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In Vitro Antimicrobial Activity of a New Antibiotic, MDL 62,879 (GE2270 A)

BETH P. GOLDSTEIN,* MARISA BERTI, FRANCA RIPAMONTI, ANNA RESCONI, ROBERTO SCOTTI, AND MAURIZIO DENARO

Lepetit Research Center, Marion Merrell Dow Research Institute, Via R. Lepetit 34, 21040 Gerenzano (Varese), Italy

Received 21 October 1992/Accepted 28 January 1993

MDL 62,879 (GE2270 A) is a new peptide antibiotic that inhibits protein synthesis through an interaction with elongation factor Tu. MDL 62,879 was very active against gram-positive clinical isolates, particularly staphylococci and enterococci, for which MICs for 90% of isolates were ≤0.13 μg/ml. It was equally active against isolates resistant to β-lactams, erythromycin, gentamicin, and glycopeptides. It also had activity against *Mycobacterium tuberculosis*. MDL 62,879 had moderate bactericidal activity against staphylococci.

The incidence of infections caused by gram-positive bacteria has been increasing during the past decade (16). Staphylococci and enterococci are troublesome nosocomial pathogens among which multiple antibiotic resistance is becoming more frequent (4, 9, 13, 15). Most recently, glycopeptide resistance has appeared in enterococci. This means that there is no longer any class of antimicrobial agents to which the major gram-positive pathogens are uniformly susceptible. Novel antibiotics are needed to have alternatives for the future.

The discovery of a new antibacterial agent, MDL 62,879 (formerly GE2270 A), which inhibits protein synthesis by interacting with protein synthesis elongation factor Tu (EF-Tu), was recently reported (1, 6, 14). None of the antibacterial agents used for the therapy of human infections has this mechanism of action, although the kirromycins, a class of EF-Tu binding antibiotics with a different chemical structure, are used in animals, mainly for treating swine dysentery (2). Preliminary studies showed that MDL 62,879 was active against gram-positive bacteria and that it was efficacious in curing *Staphylococcus aureus* septicemia in mice (14). We present additional data on the antimicrobial activity of MDL 62,879.

MATERIALS AND METHODS

Antimicrobial agents. MDL 62,879, teicoplanin, and rifampin were obtained from Lepetit. We also used ampicillin (Amplital; Carlo Erba), gentamicin (Gentalyn 80; Schering), vancomycin (Vancocin; lyophilized vials; Eli Lilly), clindamycin (Sigma), and erythromycin (Sigma). For experiments with mycobacteria, we used a stock solution of 10 mg of MDL 62,879 per ml in 0.01 M sodium phosphate-buffered saline (pH 7.4) containing 20% (vol/vol) Cremophor RH40 (BASF). For all other experiments, a 10-mg/ml solution of MDL 62,879 was prepared with dimethylformamide and diluted in water. Erythromycin and rifampin were dissolved in dimethylformamide at 10 mg/ml and further diluted in water. The other antibiotics were dissolved in water.

Bacterial isolates. Clinical isolates were used for all experiments. Staphylococcal species were identified by use of the API ID 32 Staph system; streptococcal and enterococcal species were identified by use of the API 32 Strep system. The staphylococci were chosen to include methicillin-resistant isolates (9 *S. aureus*, 12 *S. epidermidis*, 11 *S. haemolyticus*, and 6 other coagulase-negative staphylococci) and isolates with reduced susceptibility to teicoplanin (MIC, ≥16 μg/ml) (one *S. epidermidis*, eight *S. haemolyticus*, and six other coagulase-negative staphylococci). The enterococci included isolates resistant to vancomycin and/or highly resistant to gentamicin (see Table 1).

MIC determinations. With the exception of MICs for *Propionibacterium acnes* and mycobacteria, MICs were determined by broth microdilution (11) with 0.01% (wt/vol) bovine serum albumin (BSA) (fraction V; Sigma). BSA was used because preliminary experiments, done in the absence of BSA, produced variable MICs that appeared to be due to the adherence of MDL 62,879 to plastic surfaces. Such a situation could also be corrected by precoating of the microtiter wells with dilute BSA solution, but the addition of BSA to the diluent is more convenient. The MICs of the comparison antibiotics were unaffected by the addition of BSA. Streptococci were grown in Todd-Hewitt broth (Difco); staphylococci and enterococci were grown in Iso-Sensitest broth (Oxoid). Inocula were approximately 5×10^5 CFU/ml from overnight plates (11); incubation was done at 37°C for 24 h. The agar dilution technique was used for *P. acnes* and mycobacteria. For *P. acnes*, Wilkins-Chalgren agar (Oxoid) was inoculated with 10^5 CFU from overnight plates (12); incubation was done at 37°C for 48 h in N_2-CO_2-H_2 (80:10:10). Mycobacteria were tested in Difco Middlebrook 7H10 medium plus Difco OADC enrichment. Inocula were approximately 10^5 CFU from 16-day-old cultures in Difco Middlebrook 7H9 medium plus Difco ADC enrichment; incubation was done at 35°C for 14 days.

Bactericidal activity. A few colonies from an overnight plate were inoculated into cation-adjusted Difco Mueller-Hinton broth (CAMHB) and grown until the culture was turbid (10). The culture was then diluted to a density of about 10^6 CFU/ml in CAMHB, and 10-ml portions were distributed into flasks without antibiotic or with 0.25, 1, or 4 μg of MDL 62,879 per ml. Incubation was done at 37°C in a shaking water bath. At intervals, samples were removed and suitably diluted (at least 10-fold) in saline plus 0.1% peptone. Duplicate 0.1-ml samples were plated by inclusion in 2.5 ml of Todd-Hewitt soft agar (0.7% agar) on Todd-Hewitt agar plates. In our experience, this procedure is sufficient to prevent carryover effects at the concentrations used. At the

* Corresponding author.

TABLE 1. In vitro activity of MDL 62,879

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
S. aureus (15)	MDL 62,879	0.03–0.06	0.06	0.06
	Teicoplanin	0.13–8	0.25	2
	Vancomycin	0.5–2	1	2
S. epidermidis (15)	MDL 62,879	0.06–0.13	0.06	0.13
	Teicoplanin	0.5–32	8	8
	Vancomycin	1–4	2	2
S. haemolyticus (15)	MDL 62,879	0.06–0.13	0.06	0.13
	Teicoplanin	1–32	16	32
	Vancomycin	1–4	2	2
Other coagulase-negative staphylococci (26)[b]	MDL 62,879	0.016–0.13	0.06	0.13
	Teicoplanin	0.13–32	1	32
	Vancomycin	0.5–4	1	2
Enterococcus spp., vancomycin susceptible (28)[c]	MDL 62,879	0.008–1	0.016	0.06
	Ampicillin	0.25–32	1	16
	Teicoplanin	0.13–0.25	0.13	0.25
	Vancomycin	0.5–8	1	2
Enterococcus spp., vancomycin resistant (14)[d]	MDL 62,879	0.008–0.03	0.016	0.03
	Ampicillin	0.13–64	2	64
	Teicoplanin	0.13–64	32	64
	Vancomycin	>128		
Streptococcus pyogenes (14)	MDL 62,879	0.13–0.5	0.25	0.5
	Ampicillin	0.008–0.03	0.016	0.016
	Erythromycin	0.016–0.06	0.03	0.03
	Teicoplanin	0.016–0.06	0.03	0.03
	Vancomycin	0.25		
S. pneumoniae (16)	MDL 62,879	0.06–0.13	0.13	0.13
	Ampicillin	0.008–2	0.03	0.06
	Erythromycin	0.008–>128	0.03	>128
	Teicoplanin	0.016–0.06	0.03	0.06
	Vancomycin	0.25–0.5	0.25	0.5
Other streptococci (22)[e]	MDL 62,879	0.06–2	0.25	1
	Ampicillin	0.03–0.25	0.06	0.13
	Erythromycin	0.008–>128	0.016	>128
	Teicoplanin	0.016–0.25	0.03	0.13
	Vancomycin	0.25–1	0.5	0.5
P. acnes (10)	MDL 62,879	≤0.008		
	Clindamycin	≤0.008–0.06	0.03	0.06
M. tuberculosis (10)	MDL 62,879	1–8	4	8
	Rifampin	0.03–0.06	0.06	0.06
M. avium complex (10)	MDL 62,879	2–>128	>128	

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
[b] Seven *S. simulans*, five *S. hominis*, four *S. saprophyticus*, two each of *S. capitis*, *S. cohnii*, *S. sciuri*, and *S. warneri*, and one *S. lugdunensis*.
[c] Thirteen *E. faecalis* and 15 *E. faecium*. One *E. faecalis* isolate was highly resistant to gentamicin.
[d] Four *E. faecalis* and 10 *E. faecium*. Two *E. faecalis* isolates and one *E. faecium* isolate were highly resistant to gentamicin.
[e] Six *S. sanguis*, four *S. agalactiae*, three *S. bovis*, three *S. mutans*, three *S. salivarius*, two *S. acidominimus*, and one *S. mitis*.

inocula used, 99.9% killing would result in 5 to 10 colonies per plate at the 10-fold dilution. Each experiment was performed twice.

RESULTS AND DISCUSSION

On the basis of MIC determinations, MDL 62,879 had excellent in vitro activity against staphylococci, streptococci, and enterococci (Table 1). For 112 of the 113 isolates of staphylococci and enterococci, which included methicillin- and teicoplanin-resistant staphylococci and ampicillin-, gentamicin-, and vancomycin-resistant enterococci, the MICs of MDL 62,879 were ≤0.13 μg/ml; the exception was an *Enterococcus faecalis* isolate for which the MIC of MDL 62,879 was 1 μg/ml. Against these organisms, MDL 62,879 was more active than the other drugs tested. The activity of

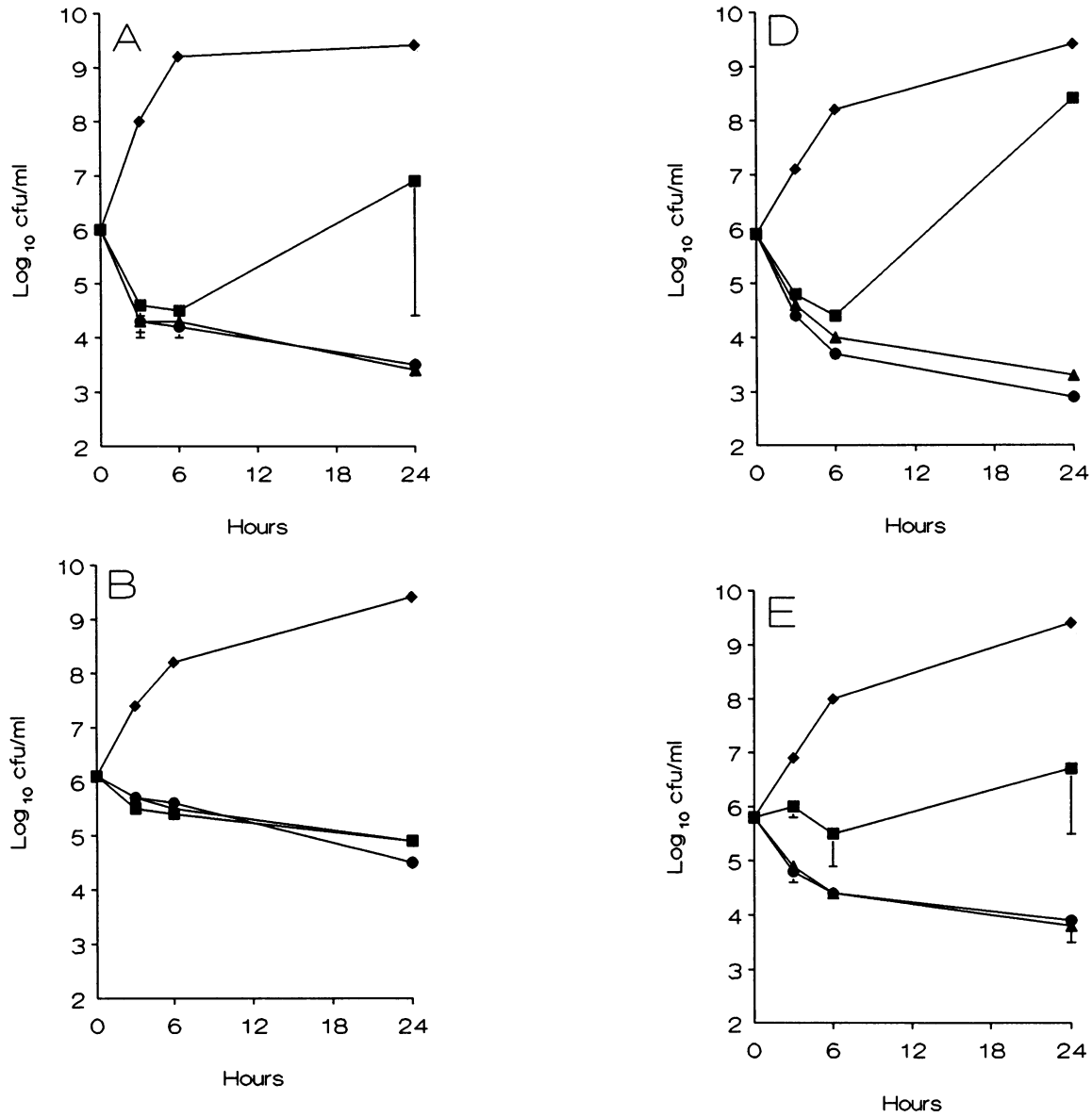
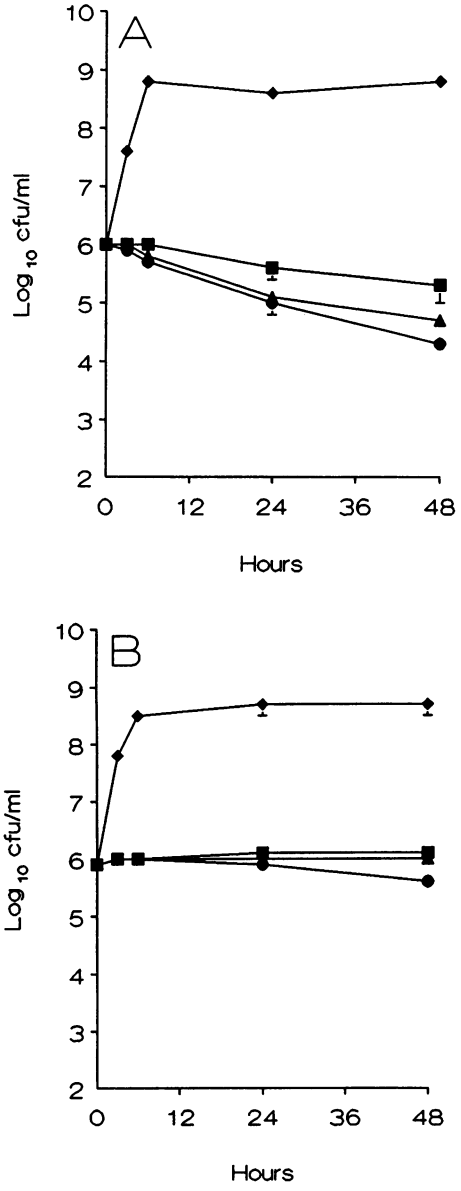


FIG. 1. Bactericidal activity of MDL 62,879 at 0.25 (■), 1 (▲), and 4 (●) μg/ml against *S. aureus* L1524 (A), *S. aureus* L1515 (B), *S. epidermidis* L1480 (C), *S. epidermidis* L1732 (D), and *S. epidermidis* L423 (E). ◆, untreated control. Except in panel D, each point is the mean count for two separate experiments; the bars indicate standard errors. Panel D shows the results of one of two experiments; in the second experiment, a population resistant to MDL 62,879 emerged after 24 h of contact with 0.25 and 1 μg/ml.

MDL 62,879 against streptococci was similar to that of vancomycin and somewhat lower than that of the other drugs tested; ampicillin had significantly lower MICs for some isolates. Several isolates of gram-positive cocci were tested additionally in CAMHB supplemented with blood or serum for the streptococci. The activity of MDL 62,879 in this medium was similar to that in Iso-Sensitest or Todd-Hewitt broth; the same was true for the reference compounds (data not shown). King et al. also reported that MDL 62,879 was highly active against gram-positive species, particularly enterococci (5).



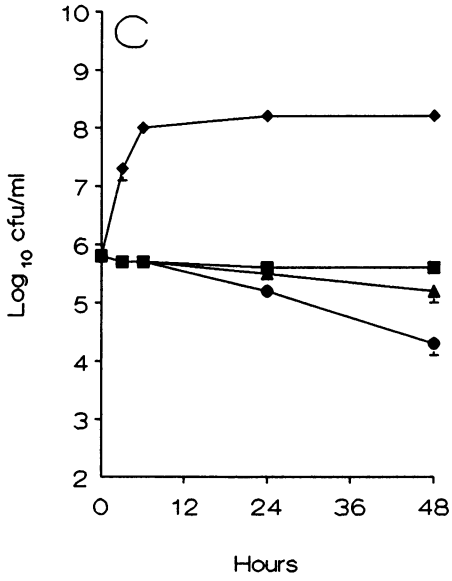


FIG. 2. Bactericidal activity of MDL 62,879 at 0.25 (■), 1 (▲), and 4 (●) μg/ml against *E. faecalis* L949 (A), *E. faecalis* L1705 (B), and *E. faecium* L1685 (C). ◆, untreated control. Each point is the mean count for two separate experiments; the bars indicate standard errors.

MDL 62,879 was also active against all isolates of *Mycobacterium tuberculosis* tested and a few *M. avium* complex isolates but at concentrations at least 10 times higher than those which inhibited other gram-positive bacteria. For 10 *P. acnes* isolates, the MICs of MDL 62,879 were ≤0.008 μg/ml, lower than those of clindamycin for most of the isolates (Table 1).

MDL 62,879 had moderate bactericidal activity against five isolates of staphylococci at concentrations of 1 and 4 μg/ml (Fig. 1). The two *S. aureus* isolates tested were killed to the extents of 98 and >99% within 24 h of contact with 4 μg/ml. The bactericidal activity of MDL 62,879 appeared to be somewhat stronger, and in some cases more rapid, against *S. epidermidis* isolates; for the three isolates tested, 24 h of contact with 4 μg/ml killed 99 to >99.9% of the inocula. In most cases, the two experiments with each isolate yielded very similar results; Fig. 1A, B, C, and E shows the mean bacterial titers. A single experiment is shown for *S. epidermidis* L1732 (Fig. 1D); in the second experiment (data not shown), bacteria resistant to MDL 62,879 (MIC, >128 μg/ml) emerged in cultures containing 0.25 and 1 μg/ml. Resistance was also observed in both experiments with *S. epidermidis* L1480 (Fig. 1C), but only at 0.25 μg/ml. None of the remaining 26 cultures of staphylococci exposed to MDL 62,879 in the time-kill experiments contained resistant organisms. Nor did we observe skipped wells in the MIC determinations with any isolate.

The conditions used in the time-kill experiments were apparently different enough from those used in the MIC determinations to allow some growth of staphylococci to occur at 0.25 μg/ml (four times the broth microdilution MIC), with the consequent emergence of resistance in 3 of the 10 cultures. Previous investigations indicated that one-step resistance to MDL 62,879 occurred in *Bacillus subtilis* and that it was mediated by an alteration of EF-Tu (6). It was therefore of interest to determine the rate of emergence of resistance. Using the Luria-Delbrück fluctuation test (8), we found the rates of mutation to MDL 62,879 resistance in *S. aureus* L165 (grown in Iso-Sensitest broth) to be 7.4×10^{-10} per cell per generation at 1 μg/ml and 3.3×10^{-10} per cell per generation at 10 μg/ml. These rates were about 1/15 the rates for rifampin, determined with the same cultures. So far, we have not observed the emergence of resistance to MDL 62,879 in animal infection experiments, under conditions in which rifampin resistance has been observed (unpublished data), but this problem has yet to be investigated systematically.

MDL 62,879 had weak bactericidal activity against one of two *E. faecalis* isolates tested and against an *E. faecium* isolate, with 97 to 98% killing being observed after 48 h of contact with 4 μg/ml (Fig. 2A and C). Against the other *E. faecalis* isolate, MDL 62,879 was bacteriostatic (Fig. 2B). No emergence of resistance was observed in these experiments; however, the lowest concentration of MDL 62,879 used (0.25 μg/ml) was 16 times its broth microdilution MIC for the three enterococci.

In conclusion, MDL 62,879 had excellent activity against gram-positive bacteria, with MICs for 90% of isolates of ≤0.13 μg/ml for staphylococci and enterococci. As expected from its novel structure and mechanism of action, it was as active against isolates resistant to ampicillin, methicillin, erythromycin, glycopeptides, and gentamicin as against susceptible bacteria. MDL 62,879 had weak (enterococci) to moderate (staphylococci) bactericidal activity. Nevertheless, in preliminary experiments, it had excellent activity against staphylococcal and streptococcal septicemia in mice and against staphylococcal and enterococcal endocarditis in rats (3). The antienterococcal activity of MDL 62,879 is of particular interest, given the increasingly limited therapeutic options available for some of these organisms. Although cell-free protein synthesis studies have demonstrated that, in contrast to the binding sites of other EF-Tu inhibitors, the EF-Tu binding site of MDL 62,879 is highly conserved among eubacteria (7), we were able to select for resistance to MDL 62,879 in staphylococci in vitro. Whether resistant mutants are at a selective disadvantage in the environment merits investigation. MDL 62,879 may be a promising antibiotic for combatting infections caused by gram-positive bacteria, particularly multiple-drug-resistant isolates.

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