Glycylcyclines: a new generation of tetracyclines

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The widespread dissemination of genes encoding tetracycline resistance has limited the utility of the tetracyclines for many clinical indications (Chopra, Hawkey & Hilton, 1992). There are three types of tetracycline resistance mechanisms described (Speer, Shoemaker & Salyers, 1992): 1) efflux, mediated by trans-membrane-spanning proteins, which results in reduction of the intracellular tetracycline concentration (Levy, 1989; Chopra, Hawkey & Hilton, 1992); 2) ribosomal protection, in which the protein synthesis machinery is rendered resistant to inhibition by a cytoplasmic protein (Salyers, Spear & Shoemaker, 1990; Burdett, 1991) and 3) chemical modification, requiring oxygen and NADPH (Speer & Salyers, 1989). Efflux, tet(A)-(E), (G) and (H), (K) and (L), and ribosomal protection, tet(M), (O) and (S), mediated by plasmid or chromosomal determinants, are the two major mechanisms of clinical significance.

Previous research with older antimicrobial agents rendered less efficacious because of drug resistance, e.g. ampicillin and nalidixic acid, has yielded important new compounds with improved activity and stability to various resistance mechanisms, i.e. amoxycillin/clavulanic acid combination and the fluoroquinolones, respectively. Based on these previous achievements, a programme was initiated at Lederle Laboratories in 1988 to develop a new tetracycline which would be active against organisms harbouring clinically important, tetracycline-resistance mechanisms.

The research programme involved a multidisciplinary approach of chemistry, molecular biology, biochemistry and microbiology. A rational database was constructed which incorporated results from a series of biochemical assays coupled with activity against a selected group of bacteria to determine structure/ function properties of the test compounds. Isogenic strains of Escherichia coli and Staphylococcus aureus, in which important tetracycline-resistant determinants were expressed, were used in an initial screening of test compounds. New tetracycline analogues were studied for their activity against tetracycline-sensitive and -resistant Gram-positive and Gram-negative bacteria; uptake across the outer membrane; binding to bacterial ribosomes and inhibition of protein synthesis. Minocycline was chosen as an appropriate starting point for chemical modifications since it has increased antimicrobial activity compared with other existing tetracyclines (Rogalski, 1985). Studies with new analogues reinforced earlier observations with the tetracycline structure that changes to the hydrophobic domain could lead to a compound with enhanced activity whereas changes in the hydrophilic domain resulted in loss of activity. Compounds where the D ring was modified were found to have new and interesting antibacterial activity (Figure). Modifications at positions 7, 8 and 9 were prepared and studied (Sum et al., 1994a; Sum, Lee & Tally, 1994a). The first major breakthrough came with the synthesis of the 9-amino series which resulted in activity against S. aureus strains carrying the tet(M) determinant, however, these compounds were unstable under various testing conditions. Subsequently, 9-formamido-minocycline was prepared which also exhibited potent activity against Gram-positive and tet(M) containing strains but poorer activity against some Gram-negative bacteria. Further structure-activity studies led to the preparation of the N,N-dimethylglycylamido- (DMG) derivatives 9-aminominoacylne (DMG-MINO), and 9-amino-6-demethyl-6-deoxytetacycline (DMG-DMDOT) (Figure). These compounds are referred to as the glycylcyclines. Both glycylamido derivatives, DMG-MINO and DMG-DMDOT, showed excellent activity against strains resistant to tetracycline due to a ribosomal protection mechanism, tet(M), and to the production of efflux pumps, tet(A), tet(B), tet(C), tet(D) and tet(K) (Testa et al., 1993). Thus modification at position 9 overcame two distinct resistant mechanisms.

The glycylcyclines exhibited potent activity against a wide diversity of Gram-positive and
Figure. Structure of tetracycline nucleus (showing hydrophobic and hydrophilic domains) and N,N-dimethylglycylamido- (DMG) derivatives of 9-aminominocycline (DMG-MINO) and 9-amino-6-demethyl-6-deoxytetracycline (DMG-DMDOT).

Gram-negative aerobic and anaerobic recent clinical isolates (Nord, Lindmark & Persson, 1993; Testa et al., 1993; Eliopoulos et al., 1994; Wexler, Molitoris & Finegold, 1994; Wise & Andrews, 1994). The DMG analogues had similar in-vitro activities, and their activity against tetracycline-resistant bacteria were generally comparable to that obtained with tetracycline-susceptible bacteria. Of significance is the good activity of the glycylcyclines against methicillin-resistant S. aureus, vancomycin-resistant enterococci (MIC<sub>90</sub> ≤ 0·5 mg/L) and penicillin-resistant Streptococcus pneumoniae (MIC<sub>90</sub> ≤ 0·12 mg/L) (Testa et al., 1993; Weiss et al., 1993a; Eliopoulos et al., 1994; Goldstein, Kitzis & Acar, 1994). The glycylcyclines exhibited potent and improved activities over minocycline against a wide spectrum of Gram-negative bacteria, including E. coli, Citrobacter freundii, Shigella spp., Salmonella spp., Morganella morgani, Proteus mirabilis and Proteus vulgaris and comparable activity to minocycline against Klebsiella pneumoniae, Klebsiella oxytoca, Serratia spp. and Enterobacter cloacae (Testa et al., 1993; Wise & Andrews, 1994). Both DMG analogues showed significantly improved activity (MIC<sub>90</sub> ≤ 0·5 mg/L) against tetracycline-susceptible and tetracycline-resistant Neisseria gonorrhoeae (Whittington et al., 1993). Similar activities were obtained with the glycylcyclines, minocycline and tetracycline against β-lactamase producing and non-producing Moraxella catarrhalis and Haemophilus influenzae. The DMG analogues showed improved activities over minocycline and tetracycline against most anaerobic clinical isolates tested, including Bacteroides fragilis group, Prevotella spp., Clostridium spp., and anaerobic Gram-positive cocci (Nord et al., 1993; Testa et al., 1993; Wexler et al., 1994; Wise & Andrews, 1994). Poor activity was noted against two strains of Mycobacterium tuberculosis, and two strains of Mycobacterium avium were not inhibited by the DMG analogues (Wise & Andrews, 1994). The glycylcyclines, like tetracycline, have good activity against bacteria lacking a cell wall such as Mycoplasma and Ureaplasma spp. and against the intracellular pathogen, Chlamydia spp. (Kenny & Cartwright, 1994; Wise & Andrews, 1994). Thus the antimicrobial activity of the glycylcyclines is restored to that of tetracycline when the latter was first introduced in 1948. The glycylcyclines inhibited ribosomal protein synthesis in cell-free preparations from either tetracycline-sensitive, wild type ribosomes or tetracycline-resistant, tet(M) protected ribosomes (Rasmussen, Gluzman & Tally, 1994).

The tet(B) pump was shown to be an efficient pump for both tetracycline and minocycline but not the glycylcyclines (Guay, Tuckman & Rothstein, 1994). The frequency of glycylcycline-resistant mutants was low in a strain containing the tet(A)B gene, tet(KS) gene, and also the mutD allele, which greatly enhances the frequency of transitions and transversions caused by the loss of the DNA polymerase proofreading function. Resistance to the glycylcyclines was associated with a single codon and each lesion was a transversion. No spontaneous glycylcycline-resistant mutants (< 1/10<sup>6</sup>) were observed. Thus it was suggested that resistance to glycylcycline is a very rare genetic event.

Good efficacy was observed with both DMG-DMDOT and minocycline against acute lethal infections with methicillin-resistant and methicillin-susceptible S. aureus strains, but DMG-DMDOT was much more efficacious than minocycline (ED<sub>50</sub> 2·9 vs 19 mg/kg, respectively) in the treatment of an infection with minocycline-resistant, methicillin-resistant...
S. aureus strain (Testa et al., 1993). Against acute lethal infections with bacteria harbouring characterized tetracycline-resistance determinants, the glycylcyclines were much more efficacious than minocycline against E. coli carrying tet(B) (efflux) (ED₉₀ 2-4 mg/kg vs > 32 mg/kg, respectively) and S. aureus carrying tet(M) (ribosomal protection) (ED₉₀ of 0.53 vs 2.2 mg/kg, respectively). DMG-DMDOT had comparable efficacy (1-4 mg/kg) to minocycline (1-1 mg/kg) against an S. aureus strain carrying tet(K) (efflux). The glycylcyclines were more effective than ampicillin or vancomycin in reducing Enterococcus faecalis counts in a murine kidney infection model than minocycline and ampicillin in reducing counts of penicillin-susceptible or penicillin-resistant S. pneumoniae in an experimental murine lung infection model (Petersen et al., 1993). In a rat granuloma pouch model in which S. aureus was the infecting organism, both DMG-MINO and DMG-DMDOT were detected in pouch fluid following intravenous dosing (Weiss, Jacobus & Testa, 1993b). A > 2 log₁₀ reduction in the initial inoculum was observed over 24 h in the infected pouch with both glycylcyclines. Against an acute lethal infection with S. aureus Smith, the glycylcyclines showed poorer efficacy when administered as a single oral dose than by the intravenous route indicating poor oral efficacy (Testa et al., 1993).

DMG-DMDOT serum levels in mice dosed intravenously with 10 mg/kg were higher and more sustained than those of minocycline over the 6 h period. Tissue (heart, lung, kidney, liver and muscle) drug concentrations in mice were also higher for DMG-DMDOT than minocycline (unpublished data). Unchanged DMG-DMDOT accounted for 75–90% of total radioactivity in the plasma of rats, dogs and mini-pigs. About 5–15% of the radioactivity was accounted for by the epimer of DMG-DMDOT. In rats and dogs, 40–45% of the intravenously administered dose was excreted in the urine, with 75–80% of the radioactivity as unchanged parent. Bile contained 23–25% and faeces 50% of the dose in rats. Unchanged parent drug is the predominant circulating or excreted material in all species tested (Chaudhary et al., 1993). A possible explanation for the differences noted with in-vivo studies may be due to the hydrophilic properties of the glycylcyclines compared with the hydrophobic properties of minocycline.

Pre-clinical toxicological studies have shown qualitatively similar profiles to those noted with minocycline and tetracycline. Phase I clinical studies to establish safety in man have begun. The development of the glycylcyclines has been accomplished by integrating the chemistry, biochemistry, molecular biology and microbiology leading to a better understanding of structure/function relationships of the tetracycline molecule. This team approach should be applied to other classes of antimicrobial agents for the development of new compounds active against the rapidly emerging resistant organisms. The glycylcyclines, if effective clinically, will be important drugs for the treatment of infections caused by resistant Gram-positive bacteria.

F. T. TALLY
G. A. ELLESTAD
R. T. TESTA
Infectious Disease and Molecular Biology Research, Medical Research Division, American Cyanamid Company, Pearl River, New York 10963, USA

References


