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In Vitro and In Vivo Antibacterial Activities of the Glycylcyclines, a New Class of Semisynthetic Tetracyclines

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***N,N*-Dimethylglycylamido (DMG) derivatives of minocycline and 6-demethyl-6-deoxytetracycline are new semisynthetic tetracyclines referred to as the glycylcyclines. The in vitro activities of the glycylcyclines were evaluated in comparison with those of minocycline and tetracycline against strains carrying characterized tetracycline resistance determinants and against 995 recent clinical isolates obtained from geographically distinct medical centers in North America. The glycylcyclines were active against tetracycline-resistant strains carrying efflux [*tet*(A), *tet*(B), *tet*(C), and *tet*(D) in *Escherichia coli* and *tet*(K) in *Staphylococcus aureus*] and ribosomal protection [*tet*(M) in *S. aureus*, *Enterococcus faecalis*, and *E. coli*)] resistance determinants. Potent activity (MIC for 90% of strains, ≤0.5 μg/ml) was obtained with the glycylcyclines against methicillin-susceptible and methicillin-resistant *S. aureus*, *E. faecalis*, *Enterococcus faecium*, and various streptococcal species. The glycylcyclines exhibited good activity against a wide diversity of gram-negative aerobic and anaerobic bacteria, most of which were less susceptible to minocycline and tetracycline. The activities of the glycylcyclines against most organisms tested were comparable to each other. The in vivo efficacies of the glycylcyclines against acute lethal infections in mice when dosed intravenously were reflective of their in vitro activities. The glycylcyclines had efficacies comparable to that of minocycline against infections with methicillin-susceptible and methicillin-resistant *S. aureus* strains, a strain carrying *tet*(K), and a tetracycline-susceptible *E. coli* strain but exceeded the effectiveness of minocycline against infections with resistant isolates, including strains harboring *tet*(M) or *tet*(B). Levels of DMG-6-demethyl-6-deoxytetracycline in serum were higher and more sustained than those of DMG-minocycline or minocycline. Our results show that the glycylcyclines have potent in vitro activities against a wide spectrum of gram-positive and gram-negative, aerobic and anaerobic bacteria, including many resistant strains. On the basis of their in vitro and in vivo activities, the glycylcyclines represent a significant advance to the tetracycline class of antibiotics and have good potential value for clinical efficacy.**

The tetracyclines, first isolated at Lederle Laboratories in 1945 from a strain of *Streptomyces aureofaciens*, represented a significant advance in the treatment of many infections (11). The activity of the tetracyclines against a wide variety of gram-positive and gram-negative aerobic and anaerobic bacteria, mycoplasmas, and rickettsiae and their efficacy against both intracellular and extracellular pathogens permitted their widespread use (12). Through modifications of the fermentation conditions and semisynthetic synthesis, several analogs, such as minocycline (MINO) and doxycycline, which exhibited improved antimicrobial activity and more favorable pharmacokinetic properties over those of the early tetracyclines were prepared (20, 27, 35). However, because of an increased incidence of resistance among many aerobic and anaerobic bacteria, the utility of the tetracycline group of antibiotics is now limited to certain indications. There are currently two major mechanisms responsible for tetracycline resistance among widely divergent bacterial species: active efflux, in which the intracellular accumulation of tetracycline is reduced by a set of membrane-associated protein pumps, and ribosomal protection, in which production of a cytoplasmic protein reduces the sensitivity of ribosomes to tetracyclines (4, 7, 18, 30).

A program was initiated to develop analogs with activity against organisms carrying these tetracycline resistance determinants while retaining activity against tetracycline-susceptible organisms, thereby restoring the broad-spectrum activity of this class of antibiotics. New semisynthetic derivatives containing the *N,N*-dimethylglycylamido (DMG) substitutent at the 9 position of MINO and 6-demethyl-6-deoxytetracycline (DMDOT) were prepared (Fig. 1) (32). Antibiotics with the 9-DMG substitutent are referred to generically as the glycylcyclines, and the two specific compounds are referred to as DMG-MINO and DMG-DMDOT.

In this study we evaluated the in vitro and in vivo efficacies of the glycylcyclines compared with those of MINO, tetracycline, and other antibiotics against a broad group of recent clinical isolates and strains harboring characterized tetracycline resistance determinants.

MATERIALS AND METHODS

Organisms. Routine clinical isolates were collected from various medical centers in the United States and Canada during 1989 to 1992. Identification of each culture was done by conventional methods: gram-negative rods by API 20E (Analytab Products, Plainville, N.Y.) and NF systems (Remel, Lenexa, Kans.), staphylococci by Staph Trac (Analytab Products), and anaerobes by the procedures outlined

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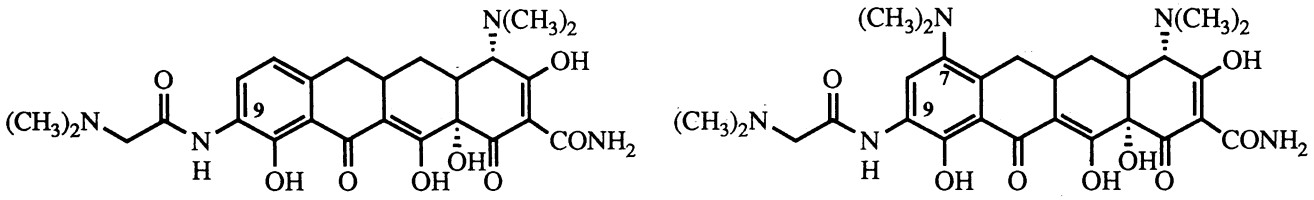

FIG. 1. Chemical structures of DMG-DMDOT and DMG-MINO.

in the *Wadsworth Anaerobic Bacteriology Manual* (33). Susceptibility of staphylococci to oxacillin was determined by the presence or absence of growth on an agar plate containing 6 μg of oxacillin per ml, as described in the *Manual of Clinical Microbiology* (31). Strains with characterized tetracycline resistance determinants (Table 1) were obtained from B. Rasmussen, Molecular Biology Department, American Cyanamid Co., and from Ian Chopra, University of Bristol, Bristol, United Kingdom. Four of the eight vancomycin-resistant enterococcal strains were obtained from the National Type Culture Collection (34); three were obtained from the Centers for Disease Control and Prevention, Atlanta, Ga.; and another was a clinical isolate sent to our laboratory. All isolates were stored frozen in skim milk at −70°C.

Antibiotics. Standard powders of DMG-MINO, DMG-DMDOT, MINO, tetracycline, and vancomycin were obtained at Lederle Laboratories, Pearl River, N.Y.; ciprofloxacin was obtained from Miles Laboratories, West Haven, Conn.; and erythromycin was obtained from Sigma Chemical Co., St. Louis, Mo.

In vitro susceptibility testing. The activities of the various antibiotics were determined by the agar dilution method following the recommendations of the National Committee for Clinical Laboratory Standards (23, 24). Mueller-Hinton II agar was used to test nonfastidious aerobic bacteria. This medium was supplemented with 5% sheep blood for the testing of *Listeria monocytogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *Streptococcus pyogenes*. GC agar supplemented with 1% hemoglobin and 1% IsoVitaleX was employed to test *Neisseria gonorrhoeae*, and *Haemophilus* test medium was used for *Haemophilus influenzae*. Anaerobic bacteria were tested on Wilkins Chalgren agar supplemented with 5% lysed sheep blood and 0.001% vitamin K. The inocula, adjusted to the recommended density (10^7 CFU/ml for aerobes and 10^8 CFU/ml for anaerobes), were applied to the surface of the agar with a Steers replicator. The test plates were incubated at 35°C for 18 h for the nonfastidious aerobic bacteria and streptococci in ambient air and in a CO_2 incubator for the *Neisseria* and *Haemophilus* species. The anaerobic bacteria were incubated in an anaerobic chamber (Coy Laboratories, Ann Arbor, Mich.) at 35°C for 48 h. The MIC was defined as the lowest concentration of antimicrobial agent that completely inhibited the growth of the organism as detected by the unaided eye.

TABLE 1. Description of tetracycline resistance determinants in *E. coli* and *S. aureus* strains

Strain and plasmid	Tetracycline resistance determinant(s)	Source or reference
E. coli		
J3272	None	29
MC4100 Tc^r	Tn*10*, *tet*(B) (inducible)	Rasmussen[a]
JC3272 Tc^r	Tn*10*, *tet*(B) (constitutive)	29
JC3272(pRP1)	*tet*(A)	29
JC3272(pBR322)	*tet*(C) (constitutive)	8
JC3272(pRA1)	*tet*(D)	8
UBMS 89-1	*tet*(M)	Chopra[b]
UBMS 90-4	*tet*(M)	Chopra
S. aureus		
649	None	6
649(pUB111)	*tet*(K)	Chopra
694(pE109)	*tet*(M)	Chopra

[a] Gift from B. Rasmussen, American Cyanamid Co., Pearl River, N.Y.
[b] Gift from I. Chopra, Bristol University, Bristol, United Kingdom.

In vivo efficacy against murine infections. The therapeutic effects of the antibiotics were determined against acute lethal infections in mice (9) with MINO-susceptible and MINO-resistant gram-positive and -negative bacteria. Female mice, strain CD-1, from Charles River Laboratories (20 ± 2 g each) were challenged by intraperitoneal injection of 0.5 ml of a bacterial suspension in either broth or 5% hog gastric mucin (10 to 100 50% lethal doses). Five to six dose levels of the antibiotics in phosphate-buffered saline (pH 7.4, 0.01 M) were administered intravenously (0.2 ml), subcutaneously (0.5 ml), or orally (0.5 ml) 0.5 h postinfection. For the infection with *Escherichia coli* JC3272 Tc^r *tet*(B) a second dose of the antibiotic was given 3 h later. In each test, five animals were treated per dose level. All the untreated controls died within 48 h of infection. The median effective dose (ED_{50}) was determined by probit analysis of the 7-day survival ratios pooled from three separate tests (13).

Antibiotic levels in mouse sera. For determination of antibiotic levels in serum, mice were bled by cardiac puncture after intravenous administration of a single bolus injection of 10 mg/kg of body weight. Seven mice were used per time point of 0.25, 0.5, 1, 2, 4, and 6 h. The blood samples were centrifuged at 10°C for 10 min to separate the serum. Serum samples were kept frozen at −70°C until assayed. The antibiotic concentration was determined by microbiological assay using *Bacillus cereus* ATCC 11778 as the test organism (1).

RESULTS

In vitro activity against tetracycline-resistant strains. Both DMG derivatives, DMG-MINO and DMG-DMDOT (the glycylcyclines), exhibited potent activity against strains carrying the two major mechanisms of tetracycline resistance, efflux and ribosomal protection (Table 2). The MICs of DMG-MINO and DMG-DMDOT were significantly lower than the MICs of tetracycline against *E. coli* strains carrying the efflux resistance determinants [*tet*(A), *tet*(B), *tet*(C), and *tet*(D)] and a *Staphylococcus aureus* strain carrying *tet*(K).

TABLE 2. In vitro activities of DMG-MINO, DMG-DMDOT, MINO, and tetracycline against strains harboring characterized tetracycline resistance determinants

Strain (plasmid)	Resistance determinant	MIC (μg/ml) of:			
		DMG-MINO	DMG-DMDOT	MINO	Tetracycline
E. coli					
JC3272 Tc^r	*tet*(B)	0.25	0.25	16	>64
MC1400 Tc^r	*tet*(B)	0.25	0.25	8	>64
JC3272(pRP1)	*tet*(A)	2	2	4	32
JC3272(pBR322)	*tet*(C)	2	2	4	64
JC3272(pRA1)	*tet*(D)	0.12	0.25	8	>64
UBMS 90-4	*tet*(M)	0.25	0.25	64	32
JC3272	None	0.25	0.5	1	1
311	None	0.25	0.5	0.5	1
S. aureus					
649(pUB111)	*tet*(K)	1	1	0.12	64
649(pE109)	*tet*(M)	0.25	0.25	8	64
UBMS 90-2	*tet*(M)	0.25	0.25	4	32
649	None	0.25	0.12	0.06	0.25
Smith	None	0.25	0.25	0.06	0.25
E. faecalis					
UBMS 90-6	*tet*(M)	0.25	1	16	>64
ATCC 29212	None	0.06	0.12	2	16

The MICs of the DMG analogs against strains carrying *tet*(B) and *tet*(D) were the same as those for the tetracycline-susceptible parent strains, while those for strains carrying *tet*(A), *tet*(C), and *tet*(K) were higher than those for the parent strain. Both DMG-MINO and DMG-DMDOT had comparable activities against tetracycline-susceptible *S. aureus* and *E. coli* strains and strains carrying the ribosomal protection determinant, *tet*(M). The MICs of tetracycline and MINO were significantly higher against these *tet*(M)-containing strains (32 to 64 and 4 to 64 μg/ml, respectively).

In vitro activity against recent clinical isolates. The activities of the glycylcyclines and comparative agents against 995 recent gram-positive and gram-negative aerobic and anaerobic clinical isolates were determined. The glycylcyclines and MINO exhibited potent activities against both methicillin-susceptible and methicillin-resistant *S. aureus* (MRSA) isolates (MICs for 90% of strains [MIC_{90}s] ≤ 0.5 μg/ml) (Table 3). The glycylcyclines were less active than MINO but similar to vancomycin against methicillin-susceptible and methicillin-resistant coagulase-negative staphylococci. Ciprofloxacin and erythromycin had poor activities against the methicillin-resistant staphylococci. Relative to the comparative agents, the glycylcyclines exhibited excellent activities (MIC_{90}s ≤ 0.25 μg/ml) against *Enterococcus faecalis*, *Enterococcus faecium*, and eight vancomycin-resistant enterococcal strains. The glycylcyclines were much more active than MINO and tetracycline against these species. All streptococcal isolates tested were inhibited by ≤0.5 μg of the glycylcyclines per ml. The MIC_{90}s against *S. agalactiae* and *S. pyogenes* were at least eightfold lower than those of MINO and comparable to those of vancomycin.

The glycylcyclines exhibited potent activities against a wide spectrum of gram-negative isolates. Against members of the family *Enterobacteriaceae*, the glycylcyclines exhibited improved activities compared with that of MINO, especially against *E. coli*, *Citrobacter freundii*, *Shigella* spp., *Salmonella* spp., and *Morganella morganii* (Table 4). Activity comparable to that of MINO was obtained against *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Serratia marcescens*, and *Enterobacter cloacae*. DMG-DMDOT showed improved activity compared with those of DMG-MINO and MINO against *Proteus mirabilis* and *Proteus vulgaris* (MIC_{90}s of 1 versus 4 and 16 μg/ml, respectively). Both DMG analogs exhibited greater activity than did MINO against *Providencia* spp.; however, the MICs against these isolates were elevated (MIC_{90}s, 8 μg/ml). Poorer activity was observed with all of the tetracycline compounds against *Pseudomonas aeruginosa* (MIC_{90}s, 32 μg/ml). The DMG compounds and MINO had improved activities compared with that of tetracycline against *Pseudomonas cepacia* and *Xanthomonas maltophilia*.

Both DMG analogs exhibited significantly improved activities (MIC_{90}s of 1 μg/ml) compared with those of MINO and tetracycline (MIC_{90}s of 64 μg/ml) against *N. gonorrhoeae*. Similar activities were obtained with both DMG compounds, MINO, and tetracycline against *Moraxella catarrhalis* and *H. influenzae*.

The DMG compounds showed improved activities compared with that of MINO against a number of anaerobic organisms (Table 5). Against *Bacteroides fragilis* and other species of the *B. fragilis* group, the MIC_{90} of the DMG analogs was 0.5 μg/ml, compared with 8 μg of MINO per ml. Similarly, the MICs of the DMG compounds against *Prevotella* species, clostridia, and anaerobic gram-positive cocci tested were at least fourfold lower than the MINO MICs.

In vivo efficacy. The in vivo efficacies of the glycylcyclines were compared with that of MINO against acute lethal infections in mice. Against an infection with the tetracycline-susceptible *S. aureus* Smith strain, the DMG analogs and MINO exhibited comparable activities when given by the intravenous or subcutaneous route (Table 6). However, when given by the oral route, the DMG analogs showed poor efficacy relative to that of MINO and relative to the efficacy obtained by the intravenous route. Because of the poor oral efficacy demonstrated in mice, comparisons against other infections were done by the intravenous route. Good efficacy was observed with the DMG analogs and MINO against infections with MRSA strains and with the DMG analogs against a MINO-resistant MRSA strain (Table 7). The ED_{50}s of the DMG analogs and MINO against a strain resistant to tetracycline because of the expression of *tet*(K) (efflux pump resistance determinant) were comparable. An *S. aureus*

TABLE 3. In vitro activities of DMG-MINO, DMG-DMDOT, and comparitive antibiotics against gram-positive isolates

Organism (no. of strains)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
MRSA (32)	DMG-MINO	0.12–2	0.25	0.5
	DMG-DMDOT	0.12–4	0.25	0.5
	MINO	0.03–4	0.06	0.12
	Tetracycline	0.12–>64	0.25	1
	Ciprofloxacin	0.12–>64	32	>64
	Vancomycin	0.5–2	1	2
	Erythromycin	0.25–>64	>64	>64
Methicillin-susceptible *S. aureus* (58)	DMG-MINO	0.12–1	0.25	0.5
	DMG-DMDOT	0.06–1	0.25	0.5
	MINO	0.03–0.12	0.06	0.12
	Tetracycline	0.12–16	0.25	0.5
	Ciprofloxacin	0.12–8	1	1
	Vancomycin	0.5–2	1	1
	Erythromycin	0.25–>64	0.25	16
Coagulase-negative staphylococci				
Methicillin resistant (90)	DMG-MINO	0.03–8	0.5	2
	DMG-DMDOT	0.06–16	1	4
	MINO	0.015–4	0.25	0.5
	Tetracycline	0.06–>64	1	64
	Ciprofloxacin	0.06–>64	0.5	64
	Vancomycin	0.12–4	2	2
	Erythromycin	0.008–>64	>64	>64
Methicillin susceptible (57)	DMG-MINO	0.06–4	0.25	1
	DMG-DMDOT	0.06–4	0.25	1
	MINO	0.015–0.5	0.12	0.25
	Tetracycline	0.12–>64	0.5	32
	Ciprofloxacin	0.12–1	0.5	0.5
	Vancomycin	0.5–4	1	2
	Erythromycin	0.06–>64	0.5	>64
E. faecalis (31)	DMG-MINO	0.015–0.25	0.12	0.12
	DMG-DMDOT	0.03–0.5	0.12	0.25
	MINO	0.03–16	8	16
	Tetracycline	0.12–64	32	64
	Ciprofloxacin	0.5–32	1	2
	Vancomycin	0.5–2	1	2
	Erythromycin	0.12–>64	1	64
E. faecium (11)	DMG-MINO	0.03–0.25	0.06	0.12
	DMG-DMDOT	0.06–0.25	0.06	0.25
	MINO	0.03–16	0.03	16
	Tetracycline	0.12–>64	0.25	32
	Ciprofloxacin	1–8	4	4
	Vancomycin	0.25–2	0.5	1
	Erythromycin	0.5–>64	8	>64
Vancomycin-resistant *Enterococcus* spp. (8)	DMG-MINO	0.015–0.06	0.03	0.06
	DMG-DMDOT	0.03–0.12	0.06	0.12
	MINO	0.03–16	0.03	16
	Tetracycline	0.12–>64	0.5	16
	Ciprofloxacin	0.5–4	2	4
	Vancomycin	>64	>64	>64
	Erythromycin	1–>64	>64	>64
S. agalactiae (32)	DMG-MINO	0.06–0.25	0.12	0.12
	DMG-DMDOT	0.12–0.25	0.25	0.25
	MINO	0.06–16	16	16
	Tetracycline	0.12–64	32	64
	Ciprofloxacin	0.25–4	0.5	1
	Vancomycin	0.25–1	0.25	0.5
	Erythromycin	0.015–4	0.03	0.06
S. pneumoniae (25)	DMG-MINO	0.03–0.25	0.25	0.25
	DMG-DMDOT	0.03–0.5	0.5	0.5
	MINO	0.06–1	0.25	0.25
	Tetracycline	0.12–16	0.5	2
	Ciprofloxacin	1–4	2	4
	Vancomycin	0.25–0.5	0.5	0.5
	Erythromycin	0.015–0.25	0.25	0.25
S. pyogenes (25)	DMG-MINO	0.06–0.25	0.25	0.25
	DMG-DMDOT	0.06–0.5	0.5	0.5
	MINO	0.03–8	0.25	4
	Tetracycline	0.12–32	0.5	16
	Ciprofloxacin	0.25–4	2	2
	Vancomycin	0.25–1	0.5	0.5
	Erythromycin	0.015–16	0.25	0.25
L. monocytogenes (8)	DMG-MINO	0.06–0.12	0.12	0.12
	DMG-DMDOT	0.06–0.12	0.12	0.12
	MINO	0.015–0.03	0.015	0.03
	Tetracycline	0.12–0.5	0.25	0.5
	Ciprofloxacin	0.5–1	0.5	1

[a] 50% and 90%, MIC_{50} and MIC_{90}.

strain (UBMS 90-2) which expresses the *tet*(M) (ribosomal protection) resistance determinant was more susceptible to treatment with the DMG analogs than to treatment with MINO.

When tested against infections caused by various *E. coli* isolates, the DMG analogs and MINO showed similar efficacies against a susceptible strain; however, against strains that were tetracycline resistant either because of *tet*(B) (efflux) or *tet*(M) (ribosomal protection) or against an uncharacterized MINO-resistant clinical isolate, the DMG analogs exhibited good efficacy while MINO did not protect mice when dosed up to 32 mg/kg.

Antibiotic levels in serum. Mice dosed intravenously with 10 mg of DMG-MINO, DMG-DMDOT, or MINO per kg showed concentrations in serum 15 min after injection of 2.7, 4.8, and 4.2 μg/ml, respectively (Fig. 2). While levels in serum of 4 μg/ml or higher were obtained for DMG-DMDOT and MINO, the peak level of DMG-DMDOT was 1.8 times higher than that of DMG-MINO. In addition, the levels of DMG-DMDOT were sustained for a longer period of time, 6 h, while the concentration of DMG-MINO declined to undetectable levels 4 h after the drug was administered.

DISCUSSION

The glycylcyclines, DMG-MINO and DMG-DMDOT, represent a quantum advance in the tetracycline class of antibiotics. They overcome the two major mechanisms responsible for tetracycline resistance in a wide variety of bacterial species, i.e., active efflux of the drug out of the bacterial cells and protection of the ribosomes (7, 8, 17, 28), thereby extending their spectrum to include multiresistant staphylococci, enterococci, many enteric bacteria, and *Neisseria* strains while maintaining excellent activity against susceptible organisms.

Tetracycline efflux is widespread and is the most studied mechanism of tetracycline resistance. To date, eight classes of tetracycline efflux genes have been described and occur in a wide variety of both gram-positive and gram-negative aerobic and anaerobic bacteria (19, 30). Both glycylcycline compounds were shown to be active against strains of *E. coli* carrying *tet*(A), *tet*(B), *tet*(C), and *tet*(D) and *S. aureus* carrying *tet*(K). Ribosomal protection, in which a cytoplasmic protein interacts or associates with the ribosome, thereby reducing the sensitivity to the tetracyclines, is also detected in a wide variety of bacteria (14, 16, 26, 28, 37). The glycylcyclines were very effective against strains of *S.*

TABLE 4. In vitro activities of DMG-MINO, DMG-DMDOT, and comparative antibiotics against gram-negative isolates

Organism (no. of strains)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
E. coli (101)	DMG-MINO	0.12–4	0.25	1
	DMG-DMDOT	0.25–4	0.5	1
	MINO	0.25–64	0.5	16
	Tetracycline	0.5–>64	1	>64
	Ciprofloxacin	≤0.008–0.5	≤0.008	≤0.008
Shigella spp. (14)	DMG-MINO	0.06–0.5	0.25	0.5
	DMG-DMDOT	0.12–0.5	0.25	0.5
	MINO	0.25–8	1	8
	Tetracycline	0.25–>64	0.5	64
	Ciprofloxacin	≤0.008	≤0.008	≤0.008
K. pneumoniae (24)	DMG-MINO	0.25–8	1	2
	DMG-DMDOT	0.25–4	1	2
	MINO	0.5–8	2	4
	Tetracycline	0.5–>64	1	4
	Ciprofloxacin	≤0.008–0.12	0.015	0.06
K. oxytoca (24)	DMG-MINO	0.25–1	0.5	1
	DMG-DMDOT	0.25–1	0.5	1
	MINO	0.5–8	1	2
	Tetracycline	0.5–>64	0.5	1
	Ciprofloxacin	≤0.008–0.12	≤0.008	0.015
C. freundii (27)	DMG-MINO	0.25–8	1	2
	DMG-DMDOT	0.5–8	1	1
	MINO	0.03–64	2	32
	Tetracycline	0.5–16	1	16
	Ciprofloxacin	≤0.008–64	≤0.008	0.12
Citrobacter diversus (14)	DMG-MINO	0.25–1	0.5	1
	DMG-DMDOT	0.25–2	0.5	1
	MINO	0.25–4	0.5	4
	Tetracycline	0.5–4	1	2
	Ciprofloxacin	≤0.008–0.03	≤0.008	0.03
Salmonella spp. (11)	DMG-MINO	0.25–0.5	0.5	0.5
	DMG-DMDOT	0.25–0.5	0.5	0.5
	MINO	0.5–16	1	16
	Tetracycline	0.5–>64	1	>64
	Ciprofloxacin	≤0.008	≤0.008	≤0.008
S. marcescens (10)	DMG-MINO	2–8	2	8
	DMG-DMDOT	2–8	2	4
	MINO	1–8	1	4
	Tetracycline	8–>64	8	64
	Ciprofloxacin	≤0.008–1	0.03	0.06
E. cloacae (31)	DMG-MINO	0.5–4	1	2
	DMG-DMDOT	0.5–2	1	2
	MINO	0.25–8	2	4
	Tetracycline	0.5–4	1	2
	Ciprofloxacin	≤0.008–0.06	≤0.008	0.015
Enterobacter aerogenes (25)	DMG-MINO	0.5–16	1	1
	DMG-DMDOT	0.25–8	0.5	1
	MINO	0.5–32	2	4
	Tetracycline	0.5–16	1	2
	Ciprofloxacin	≤0.008–0.25	≤0.008	0.015
Providencia spp. (13)	DMG-MINO	2–8	4	8
	DMG-DMDOT	1–8	2	8
	MINO	4–>64	16	>64
	Tetracycline	1–>64	64	>64
	Ciprofloxacin	≤0.008–0.12	0.06	0.12
P. mirabilis (26)	DMG-MINO	1–32	4	16
	DMG-DMDOT	0.12–2	0.5	1
	MINO	1–32	8	16
	Tetracycline	0.5–64	32	64
	Ciprofloxacin	≤0.008–0.06	0.03	0.06
P. vulgaris (18)	DMG-MINO	0.5–4	2	4
	DMG-DMDOT	0.06–1	0.5	1
	MINO	0.5–16	2	8
	Tetracycline	0.25–64	8	32
	Ciprofloxacin	≤0.008–0.25	≤0.008	0.12
M. morganii (16)	DMG-MINO	0.5–4	2	2
	DMG-DMDOT	0.5–1	1	1
	MINO	0.25–32	2	32
	Tetracycline	0.25–>64	0.5	16
	Ciprofloxacin	≤0.008	≤0.008	≤0.008
P. aeruginosa (40)	DMG-MINO	1–>64	16	32
	DMG-DMDOT	2–>64	16	32
	MINO	1–>64	16	32
	Tetracycline	2–>64	32	32
	Ciprofloxacin	0.015–8	0.12	0.5
P. cepacia (11)	DMG-MINO	1–8	4	8
	DMG-DMDOT	2–16	4	8
	MINO	0.06–8	1	4
	Tetracycline	1–>64	4	16
	Ciprofloxacin	0.06–4	2	4
X. maltophilia (23)	DMG-MINO	0.5–4	1	2
	DMG-DMDOT	1–8	4	4
	MINO	0.12–4	0.25	1
	Tetracycline	4–16	8	16
	Ciprofloxacin	1–8	2	4
M. catarrhalis (21)	DMG-MINO	0.06–0.12	0.06	0.12
	DMG-DMDOT	0.06–0.25	0.12	0.25
	MINO	0.03–0.12	0.06	0.06
	Tetracycline	0.06–0.5	0.25	0.5
	Ciprofloxacin	≤0.008–0.03	0.015	0.015
N. gonorrhoeae (32)	DMG-MINO	0.25–1	0.5	1
	DMG-DMDOT	0.5–2	0.5	1
	MINO	0.25–64	0.5	64
	Tetracycline	0.5–>64	2	64
	Ciprofloxacin[b]	≤0.008	≤0.008	≤0.008
H. influenzae (15)	DMG-MINO	0.25–0.5	0.25	0.5
	DMG-DMDOT	0.25–0.5	0.5	0.5
	MINO	0.12–0.25	0.12	0.25
	Tetracycline	0.12–8	0.25	0.5
	Ciprofloxacin	≤0.004–0.03	≤0.015	≤0.03

[a] 50% and 90%, MIC_{50} and MIC_{90}.
[b] Only 14 isolates tested.

aureus containing *tet*(M), the most prevalent resistance gene, as well as an *E. coli* isolate in which *tet*(M) was inserted. Rasmussen et al. (25) reported that the glycylcyclines show excellent inhibition of protein synthesis with cell-free ribosome studies from tetracycline-susceptible and *tet*(M)-containing strains.

The glycylcyclines and MINO were more active than vancomycin, erythromycin, and ciprofloxacin against both MRSA and methicillin-susceptible *S. aureus*. MINO, which is used to treat methicillin-resistant staphylococci fairly extensively in Japan (5) and more recently in several instances in the United States (10, 15, 36), is active against *tet*(K)- but not *tet*(M)-containing strains. The glycylcyclines had good activity against a large group of recent clinical staphylococcal isolates, including strains requiring increased MICs of both tetracycline and MINO. There is the potential for some of these strains to carry more than one of the tetracycline resistance determinants. Organisms carrying

TABLE 5. In vitro activities of DMG-MINO, DMG-DMDOT, and comparative antibiotics against anaerobic bacteria

Organism (no. of strains)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
B. fragilis (25)	DMG-MINO	0.06–2	0.25	0.5
	DMG-DMDOT	0.25–1	0.5	0.5
	MINO	≤0.008–16	4	8
	Tetracycline	0.25–>64	16	64
B. fragilis group (25)	DMG-MINO	0.06–2	0.25	0.5
	DMG-DMDOT	0.12–2	0.5	0.5
	MINO	≤0.008–8	1	8
	Tetracycline	0.25–32	2	32
Prevotella spp. (12)	DMG-MINO	0.03–1	0.5	1
	DMG-DMDOT	0.12–4	0.5	4
	MINO	0.03–32	8	16
	Tetracycline	0.25–64	32	64
Clostridium difficile (12)	DMG-MINO	0.03	0.03	0.03
	DMG-DMDOT	0.03–0.12	0.06	0.06
	MINO	0.015–16	0.03	4
	Tetracycline	0.12–32	0.25	32
Clostridium perfringens (16)	DMG-MINO	0.03–1	0.12	1
	DMG-DMDOT	0.03–2	0.12	2
	MINO	≤0.008–16	0.06	8
	Tetracycline	0.015–16	4	16
Clostridium spp. (10)	DMG-MINO	0.015–0.12	0.03	0.12
	DMG-DMDOT	0.03–0.25	0.06	0.12
	MINO	≤0.008–16	0.015	4
	Tetracycline	0.015–64	0.06	64
Gram-positive cocci (30)	DMG-MINO	0.015–0.25	0.06	0.25
	DMG-DMDOT	0.015–1	0.12	1
	MINO	0.25–32	4	8
	Tetracycline	1–>64	32	64

[a] 50% and 90%, MIC_{50} and MIC_{90}.

both efflux and ribosomal protection mechanisms have been described (3, 26, 37). The glycylcyclines exhibited good activity against other gram-positive bacteria, including various *Streptococcus* spp., *E. faecalis*, and *E. faecium*, which were less susceptible to tetracycline, MINO, and the other agents tested. Resistance determinants *tet*(M), *tet*(L), and *tet*(O) are widely distributed in these species (2, 16, 21, 37).

The majority of the *Enterobacteriaceae* were more susceptible to the glycylcyclines than to tetracycline and MINO. As noted by the MIC distribution and the large difference in the tetracycline MIC_{50} and MIC_{90}, resistance among many of these unselected, routine isolates is fairly prevalent. The DMG analogs showed especially good activities against *E. coli* and *Salmonella* and *Shigella* spp. compared with those of MINO and tetracycline.

Resistance to tetracycline among *N. gonorrhoeae* strains is widespread and is associated with the *tet*(M) determinant (22). All of the *N. gonorrhoeae* isolates in this study were susceptible to the glycylcyclines. Isolates of *M. catarrhalis* and *H. influenzae* were susceptible to all of the tetracyclines. *P. aeruginosa*, *P. cepacia*, *S. marcescens*, and some isolates of the *Proteus* group, which are inherently less susceptible to tetracycline and MINO, were also less susceptible to the glycylcyclines. Against a variety of gram-negative and gram-positive anaerobic species, the glycylcyclines had significantly improved activities (at least fourfold) compared with those of tetracycline and MINO.

The improved activity noted in vitro was also observed in vivo when the drugs were tested against acute lethal infections in mice. When dosed as a single intravenous injection, the glycylcyclines exhibited effectiveness comparable to that

TABLE 6. In vivo activities of single-dose DMG-MINO, DMG-DMDOT, and MINO against *S. aureus* Smith infection in mice challenged with 6.3×10^5 CFU

Antibiotic	Route	ED_{50} (mg/kg) (95% confidence limit)	MIC (μg/ml)
DMG-MINO	Intravenous	0.46 (0.37–0.57)	0.12
	Subcutaneous	0.55 (0.45–0.67)	
	Oral	14 (11–17)	
DMG-DMDOT	Intravenous	0.46 (0.37–0.57)	0.12
	Subcutaneous	0.60 (0.49–0.73)	
	Oral	18 (15–23)	
MINO	Intravenous	0.42 (0.34–0.52)	0.06
	Subcutaneous	0.61 (0.50–0.75)	
	Oral	0.89 (0.70–1.1)	

of MINO against infections with gram-positive or gram-negative MINO-susceptible bacteria, including an infection with an MRSA strain and an *S. aureus* strain carrying the *tet*(K) resistance determinant. Infections caused by *S. aureus* or *E. coli* carrying *tet*(M) or *E. coli* carrying the *tet*(B) resistance determinant were all more responsive to treatment with the glycylcyclines than to MINO, as was an infection caused by NEMC 87-30, a MINO-resistant *E. coli* clinical isolate. In general the in vivo results were reflective of the in vitro activity, except in the case of the infection with the *S. aureus* strain harboring the *tet*(K) determinant or the infection with a methicillin-resistant staphylococcus strain, in which the ED_{50}s obtained with the glycylcyclines were comparable to those of MINO even though MINO showed better in vitro activity against these strains.

Similar efficacy was noted against an infection caused by a tetracycline-susceptible *S. aureus* strain when the mice were treated by the intravenous or the subcutaneous route. However, when dosed orally, the glycylcyclines exhibited poor efficacy compared with that noted by the intravenous route. MINO exhibited good efficacy by both the oral and subcutaneous routes.

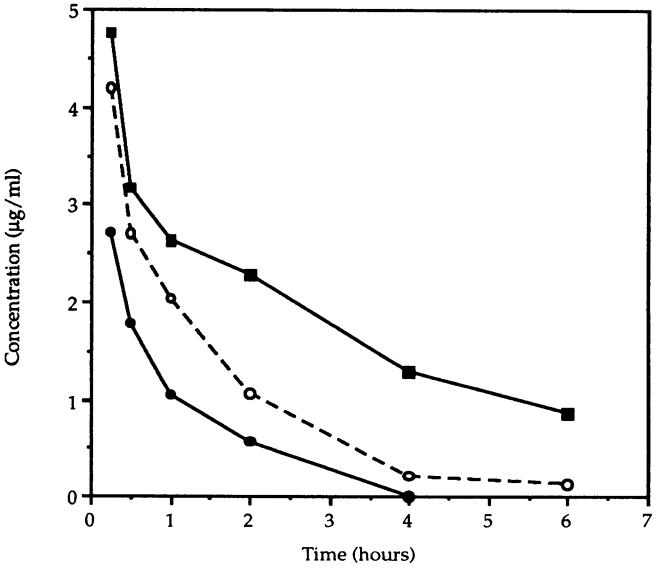

FIG. 2. Levels of DMG-MINO (●), DMG-DMDOT (■), and MINO (○) in mouse serum after a 10-mg/kg intravenous dose.

TABLE 7. In vivo activities of DMG-MINO, DMG-DMDOT, and MINO against experimental infections in mice

Strain (resistance)	Challenge dose (CFU/mouse)	Antibiotic[a]	ED_{50} (mg/kg) (95% confidence limit)	MIC (μg/ml)
S. aureus UBMS 90-2 [*tet*(M) resistance]	1.4×10^8	MG-MINO	0.56 (0.44–0.71)	0.12
		DMG-DMDOT	0.53 (0.42–0.68)	0.12
		MINO	2.2 (1.7–2.8)	2
S. aureus 649(pUB111) [*tet*(K) resistance]	4.1×10^7	DMG-MINO	1.5 (1.1–2.0)	2
		DMG-DMDOT	1.4 (0.98–1.9)	2
		MINO	1.1 (0.77–1.5)	0.25
MRSA NEMC 89-4 (MINO susceptible)	3.5×10^7	DMG-MINO	0.31 (0.23–0.40)	0.25
		DMG-DMDOT	0.23 (0.18–0.30)	0.25
		MINO	0.15 (0.11–0.20)	0.12
MRSA 2371 (MINO resistant)	1.2×10^8	DMG-MINO	2.7 (2.1–3.4)	2
		DMG-DMDOT	2.9 (2.2–3.6)	1
		MINO	19 (15–24)	8
MRSA 2794 (MINO susceptible)	1.5×10^8	DMG-MINO	3.1 (2.1–4.7)	4
		DMG-DMDOT	4.5 (3.0–6.8)	4
		MINO	6.0 (3.9–9.5)	1
E. coli UBMS 90-4 [*tet*(M) resistance]	4.4×10^7	DMG-MINO	2.5 (1.9–3.2)	0.12
		DMG-DMDOT	1.6 (1.3–2.1)	0.12
		MINO	>32	>32
E. coli JC3232 Tc^{rb} [*tet*(B) resistance]	4.2×10^7	DMG-MINO	4.5 (3.5–5.6)	0.25
		DMG-DMDOT	2.4 (1.9–3.0)	0.25
		MINO	>32	16
E. coli NEMC 87-30 (MINO resistant)	8.9×10^5	DMG-MINO	2.4 (2.0–2.8)	0.5
		DMG-DMDOT	2.0 (1.6–2.4)	0.5
		MINO	>32	32
E. coli 311 (MINO susceptible)	2.2×10^6	DMG-MINO	2.6 (2.0–3.3)	0.25
		DMG-DMDOT	1.6 (1.2–2.0)	0.25
		MINO	2.5 (2.0–3.2)	0.25

[a] Given as a single intravenous dose 30 min after infection.
[b] Second dose given 3 h after infection.

Serum drug levels in mice dosed intravenously with 10 mg/kg were higher with DMG-DMDOT and remained higher over the 6-h period of the experiment than levels of DMG-MINO or MINO. Additional studies are under way to assess whether this result is associated with differences in levels in tissue.

Thus, the glycylcyclines are a significant advance to the tetracycline class of antibiotics. The in vitro and in vivo activities of DMG-MINO and DMG-DMDOT against organisms carrying the predominant tetracycline resistance mechanisms overcome a major restriction in the use of tetracyclines. The antibacterial spectrum of the glycylcyclines is broad and includes most gram-positive and gram-negative aerobic and anaerobic organisms. Their potent in vitro and in vivo activities against the highly resistant pathogens, for which adequate therapy is currently limited, make the glycylcyclines a potential therapeutic alternative. Clinical studies evaluating their efficacies for treatment of various infections are warranted.

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REFERENCES

1. **Anhalt, J. P.** 1985. Antimicrobial assays, p. 695–709. *In* J. A. Washington II (ed.), Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York.
2. **Bentorcha, F., G. de Cespedes, and T. Horaud.** 1991. Tetracycline resistance heterogeneity in *Enterococcus faecium*. Antimicrob. Agents Chemother. **35:**808–812.
3. **Bismuth, R., R. Zilhao, H. Sakamolo, J. L. Guesdon, and P. Courvalin.** 1990. Gene heterogeneity for tetracycline resistance in *Staphylococcus* spp. Antimicrob. Agents Chemother. **34:** 1611–1614.
4. **Burdett, V.** 1986. Streptococcal tetracycline resistance mediated at the level of protein synthesis. J. Bacteriol. **165:**564–569.
5. **Chimura, T.** 1991. A therapy for methicillin-resistant *Staphylococcus aureus* (MRSA) infections in obstetrics and gynecology. 1991. Jpn. J. Antibiot. **44:**854–859.
6. **Chopra, I., P. M. Bennett, and R. W. Lacey.** 1973. A variety of staphylococcal plasmids present as multiple copies. J. Gen. Microbiol. **79:**343–345.
7. **Chopra, I., P. M. Hawkey, and M. Hilton.** 1992. Tetracyclines, molecular and clinical aspects. J. Antimicrob. Chemother. **29:** 245–277.
8. **Chopra, I., S. Shales, and P. Ball.** 1982. Tetracycline resistant determinants from groups A to D vary in their ability to confer decreased accumulation of tetracycline derivatives by *Escherichia coli*. J. Gen. Microbiol. **128:**689–692.
9. **Cleeland, R., and E. Squires.** 1991. Evaluation of new antimicrobials in vitro and in experimental animal infections, p. 752–783. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 3rd ed. The Williams & Wilkins Co., Baltimore.
10. **Darouiche, R., C. Wright, R. Hamill, M. Koza, D. Lewis, and J. Markowski.** 1991. Eradication of colonization by methicillin-resistant *Staphylococcus aureus* by using oral minocycline-rifampin and topical mupirocin. Antimicrob. Agents Chemother. **35:**1612–1615.
11. **Duggar, B. M.** 1948. Aureomycin: a product of the continuing search for new antibiotics. Ann. N.Y. Acad. Sci. **51**(1)**:**177–181.
12. **Finland, M.** 1974. Twenty-fifth anniversary of the discovery of aureomycin: the place of the tetracyclines in antimicrobial therapy. Clin. Pharmacol. Ther. **15**(1)**:**3–8.
13. **Finney, D. J.** 1971. Probit analysis, 3rd ed. Cambridge University Press, London.

14. **Goldman, R. F., T. Hasan, C. C. Hall, W. A. Strycharz, and B. S. Cooperman.** 1983. Photoincorporation of tetracycline into *E. coli* ribosomes. Identification of the major proteins photolabelled by native tetracycline and tetracycline photoproducts and implication for the inhibitory action of tetracycline on protein synthesis. Biochemistry **22**:359–368.
15. **Lawlor, M. T., M. C. Sullivan, R. E. Levitz, R. Quintillianni, and C. Nightingale.** 1990. Treatment of prosthatic valve endocarditis due to methicillin resistant *S. aureus* with minocycline. J. Infect. Dis. **161**:812–814. (Correspondence.)
16. **LeBlanc, D. J., L. N. Lee, B. M. Titmas, C. J. Smith, and F. C. Tenover.** 1988. Nucleotide sequence analysis of tetracycline resistance gene *tetO* from *Streptococcus mutans* DL5. J. Bacteriol. **170**:3618–3626.
17. **Levy, S. B.** 1984. Resistance to the tetracyclines, p. 191–240. *In* L. E. Bryan (ed.), Antimicrobial drug resistance. Academic Press, Inc., New York.
18. **Levy, S. B.** 1989. Evolution and spread of tetracycline resistance determinants. J. Antimicrob. Chemother. **24**:1–3.
19. **Levy, S. B., L. M. McMurry, V. Burdett, P. Courvalin, W. Hillen, M. C. Roberts, and D. E. Taylor.** 1989. Nomenclature for tetracycline resistance determinants. Antimicrob. Agents Chemother. **33**:1373–1374.
20. **Martell, M. J., and J. M. Boothe.** 1967. The 6-deoxytetracycline. VII. Alkylated aminotetracyclines possessing unique antibacterial activity. J. Med. Chem. **10**:359–363.
21. **McMurry, L. M., B. H. Park, V. Burdett, and S. B. Levy.** 1987. Energy-dependent efflux mediated by class L (TetL) tetracycline resistance determinant from streptococci. Antimicrob. Agents Chemother. **31**:1648–1650.
22. **Morse, S. A., S. R. Johnson, J. W. Biddle, and M. C. Roberts.** 1986. High-level tetracycline resistance in *Neisseria gonorrhoeae* is result of acquisition of streptococcal *tetM* determinant. Antimicrob. Agents Chemother. **30**:664–670.
23. **National Committee for Clinical Laboratory Standards.** 1991. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
24. **National Committee for Clinical Laboratory Standards.** 1991. Reference agar dilution procedure for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
25. **Rasmussen, B., Y. Glutzman, and F. P. Tally.** Personal communication.
26. **Roberts, M. C., and S. L. Hillier.** 1990. Genetic basis of tetracycline resistance in urogenital bacteria. Antimicrob. Agents Chemother. **34**:261–264.
27. **Rogalski, W.** 1985. Chemical modification of the tetracyclines, p. 179–316. *In* J. J. Hlavka and J. H. Boothe (ed.), The tetracyclines, handbook of experimental pharmacology, vol. 78. Springer-Verlag, New York.
28. **Salyers, A. A., B. S. Spear, and N. G. Shoemaker.** 1990. New perspectives on tetracycline resistance. Mol. Microbiol. **4**:151–156.
29. **Shales, S. W., I. Chopra, and P. R. Ball.** 1980. Evidence of more than one mechanism of plasmid-determined tetracycline resistance in *Escherichia coli*. J. Gen. Microbiol. **121**:221–229.
30. **Speer, B. S., N. B. Shoemaker, and A. A. Salyers.** 1992. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. Clin. Microbiol. Rev. **5**:387–399.
31. **Stratton, C. W., and R. C. Cooksey.** 1991. Susceptibility tests: special tests, p. 1153–1165. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
32. **Sum, P. E., V. J. Lee, R. T. Testa, J. J. Hlavka, G. A. Ellestad, J. D. Bloom, Y. Gluzman, and F. P. Tally.** Glycylcyclines. I. A new generation of potent antibacterial agents through modification of 9-aminotetracyclines. Submitted for publication.
33. **Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold.** 1985. Wadsworth anaerobic bacteriology manual, 4th ed. Star Publishers Co., Belmont, Calif.
34. **Uttley, A. H. C., C. H. Collins, J. Naidoo, and K. C. George.** 1988. Vancomycin resistant enterococci. Lancet **i**:57–58. (Letter.)
35. **Valcavi, U., A. Brandt, G. B. Corsi, F. Minoja, and G. Pascussi.** 1981. Chemical modifications in the tetracycline series. J. Antibiot. **34**:34–39.
36. **Yuk, J. H., M. C. Dignani, R. L. Harris, W. Bradshaw, and T. W. Williams, Jr.** 1991. Minocycline as an alternative antistaphylococcal agent. Rev. Infect. Dis. **13**:1023–1024. (Letter.)
37. **Zilhao, R., B. Papadopoulou, and P. Courvalin.** 1988. Occurrence of the *Campylobacter* resistance gene *tetO* in *Enterococcus* and *Streptococcus* spp. Antimicrob. Agents Chemother. **32**:1793–1796.