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In-vitro activity of RP 59500, a semisynthetic streptogramin, against staphylococci and streptococci

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The in-vitro activity of RP 59500, an injectable streptogramin derived from pristinamycin, was determined by agar dilution and compared with that of pristinamycin. Two hundred and sixty-one recent clinical isolates of Gram-positive cocci were tested against both antibiotics. The two compounds displayed similar activities. The MIC_{90}s of RP 59500 ranged from 0·5 to 2 mg/L in 114 strains of *Staphylococcus aureus* showing various phenotypes of antibiotic resistance (penicillin-susceptible; penicillin-resistant and methicillin-susceptible; methicillin-resistant; erythromycin-resistant, either inducible or constitutive; quinolone-resistant). Similar results were obtained with coagulase-negative staphylococci. RP 59500 was consistently active against streptococci, with MIC_{90}s of 0·25, 0·25 and 0·50 mg/L for *Streptococcus pyogenes* ($n = 20$), *Streptococcus agalactiae* ($n = 20$) and *Streptococcus pneumoniae* ($n = 20$), respectively. *Enterococcus faecalis* ($n = 20$) appeared to be notably less susceptible (MIC_{90}, 8 mg/L). In view of this consistent activity against all staphylococci and streptococci tested, including multiply resistant isolates, RP 59500 merits further investigation.

Introduction

There is an increasing incidence of severe infections caused by multi-resistant Gram-positive bacteria, especially staphylococci. Such infections are difficult to treat, even with newer antibiotics such as quinolones. Since pristinamycin (RP 7293) remains a very potent anti-staphylococcal agent, its efficacy was compared with that of RP 59500, a new injectable streptogramin antibiotic, against clinical isolates of Gram-positive bacteria obtained from hospitalized patients. Most of these isolates were associated with nosocomial infections, including strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae* and *Enterococcus faecalis*, and many were multi-resistant.

Materials and methods

Antimicrobial powders of known potency were provided by Rhône-Poulenc Rorer, Paris, and antibiotic solutions were prepared immediately before use. In total 244 clinical isolates were collected from patients admitted to three Swiss university hospitals. In order to avoid duplicate strains, only one sample per patient was allowed, and collection was maintained over a four-year period. A vancomycin-resistant strain of *Staphylococcus aureus* (MIC, 8 mg/L) was obtained by passage on a vancomycin-

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containing agar gradient as described previously (Michea-Hamzehpour *et al.*, 1987). Strains were kept frozen in skimmed milk at −70°C. Before study, samples were thawed, streaked on to sheep blood agar and incubated overnight at 37°C.

MICS were determined by agar dilution according to the recommendations of the National Committee for Clinical Laboratory Standards (1990). Mueller-Hinton agar (Oxoid, Basingstoke) was used throughout these experiments. A multipoint-inoculator (Cathra Systems, MTC Medical, Minnesota, USA) was used to inoculate plates with 10^4 cfu/spot, in a volume of approximately 1 μL. All plates were incubated at 37°C for 18–20 h. The MIC was defined as the lowest concentration of an agent to completely inhibit the growth of a given strain, disregarding a single colony or a faint haze caused by the inoculum.

Results

The MICs of RP 59500 and RP 7293 for various Gram-positive cocci are shown in the Table. The in-vitro activities of these two pristinamycin derivatives were similar; for each strain the MICs were within one dilution of each other. These results indicate that the injectable derivative, RP 59500, did not exert an antibacterial effect different from that of the parent product, RP 7293. Anti-staphylococcal activity did not appear to depend on either the species (*S. aureus* or *S. epidermidis*) or the resistance phenotype of the strains. These pristinamycin derivatives were more active against streptococci (*S. pyogenes, S. agalactiae* and *S. pneumoniae*), than against staphylococci, and no resistant isolates were found among these species. However, *E. faecalis* appeared to be markedly less susceptible than the other Gram-positive cocci studied.

Discussion

The most interesting finding of the present study was the fact that the in-vitro activities of the pristinamycin derivatives studied remained almost unchanged, whatever the resistance phenotype for other antibiotics. *S. aureus* exhibits a remarkable propensity for resistance to various antibiotics, and this is accounted for by numerous resistance mechanisms encoded by the chromosome, plasmids or transposons. Thus, in most parts of the world, most staphylococci are resistant to penicillin and, depending on local epidemiological conditions, a significant number of isolates are resistant to methicillin, erythromycin, aminoglycosides and/or quinolones. More worrying is the fact that some staphylococci are multi-resistant, thereby causing major therapeutic problems. A recent example is the emergence of strains resistant to both methicillin and quinolones (Schaefler, 1989).

In this context, the ability of pristinamycin derivatives to inhibit *S. aureus* strains possessing various resistance phenotypes is potentially of clinical importance. The persistent activity of RP 59500 and RP 7293 against strains expressing the MLS_B phenotype, and therefore resistant to macrolides, lincosamides and streptogramin B (Ounissi & Courvalin, 1982; Duval, 1985), is particularly remarkable. MLS_B resistance results from a dimethylation of adenine in 23S ribosomal RNA that reduces the affinity between the antibiotic and the ribosome. This alteration can be inducible, in which case the resistance is apparently dissociated; in particular, the strain will be resistant to erythromycin (inducer), but susceptible to spiramycin (non-inducer). In other cases, MLS_B resistance is constitutive, affecting all antibiotics of the group, including spiramycin. Pristinamycin derivatives contain both streptogramins A and B, which act

Table. Antimicrobial activity of RP 59500 and RP 7293 against a variety of Gram-positive organisms

Bacterial species (No. of isolates)	Antibiotic phenotype					Antibiotic activity (mg/L) RP 59500		RP 7293	
	Pen G[a]	MET	ER	SPI	PEF	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
S. aureus (20)	S[b]	S	S	S	S	⩽0·12	0·5	⩽0·12	0·5
S. aureus (20)	Ⓡ	S	S	S	S	0·5	0·5	0·25	0·25
S. aureus (20)	R	Ⓡ	S or R	S	S	0·5	0·2	1·0	2·0
S. aureus (20)	S or R	S or R	Ⓡ	S	S	0·5	1·0	0·5	1·0
S. aureus (18)	S or R	S or R	Ⓡ	Ⓡ	S	0·5	1·0	0·5	1·0
S. aureus (16)	R	S or R	S or R	S or R	Ⓡ	0·25	0·5	0·25	1·0
S. epidermidis (20)	S or R	S	S or R	S	S	0·25	0 25	0·12	0·5
S. epidermidis (20)	R	Ⓡ	S or R	S or R	S	0·25	1·0	0·25	1·0
S. epidermidis (10)	S or R	S or R	S or R	S or R	Ⓡ	0·5	1·0	0·25	0·5
S. pyogenes (20)[c]	S	S	S or R	S	S	0·12	0·12	⩽0·06	⩽0·06
S. agalactiae (20)	S	S	S	S	S	0·12	0·12	⩽0·06	⩽0·06
E. faecalis (20)	R	R	R	R	R	4·0	8·0	2·0	4·0
S. pneumoniae (20)[d]	S or R	S	S	S	S	0·5	1·0	0·25	0·5

[a]Pen G, penicillin G; MET, Methicillin, ER, Erythromycin; SPI, Spiramycin; PEF., pefloxacin.
[b]S, Susceptible; R, resistant.
[c]Including five erythromycin-resistant strains (MLS_B inducible phenotype).
[d]Including three penicillin-resistant strains.

synergically. It is interesting that resistance to streptogramin B, i.e. the MLS_B phenotype, did not affect the activity of pristinamycins. This observation confirms the findings of a previous study (Chabbert & Courvalin, 1971), in which synergy between streptogramins A and B was shown to persist against staphylococci or streptococci possessing the MLS_B phenotype. However, our collection did not include bacteria expressing the $MLS_B + S_A$ or the LS_A phenotype, which are known to be pristinamycin-resistant (Buu-Hoi, 1985; Leclercq *et al.*, 1985).

In addition to the activity of RP 59500 against both staphylococci and streptococci having the MLS_B phenotype, the lack of cross-resistance to this antibiotic in strains resistant to β-lactams (including three penicillin-resistant pneumococci), quinolones or aminoglycosides (27 strains tested, included in the Table, but not identified separately), or vancomycin (a laboratory-generated mutant) deserves full consideration in the perspective of future clinical studies.

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