for teicoplanin (range 0.03–0.5 mg/l) against these isolates were lower than those of vancomycin (range 0.5–2 mg/l); the bactericidal activity of teicoplanin was also lower than that of vancomycin recorded over the 24 hour period. An MBC range of 16– $\geq$32 mg/l teicoplanin was needed for 14 strains; the MBC for one isolate was 4 mg/l. All isolates showed a high tolerance to teicoplanin with MIC/MBC ratio of >32. With vancomycin, MBC values for ten of the fifteen isolates ranged from 16 to 128 mg/l and from 1 to 4 mg/l for four isolates. The majority (10/14) of the isolates showed a high tolerance to vancomycin with an MIC/MBC ratio $\geq$8. Synercid achieved a >99.9% kill against ten of

TABLE 3

Comparative MIC/MBC values (mg/l) for synercid, penicillin, erythromycin, teicoplanin, vanomycin and clindamycin against S.oralis

Strains	Syncercid		Penicillin		Erythromycin		Vanomycin		Teicoplanin		Clindamycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
AR3	0.5	>64	0.12	0.12	0.06	>16	0.5	64	0.03	32	0.12	>4
AR12	1	1	0.12	16	0.06	4	2	>64	0.06	32	0.12	>4
AR13	1	2	0.12	0.12	0.12	>16	2	16	0.12	4	0.12	>4
AR19	1	4	0.25	2	0.5	>16	2	16	0.25	>32	0.25	4
AR40	0.5	16	1	>16	0.5	>16	2	>64	0.03	>32	0.12	>4
23	1	2	0.25	>16	0.25	>16	4	>64	0	>32	0.25	>4
N4-1-4	1	1	0.25	0.25	2	4	2	1	0.03	>32	0.25	2
A9	1	1	0.25	0.5	0.03	>16	2	>128	0.06	>16	0.06	>1
24	1	4	0.06	<0.06	0.25	>16	1	4	0.12	>16	0.12	>1
26	1	1	0.06	8	0.12	1	2	32	0.12	>16	0.06	0.25
A6	1	2	0.06	<0.06	0.12	0.12	2	-	0.5	>16	0.12	0.12
A10	0.5	0.5	2	>2	0.12	0.5	2	>128	0.25	>16	0.06	0.5
92C17	1	2	0.06	4	0.12	>16	2	4	0.5	>16	0.12	>1
93C87	1	2	0.5	0.5	0.12	0.5	2	2	0.25	>16	0.12	>1
T8-2-12	1	4	0.25	>8	0.12	0.5	2	>128	0.5	>16	0.12	>1

the fifteen isolates at a concentration of $\leq$ 2 mg/l. Of the five remaining isolates, three required 4 mg/l, one 16 mg/l and the remaining one 64 mg/l of synercid for a 99.9% kill. The MBC 99.9% values for synercid were within the 1–4 times MIC range for all but two of the isolates. The majority of the isolates showed a relatively low tolerance to synercid (87% MIC/MBC ratio of $\leq$ 4).

Although the isolates were inhibited by lower concentrations of erythromycin than of synercid, erythromycin showed much lower bactericidal activity than synercid. Clindamycin also demonstrated lower MIC values than those for synercid, but the MBC values for ten of the fifteen isolates were between 1–4 mg/l of clindamycin, for a 99.9% kill, five isolates required >4 mg/l. All isolates showed a relatively high tolerance to clindamycin with 12/15 MIC/MBC ratio of $\geq$ 8. Two strains were tolerant to synercid show tolerance to all the antibiotics as well.

KILLING CURVE

Killing curve was performed on ten isolates from endocarditis, neutropenic and normal oral flora strains. The pattern of kill was similar within these different sources of strains. Figures 1 and 2 show the comparative bactericidal activity at 4x MIC of synercid, vancomycin and penicillin against *S. oralis* (AR12 & AR13) from a patient with endocarditis. A three-log reduction in viable count was achieved within two hours of the organism coming into contact with synercid. Figure 3 shows the comparative bactericidal activity at 4x MIC of synercid, vancomycin and penicillin against an isolate of S. Oralis (23) from the

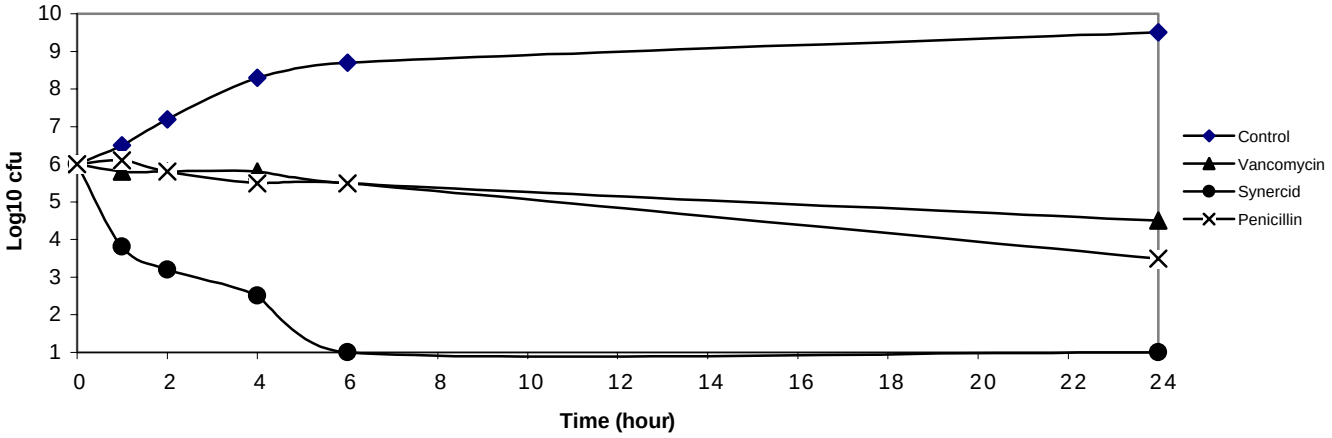

FIGURE 1. *Bacterial activity of synercid (RP 595,00), vancomycin and penicillin at 4 x MIC against S.Oralis from endocarditis patient.*

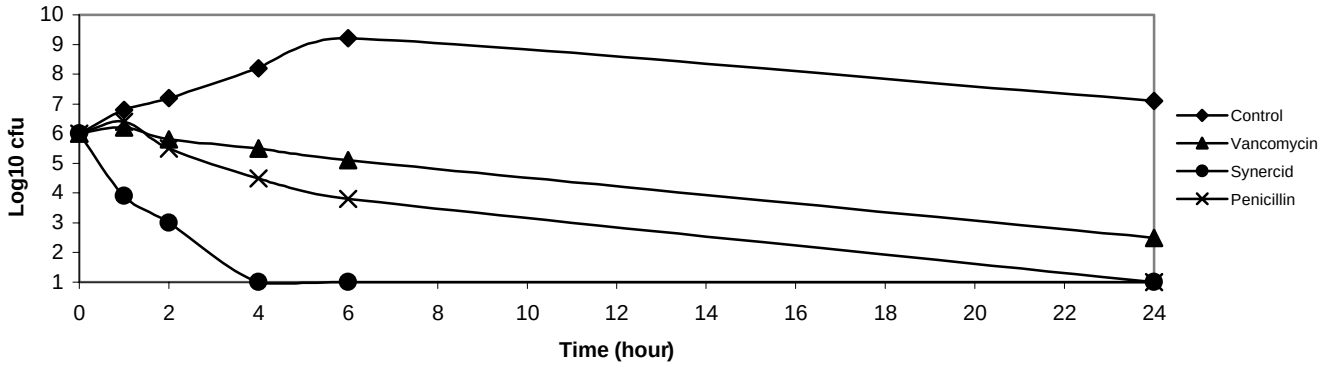

FIGURE 2. *Bacterial activity of synercid (RP 595,00), vancomycin and penicillin at 4xMIC against S.oralis from endocaridtis patient.*

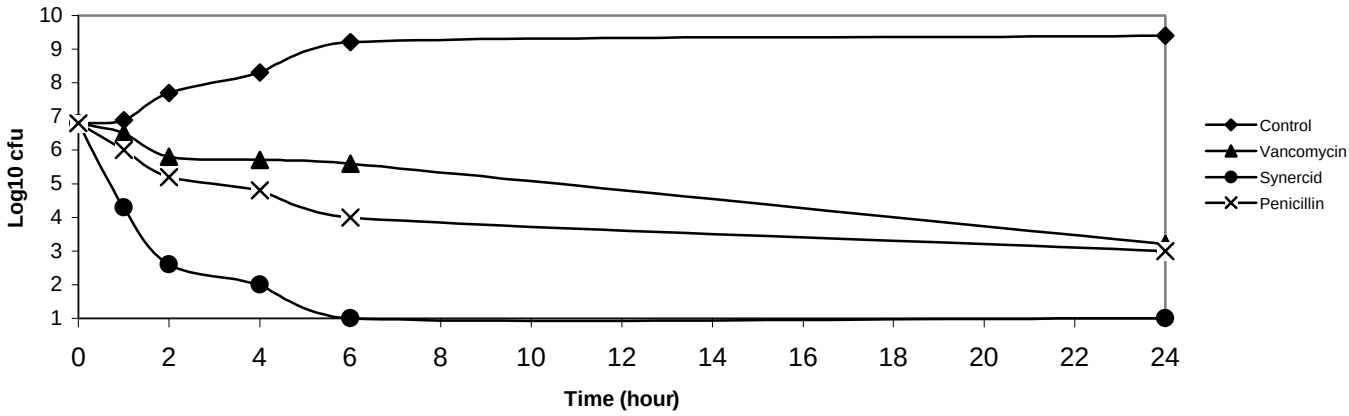

FIGURE 3. *Bacterial activity of synercid (RP 595,00), vancomycin and penicillin at 4xMIC against S.oralis from normal oral flora.*

normal oral flora. Synercid reduced the viable count of this isolate by three-$\log_{10}$ after 1.5 hour's exposure; a five-$\log_{10}$ reduction was achieved within six hours.

DISCUSSION

Susceptibility studies demonstrated that both penicillin and amoxycillin exerted similar activities against 60 isolates of *S. oralis*. These strains showed a wide range of susceptibilities but the MIC_{50} and the mode MIC values for both antibiotics were 0.125 mg/l and 0.03 mg/l respectively. Synercid, a streptogramin, demonstrated a relatively high MIC_{50} against *S. oralis* when compared with the macrolides and clindamycin. Similar findings were observed in a study reported by Williams.[13] The result of this study regarding synercid activity against *S. oralis* correlates well with the observations of Fass[14] who tested 30 viridans streptococci and found MIC_{50} of synercid to be 1 mg/l. MIC_{90} against these isolates was 2 mg/l and all isolates were inhibited by $\leq$ 4 mg/l synercid. These findings were also supported by separate studies.[14-16] There was a two-fold difference between the MIC_{50} of 0.25 mg/l and the MIC_{90} of 0.5 mg/l, compared to MIC_{90} values for clindamycin and erythromycin between 2–4 mg/l. These findings correlate well with other studies.[17-19] However, 14 % isolates required >2 mg/l clindamycin for inhibition and 28% isolates required $\geq$1 mg/l erythromycin for inhibition. Maskell[20,21] performed *in-vitro* susceptibility testing on 50 isolates of oral streptococci and obtained similar results for pristinamycin: all isolates were inhibited by < 1 mg/l pristinamycin. Susceptibility testing showed that teicoplanin demonstrated greater *in-vitro* activity than vancomycin, although it is not used as frequently as the latter.

MICs of Synercid for *S. oralis* were relatively high compared to all antibiotics tested; MBC values were $\leq$ 4 mg/l for most of the isolates, except for one at 16 mg/l and the other at > 64 mg/l. From these findings it was clear that Synercid has superior bactericidal activity against *S. oralis* compared to penicillin, erythromycin, vancomycin, teicoplanin and clindamycin. None of the *S. oralis* strains tested was tolerant to synercid. Similar findings have been demonstrated elsewhere.[16, 19]

Synercid was bactericidal (99.9% kill) against all ten isolates of *S. oralis* within six hours of contact. This correlates with the previous killing curve studies performed using Synercid against oral streptococci.[19-20] Interestingly, in this study, 99.9% kill was achieved with nine out of the ten isolates within four hours of contact. The isolates from infective endocarditis cases required two to six hours of contact with synercid for 99.9% kill, whereas for isolates from neutropenic cases and the normal oral flora, 2.5 and 1.5 hours respectively were sufficient. These results also showed that none of the ten isolates tested were tolerant to synercid. This finding correlates well with the other studies.[15,16,18] With penicillin, only four of the six infective endocarditis isolates showed a three-$\log_{10}$ reduction in viable colony count within 6 –24 hours. Vanomycin achieved a three-$\log_{10}$ reduction with only three out of the ten isolates.

CONCLUSION

There is concern about the increasing prevalence of methicillin resistant *S. aureus* (MRSA) *S. epidermidis*[21-23] and reduced vancomycin susceptibility.[24] There is great need for an agent with excellent activity against macrolide resistant strains of gram-positive organisms and the recently reported strains of *Enterococcus faecium* which are resistant to both vancomycin and gentamicin.[25] Thus synercid offers a potentially new agent for use in the treatment of infections caused by MRSA and other gram-positive bacteria.[15] Synercid might be useful against most viridans streptococci for the treatment of complicated cases of infective endocarditis.

REFERENCES

1. **Skehan, JD, Murray, M, Mills PG.** Infective endocarditis: incidence and mortality in the North Thames Region. *Brit Heart J* 1988, **59**, 62–68.
2. **Bayliss R, Clarke C, Oakley CM, Somerville W, Young JE.** The microbiology and pathogenesis of endocarditis. *Br Heart J* 1983, **50**, 513–519.
3. **Young JE, Susan**. Aetiology and epidemiology of infective endocarditis in England and Wales. *J Antimicrob Chemother* 1987, **20,** 7–14.
4. **Bouvet A, Durand A, Devine C, Etienne J, Leport C, and the Group D'enquete Sur L'endocardite en France En 1990–1991**. In vitro susceptibility to antibiotics of 200 strains of streptococci and enterococci isolated during infective endocarditis. *Lancer Publication St.Petersburg, Russia* 1994,72–3.
5. **Douglas CWI, Heath J, Hampton KK, Preston FE**. Identity of viridans streptococci isolated from cases of infective endocarditis. *J Med Microbiol* 1993, **39**, 179–82.
6. **Parker MT, Ball LC**. Streptococci and aerococci associated with systemic infections in man. *J Med Microbiol* 1976, **9**, 275–302.
7. **Barriere JC, Bouanchaud DH, Harris NV, Paris JM, Rolin O, Smith C**. The design synthesis and properties of RP59500 and related semi-synthetic streptogramin antibiotics. In: Program and abstract of the 30th Conference on Antimicrobial Agents and Chemotherapy. Atlanta. *American Society for Microbiology, Washington, DC,* 1990, 768A.
8. **Barriere JC, Bouanchaud DH, Paris JM, Rolin O, Harris NV, Smith C.** Antimicrobial activity against Staphylococcus aureus of semi synthetic injectable streptogramin: RP 59500 and related compounds. *J Antimicrob Chemother* 1992, **30**, 1–8.
9. **Aumercier M, Bouhallab S, Capmau M, Goffic FL.** RP 59500: a proposed mechanism for its bacterial activity. *J Antimicrob Chemother* 1992, **30**, 9–14.

10. **Baquero F** Gram-positive resistance: a challenge for the development of new antibiotics. 19th ICC Montreal, 1995.
11. **Beighton D, Hardie JM, Whiley RA.** A scheme for the identification of viridans streptococci. *J Med Microbiol* 1991, **35**, 367–72.
12. **Miles AA, Misra SSK, Irwin JO.** The estimation of the bactericidal power of the blood. *J Hygiene* 1938, **38**, 732–49.
13. **Williams JD, Maskell JP, Shain H, Chrysos G, Sefton AM, Fraser HY, Hardie JM**. Comparative in–vitro activity of azithromycin, macrolides (erythromycin, clarithromycin and spiramycin) and streptogramin RP 59500 against oral organisms. *J Antimicrob Chemother* 1992, **30**, 27–37.
14. **Fass RJ**. In vitro activity of RP59500, a semi-synthetic injectable prestinamycin, against staphylococci, streptococci and enterococci. *Antimicrob Agents Chemother* 1991, **35**, 553–9.
15. **Verbist L, Verhaegen J**. Comparative in-vitro activity of RP59500. *J Antimicrob Chemother* 1992, **30**, 39–44.
16. **Neu C Harold Chin, Nai-Xun, Gu, Jain-Wei**. The in-vitro activity of new streptogramin, RP 59500, RP 57667 and RP 54476, alone and in combination. *J Antimicrob Chemother* 1992, **30**, 83–94.
17. **Soussy CJ, Acar JF, Cluzel R, Courvalin P, Duval J, Fleurette J, Megraoud F, Meryaan M, Thabaut A**. A collaborative study of the in-vitro sensitivity to RP 59500 of bacteria isolated in seven hospitals in France. *J Antimicrob Chemother* 1992, **30**, 53–8.
18. **Pankuch GA, Jacobs MR, Appelbaum PC.** Study of comparative anti pneumococcal activities of penicillin G, RP59500, erythromycin, sparfloxacin, ciprofloxacin and vancomycin by using the time-kill methodology. *Antimicrob Agents Chemother* 1994, **38**, 2065–72.
19. **Pechere JC.** In-vitro activity of RP 59500, a semi synthetic streptogramin, against staphylococci and streptococci. *J Antimicrob Chemother* 1992, **30**, 15–18.
20. **Maskell JP, Willams JD**. In-vitro susceptibility of oral streptococci to pristinamycin. *J Antimicrob Chemother* 1987, **19**, 585–90.
21. **Hoban DJ, Weshnoweski B, Palatnick L.** In–vitro activity of RP59500, a new semi-synthetic streptogramin antibiotic against Staphylococcus species. In program an Abstract of the Thirteenth International conference on Antimicrobial agents and Chemotherapy, Atlanta, Georgia. *American Society for Microbiology, Washington, DC* 1990, **770**, 214.
22. **Boyce JM.** Methicillin resistant Staphylococcus aureus. Detection, epidemiology and control measures. *Infect Dis Clin North Am* 1989, **3**, 901–13.
23. **Nafziger DJ, Wenzel RP**. Coagulase-negative staphylococci epidimiology evaluation and therapy. *Infect Dis Clin North Am* 1989, **3**, 915–29.
24. **Hiramatsu K, Hanaki H, Ino T, Yabutta K, Oguri T, Tenover FC.** Methicillin-resistant Staphylococcus aureus clinical strains with reduced vancomycin susceptibility. *J Antimicrob Chemother* 1997, **40**, 135–6
25. **Cassewell MW, Seyed-Akhavani M, Wade J.** In vitro activity of RP 59500 against vancomycin resistant Enterococcus faecium also resistant to >512 mg/l of gentamicin. *33rd ICAAC, New Orleans* 1993, *Poster presentation.*