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# In Vitro Activities of Two Glycylcyclines

R. WISE* AND J. M. ANDREWS

*Department of Microbiology, Dudley Road Hospital, Birmingham, United Kingdom*

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**The in vitro activities of two glycylcyclines, CL 329,998 and CL 331,002 (two new semisynthetic tetracyclines), were evaluated in comparison with those of tetracycline and other available oral antimicrobial agents. A total of 523 recent clinical isolates were studied, including strains resistant to tetracycline. Members of the family *Enterobacteriaceae* were generally ≥16-fold more susceptible to the glycylcyclines than to tetracycline (although less difference was seen with *Proteus* spp.). *Pseudomonas aeruginosa* was modestly susceptible to both new compounds (MIC for 90% of strains tested [$MIC_{90}$], 16 μg/ml). Tetracycline- and methicillin-susceptible and -resistant strains of *Staphylococcus aureus* were all susceptible to the glycylcyclines ($MIC_{90}$ ≤ 1 μg/ml). Streptococci (including *Streptococcus pneumoniae*) and *Enterococcus faecalis* and *Enterococcus faecium* displayed a bimodal distribution of susceptibility to tetracycline yet were uniformly susceptible to the glycylcyclines ($MIC_{90}$ ≤ 0.25 μg/ml). The glycylcyclines were highly potent against *Neisseria*, *Moraxella*, *Haemophilus*, and *Bacteroides* spp. ($MIC_{90}$ ≤ 0.5 μg/ml). Strains of *Chlamydia* spp. (three *C. trachomatis* strains and one *C. pneumoniae* strain) were inhibited by ≤0.25 μg of CL 329,998 or CL 331,002 per ml. Two strains of *Mycoplasma pneumoniae* were inhibited by ≤0.12 μg of CL 331,002 per ml and by 1 μg of CL 329,998 per ml. *Mycobacterium tuberculosis* and *Mycobacterium avium* were resistant to the two glycylcyclines (MIC ≥ 8 μg/ml). These results indicate that the two glycylcyclines have potent in vitro activities against a wide range of clinically important pathogenic bacteria.**

Tetracycline antimicrobial agents have been in use for more than 40 years. They have a broad spectrum of activity against common aerobic and anaerobic pathogens, including intracellular organisms often resistant to other agents. Over the years, semisynthetic modifications have produced analogs with relatively minor modifications to the antimicrobial potency yet, as in the case of doxycycline, considerable changes in pharmacokinetic properties (7). Considerable use worldwide has been associated with increasing resistance (12).

Two new agents containing the *N,N*-dimethylglycylamido substituent at the 9 position of minocycline (known as DMG-MINO or CL 329,998) and 6-methyl-6-deoxytetracycline (known as DMG-DMDOT or CL 331,002) have been developed by American Cyanamid Laboratories, Pearl River, N.J. (9). In this study, we have evaluated the activities of these two compounds in comparison with those of tetracycline and other commonly available oral antimicrobial agents against a wide range of clinical pathogens, including those commonly resistant to tetracycline.

## MATERIALS AND METHODS

A total of 523 recent clinical isolates were studied. The following antimicrobial agents were evaluated (obtained from the indicated sources): CL 329,998 and CL 331,002 (American Cyanamid Laboratories); tetracycline, co-amoxyclav (amoxicillin-clavulanate R:1), and methicillin (SmithKline Beecham, Brentford, United Kingdom); erythromycin and vancomycin (Lilly Industries, Basingstoke, United Kingdom); cefotaxime (Roussel Laboratories, Denham, United Kingdom); and ciprofloxacin (Bayer Laboratories, Basingstoke, United Kingdom).

**Susceptibility testing.** The susceptibilities of the strains were studied by using a standard agar plate dilution method. The inocula were prepared as follows. For all strains except streptococci (including *Streptococcus pneumoniae*), enterococci, *Neisseria* spp., *Haemophilus influenzae*, and anaerobes, the organisms were grown overnight in nutrient broth (Unipath, Basingstoke, United Kingdom) to yield a viable count of about $10^9$ CFU/ml. Suspensions of streptococci, enterococci, *H. influenzae*, *Neisseria* spp., and anaerobes were prepared in nutrient broth and were further diluted in water to adjust the density equivalent to that of a 0.5 McFarland standard (approximately $10^8$ organisms per ml).

One microliter of a 1:100 dilution of an overnight culture or a 1:10 dilution of the suspension was transferred to the surface of the antibiotic-containing agar with a multipoint inoculator (Denley-Tech, Billingshurst, United Kingdom). The final size of the inoculum on the plates was therefore $10^4$ CFU. The medium used for the agar dilution procedure was Iso-Sensitest agar (pH 7.2) (CM471; Unipath) supplemented with 5% lysed blood plus 20 μg of NAD per ml to support growth of streptococci, *H. influenzae*, and *Neisseria* spp.; for anaerobes, Wilkins-Chalgren agar plus 5% horse blood was used.

All plates were incubated in air at 35 to 37°C, except that the anaerobes were grown in an anaerobic cabinet (Don Whitley, Skipton, United Kingdom) in an atmosphere of 10% hydrogen, 10% carbon dioxide, and 80% nitrogen. *H. influenzae* and *Neisseria* spp. were incubated in air enriched with 4 to 6% carbon dioxide. The MIC of the antibiotic was defined as the lowest concentration at which no more than two or three colonies were detected after overnight incubation.

The effects of 0, 20, and 70% human serum (Bradsure Biological, Market Harborough, United Kingdom) on the MIC and MBC of CL 329,998 and CL 331,002 were studied with two strains each of *Staphylococcus aureus*, *Klebsiella* spp., *Escherichia coli*, and *Pseudomonas aeruginosa* by a method based on that of Pearson et al. (5). Increasing concentrations of CL 329,998 and CL 331,002 were prepared in Iso-Sensitest broth (CM473; Unipath) and broth containing human serum. Overnight broth cultures were diluted to give a final inoculum of $10^5$ CFU/ml. After overnight incubation at 35 to 37°C, the MIC

* Corresponding author. Mailing address: Microbiology Department, Dudley Road Hospital, Birmingham, United Kingdom. Phone: 021-507-4255. Fax: 021-551-7763.

TABLE 1. Activities of CL 329,998 and CL 331,002 in comparison with those of other antimicrobial agents

| Organism (no. of isolates) | Antibiotic | MIC (μg/ml)[a] | | |
|---|---|---|---|---|
| | | 50% | 90% | Range |
| *Escherichia coli* (15) | CL 329,998 | 0.5 | 1 | 0.25–2 |
| | CL 331,002 | 0.5 | 0.5 | 0.25–2 |
| | Tetracycline | 2 | 32 | 1–128 |
| | Co-amoxyclav | 2 | 8 | 0.5–16 |
| | Cefotaxime | 0.03 | 0.03 | 0.008–0.06 |
| | Ciprofloxacin | 0.015 | 0.03 | 0.004–0.03 |
| *Klebsiella* spp. (15) | CL 329,998 | 1 | 4 | 0.5–32 |
| | CL 331,002 | 0.5 | 4 | 0.25–32 |
| | Tetracycline | 2 | 128 | 1–>128 |
| | Co-amoxyclav | 2 | 16 | 1–16 |
| | Cefotaxime | 0.03 | 0.5 | 0.015–2 |
| | Ciprofloxacin | 0.03 | 0.25 | 0.008–0.25 |
| *Proteus mirabilis* (15) | CL 329,998 | 8 | 16 | 2–32 |
| | CL 331,002 | 1 | 2 | 0.5–2 |
| | Tetracycline | 32 | 64 | 16–128 |
| | Co-amoxyclav | 0.5 | 1 | 0.5–1 |
| | Cefotaxime | 0.015 | 0.015 | 0.008–0.03 |
| | Ciprofloxacin | 0.03 | 0.03 | 0.03–0.06 |
| Indole-positive *Proteus* spp. (15) | CL 329,998 | 4 | 8 | 1–16 |
| | CL 331,002 | 1 | 2 | 0.5–2 |
| | Tetracycline | 32 | 64 | 1–64 |
| | Co-amoxyclav | 4 | 64 | 1–128 |
| | Cefotaxime | 0.03 | 0.06 | 0.008–0.06 |
| | Ciprofloxacin | 0.03 | 0.06 | 0.008–0.12 |
| *Serratia* spp. (15) (6 *S. marcescens* and 9 *S. liquefaciens* strains) | CL 329,998 | 4 | 4 | 1–32 |
| | CL 331,002 | 4 | 8 | 1–16 |
| | Tetracycline | 64 | >128 | 4–>128 |
| | Co-amoxyclav | 64 | 128 | 4–>128 |
| | Cefotaxime | 0.25 | 4 | 0.12–16 |
| | Ciprofloxacin | 0.06 | 16 | 0.008–16 |
| *Providencia* spp. (15) | CL 329,998 | 4 | 8 | 0.12–8 |
| | CL 331,002 | 4 | 8 | 0.008–8 |
| | Tetracycline | 128 | 128 | 1–>128 |
| | Co-amoxyclav | 64 | 128 | 1–128 |
| | Cefotaxime | 0.03 | 0.25 | 0.004–0.5 |
| | Ciprofloxacin | 0.03 | 0.5 | 0.004–8 |
| *Salmonella enteritidis* (30) | CL 329,998 | 2 | 2 | 0.5–2 |
| | CL 331,002 | 1 | 1 | 0.5–1 |
| | Tetracycline | 2 | 64 | 2–128 |
| | Co-amoxyclav | 1 | 1 | 0.5–16 |
| | Cefotaxime | 0.12 | 0.25 | 0.06–0.25 |
| | Ciprofloxacin | 0.03 | 0.03 | 0.03 |
| *Pseudomonas aeruginosa* (29) | CL 329,998 | 8 | 16 | 2–32 |
| | CL 331,002 | 4 | 16 | 2–16 |
| | Tetracycline | 16 | 32 | 4–64 |
| | Co-amoxyclav | 128 | >128 | 2–>128 |
| | Cefotaxime | 16 | 64 | 0.06–>128 |
| | Ciprofloxacin | 0.25 | 2 | 0.12–8 |
| *Acinetobacter calcoaceticus* (14) | CL 329,998 | 1 | 1 | 0.25–4 |
| | CL 331,002 | 1 | 1 | 0.5–1 |
| | Tetracycline | 2 | 4 | 1–32 |
| | Co-amoxyclav | 4 | 8 | 2–16 |
| | Cefotaxime | 8 | 16 | 4–32 |
| | Ciprofloxacin | 0.25 | 0.5 | 0.06–0.5 |
| *Staphylococcus aureus* (50) (including 4 methicillin-resistant strains) | CL 329,998 | 0.25 | 1 | 0.06–2 |
| | CL 331,002 | 0.06 | 0.5 | 0.03–4 |
| | Tetracycline | 0.25 | 64 | 0.06–128 |
| | Co-amoxyclav | 0.5 | 1 | 0.12–32 |

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TABLE 1—*Continued*

| Organism (no. of isolates) | Antibiotic | MIC (μg/ml)[a] | | |
|---|---|---|---|---|
| | | 50% | 90% | Range |
| | Cefotaxime | 2 | 2 | 0.5–128 |
| | Erythromycin | 0.12 | >128 | 0.06–>128 |
| | Ciprofloxacin | 0.5 | 4 | 0.06–32 |
| | Vancomycin | 2 | 2 | 0.5–2 |
| | Methicillin | 1 | 4 | 0.5–32 |
| *Staphylococcus saprophyticus* (20) | CL 329,998 | 0.5 | 0.5 | 0.06–1 |
| | CL 331,002 | 0.12 | 0.12 | 0.03–0.5 |
| | Tetracycline | 0.25 | 0.5 | 0.06–32 |
| | Co-amoxyclav | 0.25 | 0.5 | 0.06–0.5 |
| | Cefotaxime | 2 | 4 | 0.5–4 |
| | Erythromycin | 0.12 | 0.12 | 0.03–128 |
| | Ciprofloxacin | 0.25 | 0.5 | 0.06–0.5 |
| | Vancomycin | 2 | 2 | 0.5–2 |
| | Methicillin | 2 | 4 | 0.5–4 |
| Coagulase-negative staphylococci (20) (9 *S. warneri*, 4 *S. haemolyticus*, 2 *S. auricularis*, and 2 *S. hominis* strains and 1 *S. capitis*, 1 *S. epidermidis*, and 1 *S. simulans* strain) | CL 329,998 | 0.5 | 4 | 0.25–4 |
| | CL 331,002 | 0.5 | 4 | 0.03–4 |
| | Tetracycline | 1 | 128 | 0.06–128 |
| | Co-amoxyclav | 0.5 | 8 | 0.06–16 |
| | Cefotaxime | 2 | 16 | 0.25–128 |
| | Erythromycin | 4 | >128 | 0.06–>128 |
| | Ciprofloxacin | 0.12 | 0.5 | 0.06–16 |
| | Vancomycin | 2 | 2 | 0.5–2 |
| | Methicillin | 1 | 16 | 0.5–32 |
| Group A streptococci (18) | CL 329,998 | 0.06 | 0.06 | 0.03–0.06 |
| | CL 331,002 | 0.06 | 0.12 | 0.06–0.12 |
| | Tetracycline | 0.25 | 32 | 0.12–32 |
| | Co-amoxyclav | 0.015 | 0.015 | 0.015 |
| | Cefotaxime | 0.015 | 0.015 | 0.008–0.015 |
| | Erythromycin | 0.06 | 8 | 0.06–16 |
| | Ciprofloxacin | 0.5 | 1 | 0.12–1 |
| | Penicillin | 0.008 | 0.008 | 0.008–0.015 |
| Group B streptococci (19) | CL 329,998 | 0.06 | 0.06 | 0.06–0.12 |
| | CL 331,002 | 0.12 | 0.12 | 0.06–0.12 |
| | Tetracycline | 32 | 64 | 0.12–64 |
| | Co-amoxyclav | 0.06 | 0.06 | 0.06 |
| | Cefotaxime | 0.03 | 0.03 | 0.03 |
| | Erythromycin | 0.06 | 0.06 | 0.06–128 |
| | Ciprofloxacin | 0.5 | 1 | 0.5–2 |
| | Penicillin | 0.03 | 0.03 | 0.03 |
| *Streptococcus pneumoniae* (20) | CL 329,998 | 0.03 | 0.03 | 0.03–0.06 |
| | CL 331,002 | 0.06 | 0.06 | 0.06 |
| | Tetracycline | 16 | 32 | 0.12–32 |
| | Co-amoxyclav | 0.03 | 1 | 0.008–1 |
| | Cefotaxime | 0.015 | 0.5 | 0.008–1 |
| | Erythromycin | 0.12 | 16 | 0.06–>128 |
| | Ciprofloxacin | 1 | 2 | 0.5–4 |
| | Penicillin | 0.015 | 1 | 0.008–1 |
| Enterococci (30) (20 *E. faecalis* and 10 *E. faecium* strains) | CL 329,998 | 0.12 | 0.12 | 0.03–0.25 |
| | CL 331,002 | 0.25 | 0.25 | 0.06–0.5 |
| | Tetracycline | 16 | 64 | 0.12–>128 |
| | Co-amoxyclav | 0.5 | 8 | 0.06–8 |
| | Cefotaxime | 32 | >128 | 0.5–>128 |
| | Erythromycin | 4 | 128 | 0.5–>128 |
| | Ciprofloxacin | 2 | 2 | 1–4 |
| | Penicillin | 2 | 64 | 0.25–64 |
| *Haemophilus influenzae* (35) (including 16 β-lactamase-producing strains) | CL 329,998 | 0.12 | 0.25 | 0.06–0.5 |
| | CL 331,002 | 0.25 | 0.5 | 0.12–0.5 |
| | Tetracycline | 0.5 | 8 | 0.12–16 |
| | Co-amoxyclav | 0.5 | 1 | 0.25–1 |

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TABLE 1—*Continued*

| Organism (no. of isolates) | Antibiotic | MIC (μg/ml)[a] | | |
|---|---|---|---|---|
| | | 50% | 90% | Range |
| | Cefotaxime | 0.008 | 0.015 | 0.004–0.25 |
| | Erythromycin | 8 | 16 | 0.03–16 |
| | Ciprofloxacin | 0.015 | 0.015 | 0.008–0.015 |
| | Penicillin | 4 | 16 | 0.12–32 |
| *Moraxella catarrhalis* (30) | CL 329,998 | 0.06 | 0.12 | 0.06–0.12 |
| | CL 331,002 | 0.12 | 0.25 | 0.12–0.25 |
| | Tetracycline | 0.25 | 0.25 | 0.12–0.5 |
| | Co-amoxyclav | 0.06 | 0.25 | 0.015–1 |
| | Cefotaxime | 0.12 | 0.5 | 0.06–0.5 |
| | Erythromycin | 0.12 | 0.25 | 0.12–0.25 |
| | Ciprofloxacin | 0.03 | 0.03 | 0.03 |
| | Penicillin | 1 | 8 | 0.03–8 |
| *Neisseria meningitidis* (19) | CL 329,998 | 0.12 | 0.12 | 0.06–0.12 |
| | CL 331,002 | 0.12 | 0.12 | 0.12–0.25 |
| | Tetracycline | 0.25 | 0.25 | 0.12–0.5 |
| | Co-amoxyclav | 0.06 | 0.12 | 0.03–0.12 |
| | Cefotaxime | 0.004 | 0.004 | 0.004 |
| | Erythromycin | 0.25 | 0.5 | 0.12–0.5 |
| | Ciprofloxacin | 0.004 | 0.004 | 0.004 |
| | Penicillin | 0.03 | 0.06 | 0.015–0.06 |
| *Neisseria gonorrhoeae* (42) (including 5 β-lactamase-producing strains) | CL 329,998 | 0.03 | 0.12 | 0.004–0.25 |
| | CL 331,002 | 0.06 | 0.25 | 0.008–0.5 |
| | Tetracycline | 0.12 | 0.5 | 0.008–2 |
| | Co-amoxyclav | 0.25 | 0.5 | 0.004–0.5 |
| | Cefotaxime | 0.004 | 0.008 | 0.004–0.015 |
| | Erythromycin | 0.06 | 0.5 | 0.03–0.5 |
| | Ciprofloxacin | ≤0.002 | 0.004 | 0.002–0.008 |
| | Penicillin | 0.06 | 2 | 0.004–4 |
| *Bacteroides fragilis* (24) | CL 329,998 | 0.12 | 0.25 | 0.015–0.25 |
| | CL 331,002 | 0.25 | 0.5 | 0.03–0.5 |
| | Tetracycline | 1 | 32 | 0.12–32 |
| | Co-amoxyclav | 0.5 | 4 | 0.25–4 |
| | Cefotaxime | 16 | >128 | 0.5–>128 |
| | Erythromycin | 0.5 | 0.5 | 0.06–2 |
| | Ciprofloxacin | 2 | 4 | 2–4 |
| *Clostridium perfringens* (8) | CL 329,998 | 0.06 | | 0.03–0.25 |
| | CL 331,002 | 0.12 | | 0.03–0.5 |
| | Tetracycline | 0.25 | | 0.12–32 |
| | Co-amoxyclav | 0.03 | | 0.015–0.12 |
| | Cefotaxime | 0.12 | | 0.004–4 |
| | Erythromycin | 0.5 | | 0.25–1 |
| | Ciprofloxacin | 0.25 | | 0.12–0.25 |
| *Clostridium difficile* (8) | CL 329,998 | 0.06 | | 0.06–0.25 |
| | CL 331,002 | 0.12 | | 0.12–0.5 |
| | Tetracycline | 0.25 | | 0.25–128 |
| | Co-amoxyclav | 1 | | 0.5–2 |
| | Cefotaxime | 128 | | 4–128 |
| | Erythromycin | 0.5 | | 0.25–>128 |
| | Ciprofloxacin | 8 | | 4–8 |
| Peptostreptococci (17) | CL 329,998 | 0.06 | 0.06 | 0.03–0.25 |
| | CL 331,002 | 0.12 | 0.25 | 0.03–0.5 |
| | Tetracycline | 16 | 64 | 0.12–128 |
| | Co-amoxyclav | 0.12 | 2 | 0.015–8 |
| | Cefotaxime | 0.25 | 8 | 0.06–16 |
| | Erythromycin | 0.06 | 1 | 0.03–>128 |
| | Ciprofloxacin | 1 | 1 | 0.12–4 |

[a] 50% and 90%, MICs for 50 and 90% of isolates, respectively.

TABLE 2. MICs and MLCs for four strains of *Chlamydia* spp.[a]

| Strain | CL 329,998 | | CL 331,002 | | Ciprofloxacin | | Erythromycin | | Tetracycline | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MIC | MLC | MIC | MLC | MIC | MLC | MIC | MLC | MIC | MLC |
| *C. trachomatis* | | | | | | | | | | |
| 712 | 0.12 | 0.25 | 0.12 | 0.25 | 2.0 | 2.0 | 0.25 | 2.0 | 0.5 | 2.0 |
| 300 | 0.25 | 0.5 | 0.06 | 0.25 | 2.0 | 2.0 | 0.5 | 1.0 | 1.0 | 1.0 |
| 815 | 0.25 | 0.5 | 0.06 | 0.12 | 2.0 | 2.0 | 0.5 | 2.0 | 0.5 | 2.0 |
| *C. pneumoniae* TW183 | 0.12 | 0.5 | 0.06 | 0.12 | 2.0 | 2.0 | 0.5 | 1.0 | 1.0 | 1.0 |

[a] All values are expressed as micrograms per milliliter.

was defined as the lowest concentration which gave no visible growth. Ninety-nine percent lethality was determined by subculture of 0.1 ml into antibiotic-free media.

**Activity against *Chlamydia* spp.** The activities of CL 329,998, CL 331,002, ciprofloxacin, erythromycin, and tetracycline against three isolates of *Chlamydia trachomatis* and one isolate of *Chlamydia pneumoniae* were determined by the method of Webberley et al. (11). Briefly, McCoy cell coverslip cultures were infected with approximately 1,000 inclusion-forming units of chlamydiae, exposed to the various dilutions of the antimicrobial agents for 48 h, and then stained with a fluorescein-labelled monoclonal antibody (Imagen *Chlamydia* test; Dako Diagnostics Ltd., Ely, United Kingdom). The MIC was the lowest concentration that inhibited all inclusion development. The minimum lethal concentration (MLC) was defined as the lowest concentration that inhibited inclusion development in cell sheets exposed to an antimicrobial agent for 48 h and then reincubated in antimicrobial agent-free medium for an additional 48 h. The MIC was defined as the lowest concentration at which no inclusions were observed.

**Activity against *Mycoplasma pneumoniae*.** Two strains of *M. pneumoniae*, NCTC 10119 and a clinical isolate (DRH NB1), were studied on the basis of a method described by Waites et al. (10). An inoculum of $5 \times 10^3$ to $5 \times 10^4$ CFU in *M. pneumoniae* broth (Unipath) supplemented with horse serum, yeast extract, 1% glucose, and 0.001% phenol red was prepared. This suspension was further diluted in antibiotic-containing media (175 μl of organism suspension plus 25 μl of antibiotic). The initial MIC was determined by comparison of the test with the antibiotic-free control, and the final MIC was determined following a further 3-day incubation.

**Activity against *Mycobacteria* spp.** The activities against two strains of *Mycobacteria tuberculosis* (NCTC 7416 and clinical isolate T523) and one clinical isolate of *Mycobacterium avium-M. intracellulare* were assessed by a method based on that of McClatchy (2). An organism suspension was prepared (equivalent to a 1 McFarland standard). This was further diluted 100-fold in sterile distilled water, and 10 μl of the dilution was inoculated onto the surface of antibiotic-containing supplemented Middlebrook agar (pH 10; Difco, East Molsey, United Kingdom). Plates were incubated at 35 to 37°C in an atmosphere of 5 to 6% $CO_2$ and examined for up to 3 weeks. The MIC was defined as the lowest concentration inhibiting viable growth. Incubation was at 37°C in a microaerophilic atmosphere (*Campylobacter* system GasPak; Unipath).

## RESULTS

The activities of CL 329,998 and CL 331,002 and the other agents studied against the 523 strains are shown in Table 1. Members of the family *Enterobacteriaceae* were generally two- to fourfold more susceptible to CL 331,002 than to CL 329,998, which were both markedly more active than tetracycline, the MIC for 90% of strains tested ($MIC_{90}$) of the latter being ≥32 μg/ml. Those strains which were less susceptible to CL 329,998 and CL 331,002 also were less susceptible to tetracycline; for example, a strain of *Klebsiella pneumoniae* was susceptible to 32 μg of CL 329,998 per ml and to >128 μg of tetracycline per ml. The converse was not necessarily the case, in that the strains less susceptible to tetracycline (MIC > 64 μg/ml) were often susceptible to the two glycylcyclines (MIC ≤ 2 μg/ml). No cross-resistance to β-lactams or ciprofloxacin was detected; however, those strains which were less susceptible to one glycylcycline were less susceptible to the other.

The glycylcyclines had similar activities against the *Acinetobacter* spp. ($MIC_{90}$, 1 μg/ml) and *P. aeruginosa* ($MIC_{90}$, 16 μg/ml) and were two- to fourfold more active than tetracycline. There was a bimodal distribution of susceptibility of *S. aureus* to tetracycline, with MICs of either ≤0.25 or >8 μg/ml. This was not observed for the two glycylcyclines, the range of susceptibilities being narrow and CL 331,002 being fourfold more active than CL 329,998. The four methicillin-resistant strains (MIC ≥ 8 μg/ml) were uniformly susceptible to the new compounds (for example, all were susceptible to ≤0.5 μg of CL 331,002 per ml). The glycylcyclines were two- to fourfold more active than vancomycin. The methicillin-resistant strains did demonstrate cross-resistance with erythromycin and ciprofloxacin.

*Staphylococcus saprophyticus* was generally equally susceptible to CL 329,998 and tetracycline but CL 331,002 was fourfold more active against the organism. The coagulase-negative staphylococci were more susceptible to the glycylcyclines than to tetracycline. Three of the four strains most resistant to CL 329,998 and CL 331,002 (MIC, 4 μg/ml) were *Staphylococcus haemolyticus*.

Lancefield group A and B streptococci and *S. pneumoniae* were uniformly susceptible to the two glycylcyclines, but a tetracycline-susceptible population (MIC ≤ 0.25 μg/ml) and a tetracycline-resistant population (MIC ≥ 16 μg/ml) were observed. All strains of *Enterococcus faecalis* and *Enterococcus faecium* were susceptible ($MIC_{90}$ ≤ 0.25 μg/ml) to the glycylcyclines, but the majority were resistant to tetracycline ($MIC_{90}$, 64 μg/ml). Strains of *Moraxella catarrhalis* and *Neisseria* spp. were susceptible to CL 329,998 and CL 331,002 ($MIC_{90}$ ≤ 0.25 μg/ml).

TABLE 3. Activities of CL 322998 and CL 331,002 against two strains of *M. pneumoniae*

| Strain | Activity (μg/ml) | | | |
|---|---|---|---|---|
| | CL 329,998 | CL 331,002 | Tetracycline | Erythromycin |
| NCTC 10119 | 1.0 | 0.06 | 0.25 | 0.03 |
| M2629 | 1.0 | 0.12 | 0.12 | 0.03 |

TABLE 4. Effects of increasing amounts of serum on the activity of CL 331,002[a]

| Organism[b] | Substrate and activity | | | | | | |
|---|---|---|---|---|---|---|---|
| | Agar, MIC | Iso-Sensitest broth alone | | Iso-Sensitest broth + 20% HS[c] | | Iso-Sensitest broth + 70% HS | |
| | | MIC | MBC | MIC | MBC | MIC | MBC |
| *E. faecalis* | 0.25 | 0.25 | 8 | 0.12 | 4 | 0.25 | 4 |
| | 0.25 | 0.5 | 8 | 0.25 | 8 | 0.5 | 8 |
| *S. pneumoniae* | 0.06 | 0.12 | 0.5 | 0.12 | 1 | 0.12 | 2 |
| | 0.06 | 0.12 | 0.25 | 0.06 | 1 | 0.25 | 2 |
| *H. influenzae* | 0.25 | 0.25 | 2 | 0.25 | 4 | 0.5 | 4 |
| | 0.12 | 0.5 | 4 | 0.5 | 4 | 1 | 4 |
| *S. aureus* | 0.03 | 0.5 | 1 | 0.5 | 1 | 0.5 | 1 |
| | 0.12 | 0.5 | 2 | 0.25 | 1 | 0.12 | 0.25 |

[a] All values are expressed as micrograms per milliliter.
[b] Two strains of each species were tested.
[c] HS, human serum.

The glycylcyclines were the most active agents tested against *Bacteroides fragilis*, with a narrow range of MICs noted, but in the case of tetracycline a bimodal distribution was demonstrated (mode MICs, 0.5 and 32 μg/ml). No evidence of cross-resistance between the glycylcyclines and tetracycline was found. Whereas tetracycline had little activity against *Clostridium difficile* (the MICs for three of eight strains were ≥128 μg/ml), there was uniform susceptibility to the two new agents. The peptostreptococci were >64-fold more susceptible to the glycylcyclines than to tetracycline.

In Table 2, the MICs and MLCs for three strains of *C. trachomatis* and one strain of *C. pneumoniae* are shown. CL 329,998 and CL 331,002 were four- to eightfold more active than tetracycline, ciprofloxacin, or erythromycin.

In Table 3, the activities of tetracycline and erythromycin against two *M. pneumoniae* strains are compared with those of the new agents. CL 331,002 was 8- to 16-fold more active than CL 329,998, and the former displayed activity similar to those of erythromycin and tetracycline.

The two strains of *M. avium* were not inhibited by 128 μg of either glycylcycline per ml. The two strains of *M. tuberculosis* were inhibited by 16 and 32 μg of CL 329,998 per ml and by 8 and 16 μg of CL 331,002 per ml.

In Tables 4 and 5, the effects of serum and medium on the activities of the two compounds against eight selected strains are shown. There was little difference between broth- and agar-derived MICs. Although there was little difference between the MIC and MBC for *S. pneumoniae*, a 4- to 32-fold difference was seen for the other strains tested. The addition of serum to the medium had little effect on the MIC or MBC of either compound.

TABLE 5. Effects of increasing amounts of serum on the activity of CL 329,998[a]

| Organism[b] | Substrate and activity | | | | | | |
|---|---|---|---|---|---|---|---|
| | Agar, MIC | Iso-Sensitest broth alone | | Iso-Sensitest broth + 20% HS[c] | | Iso-Sensitest broth + 70% HS | |
| | | MIC | MBC | MIC | MBC | MIC | MBC |
| *E. faecalis* | 0.12 | 0.25 | 4 | 0.12 | 4 | 0.25 | 4 |
| | 0.25 | 0.25 | 8 | 0.25 | 4 | 0.25 | 4 |
| *S. pneumoniae* | 0.03 | 0.06 | 0.12 | 0.06 | 0.25 | 0.06 | 0.5 |
| | 0.03 | 0.06 | 0.06 | 0.06 | 0.12 | 0.06 | 0.5 |
| *H. influenzae* | 0.12 | 0.12 | 0.5 | 0.25 | 2 | 0.25 | 2 |
| | 0.06 | 0.25 | 2 | 0.25 | 2 | 0.25 | 2 |
| *S. aureus* | 0.25 | 2 | 8 | 0.5 | 4 | 0.5 | 16 |
| | 1 | 2 | 16 | 0.25 | 0.5 | 0.06 | 2 |

[a] All values are expressed as micrograms per milliliter.
[b] Two strains of each species were tested.
[c] HS, human serum.

## DISCUSSION

Currently available tetracyclines such as minocycline, chlortetracycline, and tetracycline act by preventing protein synthesis following binding to the 30S ribosomal subunit (6), although new analogs were found which have a different bacterial target (4) and affect bacterial membrane permeability. Tetracycline analogs enjoy considerable use on a worldwide basis, but increasing resistance militates against their use in many countries. The *tetK* determinant in *S. aureus* and the widespread *tetM* determinant in streptococci and other species have been well studied (3, 8).

The two glycylcyclines CL 329,998 and CL 331,002 possess a number of novel properties. First, we have confirmed the finding of others (9) that they show enhanced activities (in comparison with that of tetracycline) against members of the *Enterobacteriaceae*, and activity against strains with acquired tetracycline resistance suggests that these new compounds may have a potential role, depending upon their pharmacokinetic and safety profiles, in the treatment of infections caused by these pathogens. Generally, but not invariably, CL 331,002 was more active than CL 329,998, and this again reflects the earlier findings (9). It was of interest that intergenus differences were seen among the *Enterobacteriaceae*; for example, *E. coli* and *Salmonella* spp. were more susceptible than *Serratia* and *Providentia* spp. Second, although there was a less marked difference in activity between the glycylcyclines and tetracycline against strains of staphylococci and streptococci normally susceptible to tetracycline, the two new agents were active against the strains which were tetracycline resistant, which suggests either a different mechanism(s) of action of these new compounds or a differential mechanism of resistance. Against these genera, CL 329,998 was usually marginally more active than CL 331,002.

CL 329,998 and CL 331,002 were also active against the common respiratory pathogens *H. influenzae*, *Moraxella catarrhalis*, and *S. pneumoniae* as well as atypical pathogens *M. pneumoniae* and *C. pneumoniae*, which were inhibited by 1 μg of CL 329,998 per ml. It therefore appears that these agents have considerable potential in the treatment of these common infections. Similarly, the pathogens responsible for the majority of sexually transmitted diseases, strains of *Neisseria gonorrhoeae* and *C. trachomatis*, were all inhibited by 0.5 μg of either compound per ml; however, we have not studied tetracycline-resistant *N. gonorrhoeae* to determine whether cross-resistance exists. No useful antimycobacterial activity was demonstrated.

Human serum had only a minimal effect on activity; the difference seen between the MIC and the MBC tended to be strain dependent and more marked for enterococci than for *S. pneumoniae*. This reflects the relative bacteriostatic activity noted for the earlier tetracyclines (1).

The broad spectrum of activity of these new agents and their activities against strains resistant to the earlier tetracyclines suggest that they could be tested in widespread clinical trials once pharmacokinetic and toxicological studies are available.

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