

Activity of glycylycylines CL 329998 and CL 331002 against minocycline-resistant and other strains of methicillin-resistant *Staphylococcus aureus*

J. M. T. Hamilton-Miller* and Saroj Shah

Department of Medical Microbiology, Royal Free Hospital School of Medicine,
London NW3 2QG, UK

Two glycylycylines have been tested against 191 strains of methicillin-resistant *Staphylococcus aureus*, 72 of which were resistant to minocycline, isolated from many parts of the world. MICs of CL 329998 ranged from 0.06 to 4 mg/L, with MIC₅₀ and MIC₉₀ 0.5 and 2 mg/L, respectively. CL 331002 was slightly less potent, having an MIC range 0.12–16 mg/L and MIC₅₀ and MIC₉₀ values of 1 and 4 mg/L, respectively.

Introduction

The tetracyclines have been available for more than 40 years, during which time bacterial resistance has eroded their clinical usefulness (Speer, Shoemaker & Salyers, 1992). Chemical manipulation at the 5, 6 and 7 positions resulted in two important derivatives, doxycycline and minocycline, with markedly improved pharmacokinetic and microbiological properties compared with the natural products. However, for the past 20 years there have, until now, been no significant advances in this field.

Recently, modifications of the D ring of tetracyclines in the 9 position have given rise to a series of compounds referred to as the glycylycylines (Testa *et al.*, 1993). Two of these, the N-N-dimethylglycylamido derivatives of minocycline (CL 329998) and of 6-demethyl-6-deoxytetracycline (CL 331002), have excited considerable interest, as they are active *in vitro* against tetracycline-resistant strains harbouring efflux (*tet A–tet D*, and *tet K*) and ribosomal protection (*tetM*) genes (Testa *et al.*, 1993; Eliopoulos *et al.*, 1994; Goldstein, Kitzis & Acar, 1994). If they live up to the expectations raised by these *in-vitro* results, the glycylycylines could rejuvenate the tetracyclines as a therapeutic class.

A pathogen that is causing considerable problems in terms of treatment options is methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is found world wide, and some isolates may be resistant to antibiotics from as many as 11 different chemical classes (Maple, Hamilton-Miller & Brumfitt, 1989). While there is a perception that MRSA strains are almost always sensitive to minocycline (Freeman, Nightingale & Quintiliani, 1994), this is based on results obtained with isolates from single locations, and in many reports on susceptibility patterns of MRSA neither tetracycline nor minocycline was tested. On the contrary, we (Maple *et al.*, 1989) found a high incidence

*Tel: +44-(171)-794-0500; Fax: +44-(171)-435-9694.

of resistance to minocycline (72% of strains having MIC \geq 16 mg/L) among 106 strains of MRSA collected from 21 countries. Although several groups have tested glycylicyclines against MRSA (Testa *et al.*, 1993; Eliopolous *et al.*, 1994; Goldstein *et al.*, 1994; Weiss, Jacobus, Petersen & Testa, 1995), many of the strains were sensitive to tetracycline and almost all were sensitive to minocycline, so the observation that they were sensitive to the glycylicyclines was entirely predictable. The question thus remains as to whether glycylicyclines are active against MRSA that are minocycline-resistant. To address this, we have tested two glycylicyclines against a large number of MRSA with varying patterns of susceptibility to tetracycline and minocycline, and have analysed our results separately for strains of differing resistance phenotypes.

Materials and methods

Microbiological methods

One hundred and ninety one strains of MRSA, from 26 countries, were tested. The plate dilution method, with IsoSensitest agar (Unipath, Basingstoke, UK) and an inoculum of 10^6 cfu per spot, was used to determine MICs of tetracycline, minocycline and two glycylicyclines, CL 329998 and CL 331002 (Lederle Laboratories, Hants, UK).

Analysis of data

For the purposes of analysis the strains were divided into four phenotypic groups, according to their susceptibility to tetracycline and minocycline: group A, tetracycline-sensitive, minocycline-sensitive (34 strains); group B, tetracycline-resistant, minocycline-sensitive (42 strains); group C, tetracycline-resistant, minocycline-intermediate (43 strains); and group D, tetracycline-resistant, minocycline-resistant (72 strains).

MIC findings were summarized in terms of the MIC₅₀, MIC₉₀, I₅₀, I₉₀ (calculated by cumulation and interpolation), and geometric mean MICs.

Breakpoints

There do not appear to be any definitive breakpoints for minocycline when (as recommended by the Working Party of the BSAC, 1991) IsoSensitest agar is used. We therefore took as working values those recommended by The National Committee for Clinical Laboratory Standards (NCCLS, 1993) and the Comité de l'Antibiogramme de la Société Française de Microbiologie (Soussy *et al.*, 1994). Thus, sensitive, intermediate and resistant strains were defined as having MICs \leq 4, 8 and \geq 16 mg/L, respectively. The same breakpoints were taken for tetracycline, as recommended when using IsoSensitest agar (Oxoid, 1980).

Results and discussion

The results (Table) make it clear that both glycylicyclines are active against minocycline-resistant MRSA. Combining findings for all 191 MRSA strains, CL 329998 was slightly more active than CL 331002 (geometric mean MIC 0.75 mg/L compared with 1.14 mg/L, respectively).

Table. Activities of tetracycline, minocycline and two glycytyclines against 191 strains of MRSA

Resistance phenotype*	number of strains	antibiotic	MIC parameters (mg/L)				
			I ₅₀	I ₉₀	50%	90%	geometric mean
Tetracycline S, minocycline S	34	tetracycline	1.2	2.9	2	4	1.7
		minocycline	0.32	0.56	0.5	1	0.42
		CL 329998	0.3	0.46	0.5	0.5	0.39
		CL 331002	0.47	0.9	0.5	1	0.64
Tetracycline R, minocycline S	42	tetracycline	34	150	64	256	61.7
		minocycline	2.3	3.6	4	4	2.2
		CL 329998	0.23	1	0.25	1	0.45
		CL 331002	0.42	1.7	0.5	2	0.59
Tetracycline R, minocycline I	43	tetracycline	52	200	64	256	85.7
		minocycline	5.6	7.7	8	8	8
		CL 329998	0.37	1.5	0.5	2	0.61
		CL 331002	0.58	2.3	1	4	0.95
Tetracycline R, minocycline R	72	tetracycline	170	270	256	512	209
		minocycline	12	17	16	32	17.4
		CL 329998	1.3	2.2	2	4	1.67
		CL 331002	1.7	3.4	2	4	2.2

*S, Sensitive; I, intermediate; R, resistant.

Strains in group B, being sensitive to minocycline but resistant to tetracycline, will probably be of genotype *tet* K, while those in group D (resistant to both tetracycline and minocycline) are likely to be of genotype *tet* M (Speer *et al.*, 1992). Group C strains, although of "intermediate" susceptibility to minocycline, were only slightly more resistant to tetracycline and the glycylicyclines compared with strains in group B. However, group D strains were approximately 3-fold less susceptible to all four compounds than those in group C. Unlike the situation with coagulase-negative staphylococci, as described by Archer & Climo (1994), both glycylicyclines were more active than minocycline against MRSA of assumed genotype *tet* K.

There was a clear geographical difference in the incidence of minocycline resistance among the MRSA tested. For example, while 62% (18/29) of strains from the Americas were sensitive (MIC \leq 4 mg/L), only 27% (31/115) of European and Asiatic strains were susceptible ($P < 0.001$). It is important to bear in mind the possibility of such differences when assessing the potential value of new antibiotics against MRSA.

On the basis of our findings, CL 329998, the glycylicycline derived from minocycline, can be expected to be active *in vitro* against all MRSA; the only other freely available antibiotic class for which such a claim can be made is the glycopeptides.

Acknowledgement

We thank Lederle Laboratories UK for supplying antibiotics and for financial assistance.

References

- Archer, G. L. & Climo, M. W. (1994). Antimicrobial susceptibility of coagulase-negative staphylococci. *Antimicrobial Agents and Chemotherapy* **38**, 2231–7.
- Eliopolous, G. M., Wennersten, C. B., Cole, G. & Moellering, R. C. (1994). *In vitro* activities of two glycylicyclines against gram-positive bacteria. *Antimicrobial Agents and Chemotherapy* **38**, 534–41.
- Freeman, C. D., Nightingale, C. H. & Quintiliani, R. (1994). Minocycline: old and new therapeutic uses. *International Journal of Antimicrobial Agents* **4**, 325–35.
- Goldstein, F. W., Kitzis, M. D. & Acar, J. F. (1994). N,N-dimethylglycyl-amido derivative of minocycline and 6-demethyl-6-desoxytetracycline, two new glycylicyclines highly effective against tetracycline-resistant gram-positive cocci. *Antimicrobial Agents and Chemotherapy* **38**, 2218–20.
- Maple, P. A. C., Hamilton-Miller, J. M. T. & Brumfitt, W. (1989). World-wide antibiotic resistance in methicillin-resistant *Staphylococcus aureus*. *Lancet* *i*, 537–40.
- National Committee for Clinical Laboratory Standards (1993). *Performance Standards for Antimicrobial Disk Susceptibility Tests—Fifth Edition; Approved Standard. Document M2-A5*. NCCLS. Villanova, PA.
- Oxid. (1980). *Error Minimization in Susceptibility Testing by Means of the Agar Diffusion Test*. Oxid Ltd. Basingstoke, UK.
- Soussy, C. J., Cluzel, R., Courvalin, P. and the Comité de l'Antibiogramme de la Société Française de Microbiologie. (1994). Definition and determination of *in vitro* antibiotic susceptibility breakpoints for bacteria in France. *European Journal of Clinical Microbiology and Infectious Diseases* **13**, 238–46.
- Speer, B. S., Shoemaker, N. B. & Salyers, A. A. (1992). Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. *Clinical Microbiology Reviews* **5**, 387–99.
- Testa, R. T., Petersen, P. J., Jacobus, N. V., Sum, F.-E., Lee, V. J. & Tally, F. P. (1993). *In vitro* and *in vivo* antibacterial activities of the glycylicyclines, a new class of semisynthetic tetracyclines. *Antimicrobial Agents and Chemotherapy* **37**, 2270–7.

- Weiss, W. J., Jacobus, N. V., Petersen, P. J. & Testa, R. T. (1995). Susceptibility of enterococci, methicillin-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae* to the glycylicyclines. *Journal of Antimicrobial Chemotherapy* **36**, 225–30.
- Working Party of the British Society for Antimicrobial Chemotherapy. (1991). A guide to sensitivity testing. *Journal of Antimicrobial Chemotherapy* **27**, Suppl. D.

(Received 10 January 1996; accepted 19 February 1996)