IN VITRO AND *IN VIVO* EVALUATION OF MDL 19,592, AN ORAL CEPHALOSPORIN

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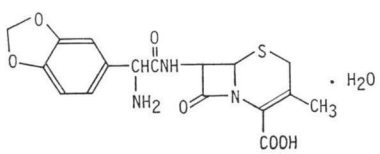
MDL 19,592 is a new semisynthetic cephalosporin with a good therapeutic potential against Gram-positive bacterial infections when administered orally or parenterally. In the oral treatment of benzylpenicillin-resistant *Staphylococcus aureus* infections in mice, MDL 19,592 was superior to cephalexin, cephradine, cefaclor, cefadroxil and cefroxadine. These *in vivo* results reflect the *in vitro* superiority expressed by MDL 19,592 over the other oral cephalosporins against staphylococci. Additionally, MDL 19,592 orally was superior to cefazolin and cephalothin administered subcutaneously and to a number of penicillinase-resistant penicillins given orally or subcutaneously in the treatment of *S. aureus* mouse infections. MDL 19,592 killed *S. aureus* cells at the same or faster rate than did cephalexin or cephradine. As compared to cephalexin, MDL 19,592 was marginally superior *in vitro* against *Streptococcus pyogenes* and *Streptococcus pneumoniae*. *In vivo*, MDL 19,592 was significantly the more effective of the two against *S. pyogenes* and marginally more effective against *S. pneumoniae*. Against Gram-negative organisms, with the exception of *Haemophilus influenzae*, cephalexin was the more potent of the two antibiotics both *in vitro* and *in vivo*. Administered orally to mice, MDL 19,592 was absorbed as rapidly as cephalexin with both drugs attaining similar concentrations in the blood. MDL 19,592, like cephalexin, was minimally bound by mouse serum.

As compared to the number of parenteral cephalosporins and cephalosporin-like antibiotics available or being developed for clinical use, the number of orally effective compounds is small. The older oral drugs, cephalexin and cephradine, are widely used, and in general are only marginally different from each other with respect to their *in vitro* and *in vivo* properties. The newer oral cephalosporins on the market, cefaclor and cefadroxil, have the same spectrum of activity as the older drugs, but cefaclor possesses potency superiority with respect to *Haemophilus influenzae*[1], while cefadroxil has the pharmacokinetic advantages of a longer serum half-life and a greater area under the serum level vs. time curve[2]. This paper describes the results of our studies of a semisynthetic cephalosporin, MDL 19,592 (Fig. 1), which was found to be effective after oral administration against experimental systemic mouse infections, in particular, those caused by staphylococci or streptococci. The results to be described indicated that MDL 19,592 had potential use in humans. Accordingly, MDL 19,592 was compared to cephalexin in a double-blind crossover study employing single oral doses of 100 to 1,000 mg[3]. The findings of that study were that the drug was well tolerated, its pharmacokinetics were essentially identical to those of cephalexin, and 86% of it was excreted in the urine. Subsequently, employing 8 subjects per group, MDL 19,592 was administered at dosage levels of 250, 500 or 1,000 mg orally per subject every 6 hours for 10 days, and at the 500 mg level, also for 7 days. During the study the 32 subjects

Fig. 1. Structure of MDL 19,592.



tolerated the drug as well as other subjects in the studies tolerated cephalexin, and while some pharmacokinetic parameters of MDL 19,592 and cephalexin were different, both serum clearance and percent recovery of drug in the urine were essentially the same for the two cephalosporins.[4] Two of the 8 subjects who had received 4 g of MDL 19,592 per day developed rashes after the tenth day of treatment.

Materials and Methods

Antibiotics

MDL 19,592 was prepared in our laboratories and is a white crystalline powder that is slightly soluble in water. Cephalexin, cefaclor, cephalothin, and cefazolin were obtained from Eli Lilly and Co.; methicillin, oxacillin, cloxacillin, and dicloxacillin from Bristol Laboratories; cephradine from E. R. Squibb & Sons, Inc.; cefroxadine (CGP 9000) from Ciba-Geigy Corp.; cefadroxil from Mead Johnson Laboratories; and nafcillin from Wyeth Laboratories.

Antibiotic Spectrum

All bacterial strains were clinical isolates. Minimal inhibitory concentrations (MICs) were determined by diluting antibiotics in a suitable agar medium and inoculating the agar surface with 0.003 ml of diluted culture using the multiple inoculator of STEERS *et al.*[5]. Serial twofold dilutions of antibiotic were used for all MIC determinations except where streptococci were concerned. For those organisms, the incremental difference in concentrations employed in a series was 0.1 μg/ml. For routine MIC determinations, all cultures except *Streptococcus pneumoniae, Streptococcus pyogenes, Neisseria gonorrhoeae, H. influenzae* and anaerobes were grown for 18 hours in Trypticase soy broth (TSB; Difco) and diluted in Mueller-Hinton broth (MHB; BBL) to obtain approximately 2×10^5 colony-forming units (cfu) of staphylococci and 4×10^4 cfu of the other organisms as inocula. *S. pneumoniae* and *S. pyogenes* were grown for 24 hours in brain heart infusion broth (BHIB; Difco) and diluted in BHIB to obtain about 2×10^6 cfu as inocula. *H. influenzae* cultures were grown 24 hours in BHIB containing 1% Supplement C (Difco) and diluted 1/100 in the same broth. Strains of *N. gonorrhoeae* were grown for 24 hours as spread plates on GC agar base (BBL) supplemented with 1% hemoglobin (BBL) and 1% Isovitalex (BBL). Surface growth from one plate suspended in 100 ml TSB served as inoculum. Anaerobes were grown for 48 hours in thioglycolate broth (BBL) and diluted 1/10 in MHB. MICs for *H. influenzae* and *N. gonorrhoeae* were determined using the supplemented GC agar described above. MICs for anaerobes were obtained using Mueller-Hinton agar (MHA; BBL) containing 1% Supplement C. MICs for all other cultures were determined using MHA. Plates containing *H. influenzae* and *N. gonorrhoeae* were incubated for 24 hours in an atmosphere of 6% carbon dioxide in air; those containing anaerobes were incubated for 48 hours in a Gas Pak 100 Anaerobic System (BBL); all other plates were incubated for 18 hours in air. All cultures were grown at 37°C, and after incubation, the lowest concentration of antibiotic allowing no or essentially no growth was considered to be the MIC.

Antibiotic Kill Rate

Staphylococcus aureus killing curves were obtained by inoculating antibiotic-containing MHB with 18-hour cultures. Samples taken were diluted and added to MHA to make pour plates. After 18 to 24 hours incubation at 37°C, the cfu were counted.

Susceptibility to *S. aureus* Penicillinases

Cultures were grown and induced their β-lactamases with methicillin according to the method of RICHMOND[6]. Cells were removed by centrifugation and ammonium sulfate added to the centrifugate until 60% saturation was achieved. The precipitate was collected by centrifugation, dissolved in a minimal amount of 0.1 M sodium acetate buffer, pH 5.9, and dialyzed against the same buffer overnight at 4°C. The dialysate was frozen and stored at −20°C until used. The concentrated enzyme preparation was diluted in 0.1 M sodium - potassium phosphate buffer, pH 7.0, so that when benzylpenicillin was added to saturate the enzyme, 2 to 3 μmol were hydrolyzed at a linear rate over 15 minutes at 37°C. The cephalosporins tested were added to the same enzyme preparations and the reaction mixtures incubated 3 hours. Samples were taken at prescribed intervals, fast frozen in acetone chilled with dry ice and

stored at −20°C until assayed. Samples to be assayed were thawed and kept in an ice bath until assayed by an agar diffusion method using antibiotic medium 2 (Difco) seeded with *Micrococcus luteus* ATCC 9341. Standard curves were prepared from zone of inhibition responses obtained with known concentrations of phosphate-buffered antibiotics applied to 6 mm discs. Responses obtained with samples diluted in buffer were referred to standard curves to determine antibiotic concentrations.

Treatment of Experimental Infections in Mice

Charles River albino CD-1 male mice weighing 20 ±1 g were infected by intraperitoneal injection of a bacterial suspension containing a sufficient number of organisms to produce uniformly lethal infections within 72 hours. The suspensions, prepared in BHIB from overnight cultures, were 0.1 to 1.0 ml in volume, depending upon the organism. Those of *Escherichia coli* and *S. aureus* strains M202, M238, and M258 contained 2.5% hog gastric mucin. Groups of ten mice each were treated orally or subcutaneously with appropriate concentrations of antibiotic (representing a series of two or four-fold dilutions) at 1 and 4 hours after infection. The number of mice in each group surviving the challenge for 4 days was recorded and the ED_{50} (the dose in mg/kg required to protect 50% of the infected mice) determined by BERKSON's minimum chi square method[7].

Antibiotic Levels in Mouse Serum

MDL 19,592 and cephalexin were administered in water *per os* to albino CD-1 male mice weighing 25 ±1 g. Four mice were bled from their orbital sinuses for each sampling time and sacrificed. The blood was pooled and the serum samples collected were bioassayed by the agar diffusion method using *M. luteus*. Samples were diluted in serum and assayed against standard curves plotted from results obtained with known concentrations of antibiotics added to serum.

Urinary Excretion of MDL 19,592

Urine samples were collected from five albino CD-1 male mice weighing 20 ±1 g 60 minutes after *per os* dosing with MDL 19,592 at 80 mg/kg; each mouse was dosed with antibiotic in 0.25 ml of water. Pooled urine samples from dosed and non-dosed mice were chromatographed on cellulose Chromogram sheets (Eastman 13254) previously impregnated with 0.1 M potassium citrate buffer, pH 4.5, and dried. The chromatograms were developed ascendingly with acetone - H_2O (3:1), examined under ultraviolet light, and then bioautographed on antibiotic medium #1 (Difco) seeded with *Bacillus subtilis* ATCC 6633.

Mouse Serum Binding

Twenty albino CD-1 male mice, each weighing 24 ±1 g were dosed with MDL 19,592 or cephalexin at 40 mg/kg and bled from the orbital sinuses at 30 to 40 minutes after dosing. The blood samples from each group were pooled and the serum collected. Additionally, the cephalosporins were added to mouse serum at a concentration of 25 μg/ml and the samples incubated for 30 minutes at 37°C. An aliquot of each sample was transferred to an Amicon Centriflo ultrafiltration membrane cone (2100 CF50) and the protein-free filtrate obtained by centrifugation for 20 minutes at 500×*g* at 4°C. The antibiotic concentrations in protein-free filtrates and sera were determined by bioassay using *M. luteus*. Serum samples were assayed as described above. Protein-free filtrates were diluted in 0.1 M potassium phosphate buffer, pH 7.4, and assayed against standard curves established from results obtained with antibiotics dissolved in phosphate buffer (protein-free filtrate had no effect on the response of *M. luteus* to these antibiotics).

Results and Discussion

Studies *In Vitro*

When compared to cephalexin against a selected group of organisms, MDL 19,592 was more potent for Gram-positive and cephalexin more potent for Gram-negative bacteria (Table 1). Limited testing against other members of the Enterobacteriaceae not shown found cephalexin to be the more potent of the two compounds wherever activity was observed; neither showed noteworthy activity against *Serratia*

marcescens, Proteus vulgaris or *Proteus inconstans*. Against *H. influenzae*, MDL 19,592 was essentially as effective as cephalexin. Both compounds had MICs of 12.5 μg/ml for 8 out of 11 ampicillin-sensitive and 10 out of 12 ampicillin-resistant strains. The other 5 strains were inhibited at 25 μg/ml or higher, depending upon the strain and the compound. With respect to other organisms, neither compound was effective or significantly so against strains of enterococci, *Pseudomonas aeruginosa, N. gonorrhoeae* or *Bacteroides fragilis*.

Table 1. Comparative *in vitro* activity of MDL 19,592 and cephalexin against selected Gram-positive and Gram-negative organisms.

Organism		MIC (μg/ml)	
		MDL 19,592	Cephalexin
Staphylococcus aureus	M240[a]	0.5	1.0
S. aureus	M238[b]	2	4
S. epidermidis	M111[b]	0.5	2
Streptococcus pneumoniae	D137	1.6	2
S. pyogenes	St139	0.4	0.6
Enterobacter cloacae	Ae10	>100	25
E. aerogenes	Ae17	>100	25
Escherichia coli	Es59	25	6.2
Klebsiella pneumoniae	K39	25	6.2
Proteus mirabilis	P6	>100	25
Salmonella schottmuelleri	Sa27	12.5	3.1

[a] Benzylpenicillin-sensitive.
[b] Benzylpenicillin-resistant.

Table 2. Effect of inoculum level of seven benzylpenicillin-resistant *S. aureus* isolates on the *in vitro* potency of MDL 19,592 and five reference cephalosporins.

Antibiotic	cfu as inoculum	MIC (μg/ml)							Geometric mean MIC	Potency superiority of MDL 19,592 over references[a]
		Strain								
		M237	M238	M247	M255	M258	M260	M262		
MDL 19,592	2×10^4	2	2	2	2	2	1	2	1.8	
	2×10^5	2	2	2	2	4	1	4	2.1	
	2×10^6	8	8	8	8	4	4	8	6.6	
Cephalexin	2×10^4	4	4	4	4	4	2	4	3.6	2
	2×10^5	8	4	8	4	8	4	8	6.0	2.9
	2×10^6	16	16	16	16	32	8	32	17.7	2.7
Cephradine	2×10^4	4	4	4	4	8	2	4	4.0	2.2
	2×10^5	8	4	8	4	8	4	8	6.0	2.9
	2×10^6	16	16	16	16	32	8	32	17.7	2.7
Cefadroxil	2×10^4	4	4	4	4	4	4	4	4	2.2
	2×10^5	8	4	8	8	8	8	8	7.3	3.5
	2×10^6	16	16	16	32	8	16	32	17.7	2.7
Cefroxadine	2×10^4	4	4	4	4	4	4	8	4.4	2.4
	2×10^5	16	32	8	16	32	8	16	16.0	7.6
	2×10^6	64	64	32	64	64	32	>64	50.8[b]	7.7
Cefaclor	2×10^4	8	4	4	8	8	4	8	5.9	3.3
	2×10^5	16	32	8	16	16	8	16	14.5	6.9
	2×10^6	>64	>64	32	>64	>64	>64	>64	NC[c]	NC

[a] Geometric mean MIC of reference cephalosporin ÷ geometric mean MIC of MDL 19,592.
[b] Excluding strain M262.
[c] NC: Not calculable.

The initial results obtained *in vitro* suggested that further investigations should be directed towards determining the potential of MDL 19,592 for the treatment of Gram-positive coccal infections, particularly those of staphylococcal origin. Seven representative benzylpenicillin-resistant strains of *S. aureus* were used in a study to determine the effect of inoculum size on the MICs of MDL 19,592 and other orally absorbed cephalosporins (Table 2). MDL 19,592 was two to three times more potent than cephalexin, cephradine, and cefadroxil at all three inoculum levels tested. As compared to cefroxadine and cefaclor, MDL 19,592 also was two to three times more potent at the lowest inoculum level, but at the two higher levels, it was more potent than the other two compounds by a factor equal to or greater than seven. The *in vivo* results described later illustrate the fact that MICs obtained using an inoculum of 2×10^4 cfu, a level that is recommended for agar dilution tests[5], are not predictive of *in vivo* efficacy in our studies. Results of additional studies using a larger number of resistant strains of *S. aureus*, including the seven shown in Table 2, confirmed the superiority of MDL 19,592 over the other orals tested (Table 3). Although not shown, this superiority was observed for each strain tested. The results obtained with cefaclor and cefroxadine suggest that they are relatively labile to *S. aureus* penicillinase. As shown in Table 4, MDL 19,592 was the most potent of the six compounds tested against representative benzylpenicillin-resistant strains of *Staphylococcus epidermidis* and sensitive strains of *S. aureus*. Against *S. pyogenes* and *S. pneumoniae*, MDL 19,592 was slightly more potent than cephalexin (Table 5).

Because MDL 19,592 was more potent *in vitro* than the other oral cephalosporins against staphylococci, it was of interest to compare it to cephalexin and cephradine (the next most potent compounds) to assess their respective abilities to kill cells of four penicillin-resistant strains of *S. aureus*. As shown in Fig. 2a, MDL 19,592 and cephalexin were more bactericidal than cephradine over the first 3 hours at 50 μg/ml and 6 hours at 20 μg/ml for strain M238. The rate of kill between 6 and 24 hours was greater at the lower concentration for all three compounds. When tested against strain M235, all compounds

Table 3. Comparative *in vitro* activity of MDL 19,592 and other oral cephalosporins against 22 benzylpenicillin-resistant isolates of *S. aureus*.

Comparison	Antibiotic	cfu as inoculum	No. of strains with MIC (μg/ml) of: 0.5	1	2	4	8	16	32	64	>64	Geometric mean MIC	Potency superiority of MDL 19,592 over references[a]
1	MDL 19,592	2×10^5	1	3	10	8						2.2	
		2×10^6		1	3	9	8	1				4.7	
	Cephalexin	2×10^5			1	10	9	2				5.8	2.6
		2×10^6				4	4	9	5			12.8	2.7
	Cephradine	2×10^5			4	6	11	1				5.3	2.4
		2×10^6			1	3	3	10	5			12.8	2.7
2	MDL 19,592	2×10^5		9	9	4						1.7	
		2×10^6		1	1	8	11	1				5.5	
	Cefadroxil	2×10^5			1	10	11					5.5	3.2
		2×10^6				1	7	12	2			12.8	2.3
	Cefroxadine	2×10^5			1	3	12	3	3			9.1	5.4
		2×10^6				3	3	4	7	4	1	19.5[b]	3.5
	Cefaclor	2×10^5			2	8	6	4	2			7.1	4.2
		2×10^6				2	4		5	2	9	NC[c]	NC

[a] Geometric mean MIC of reference cephalosporin ÷ geometric mean MIC of MDL 19,592.
[b] Based on MICs for 21 strains.
[c] NC: Not calculable.

Table 4 Comparative *in vitro* activity of MDL 19,592 and other oral cephalosporins against benzylpenicillin-resistant *S. epidermidis* and benzylpenicillin-sensitive *S. aureus*.

Antibiotic	cfu as inoculum	MIC (μg/ml)											
		S. epidermidis strains					Geometric mean MIC	*S. aureus* strains					Geometric mean MIC
		M108	M109	M111	M112	M113		M202	M204	M224	M240	M241	
MDL 19,592	2×10^5	2	8	0.5	0.5	0.5	1.2	0.5	0.5	2	1	0.5	0.76
	2×10^6	4	16	2	2	1	3	1	1	2	2	2	1.5
Cephalexin	2×10^5	4	16	2	2	2	3.5	2	1	4	2	2	2
	2×10^6	32	32	8	4	2	9.2	2	4	4	4	4	3.5
Cephradine	2×10^5	4	16	2	1	1	2.6	1	1	4	2	2	1.7
	2×10^6	16	32	8	4	2	8	2	4	4	4	4	3.5
Cefadroxil	2×10^5	4	8	1	2	2	2.6	2	2	4	2	2	2.3
	2×10^6	8	32	4	4	4	7	4	4	4	4	4	4
Cefroxadine	2×10^5	4	16	2	2	2	3.5	0.5	1	2	2	2	1.3
	2×10^6	16	16	4	4	2	6.1	1	2	4	2	2	2
Cefaclor	2×10^5	4	8	1	1	1	2	0.5	1	2	1	1	1
	2×10^6	16	32	2	2	1	4.6	1	1	4	2	2	1.7

Fig. 2a. Time-kill curve of MDL 19,592 (●), cephalexin (○), and cephradine (□) against benzylpenicillin-resistant *S. aureus* M238 at 20 μg/ml (---) and 50 μg/ml (—).

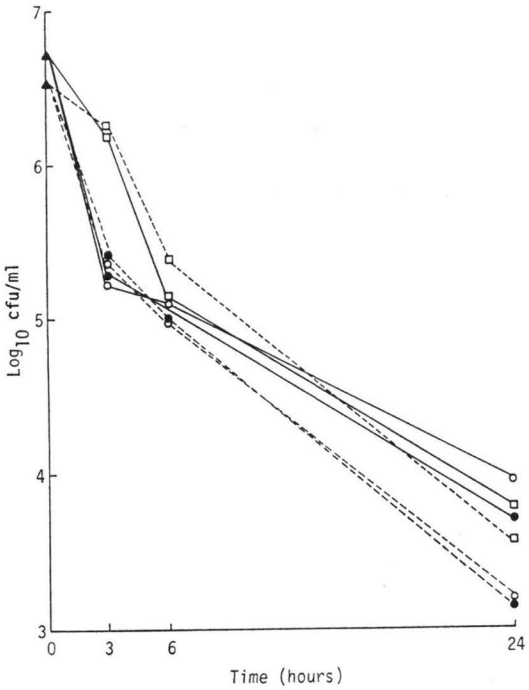


Fig. 2b. Time-kill curve of MDL 19,592 (●), cephalexin (○), and cephradine (□) against benzylpenicillin-resistant *S. aureus* M235 at 20 μg/ml (---) and 50 μg/ml (—).

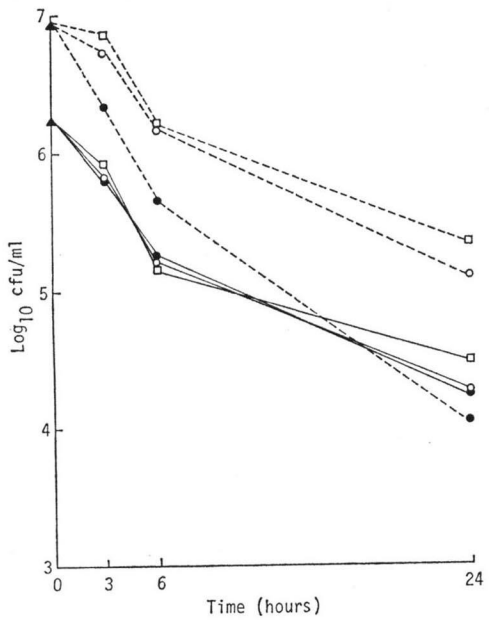


Fig. 2c. Time-kill curve of MDL 19,592 (●), cephalexin (○), and cephradine (□) against benzylpenicillin-resistant *S. aureus* M261 at 50 μg/ml.

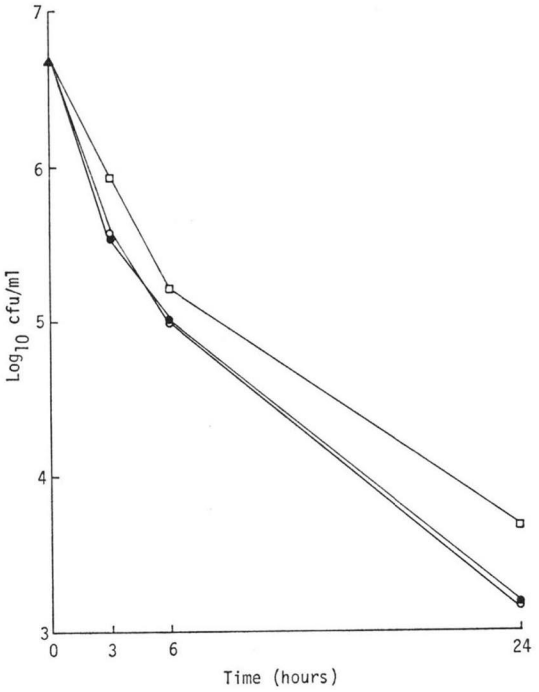
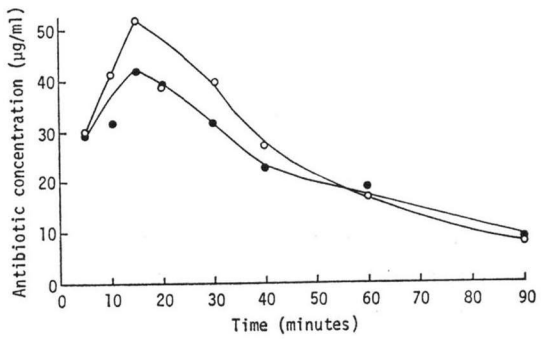


Fig. 3. Antibiotic concentration in mouse blood serum after oral administration of 40 mg/kg. Symbols: MDL 19,592 (○), cephalexin (●).



showed similar killing rates at 50 μg/ml; however, at 20 μg/ml only MDL 19,592 reduced the population at a much faster rate than it did at 50 μg/ml (Fig. 2b). The more rapid kill rates observed for strains M238 and M235 at the lower concentrations of cephalosporin may be a reflection of

Table 5. Comparative *in vitro* activity of MDL 19,592 and cephalexin against 15 strains of *S. pyogenes* and 11 strains of *S. pneumoniae*.

Antibiotic	Organism	No. of strains with MIC (μg/ml) of:									
		0.3	0.4	0.5	0.6	0.7	0.8	1.4	1.6	2.0	3.1
MDL 19,592	*S. pyogenes*	6	7	2							
Cephalexin			7	4	4						
MDL 19,592	*S. pneumoniae*		1			1	2	2	4		1
Cephalexin			1				1		4	1	4

Table 6. Comparative oral activity of MDL 19,592 and other cephalosporins against experimental *S. aureus* infections in mice.

Comparison	Strain	Challenge (cfu)	Compound	ED_{50} (mg/kg/dose)[a]	
				Test	
				1	2
1	M238[b]	6×10^7	MDL 19,592	3.1	9.8
			Cephalexin	8.9	29
			Cephradine	10	35
	M258[b]	1×10^8	MDL 19,592	16	16
			Cephalexin	51	41
			Cephradine	24	29
	M240[c]	4×10^8	MDL 19,592	0.6	0.26
			Cephalexin	2.6	0.75
	M202[c]	4×10^7	MDL 19,592	0.4	—
			Cephalexin	1.8	—
			Cephradine	1.0	—
2	M238	6×10^7	MDL 19,592	3.3	7.9
			Cefadroxil	5.9	13.2
			Cefroxadine	15.2	26.8
			Cefaclor	25	137
	M258	1×10^8	MDL 19,592	5.3	7.7
			Cefadroxil	22	19
			Cefroxadine	40	45
			Cefaclor	84	>160

[a] Compounds were dissolved or suspended in water and administered 1 and 4 hours after infection.
[b] Benzylpenicillin-resistant.
[c] Benzylpenicillin-sensitive.

the Eagle effect[9,10] in which a relatively low as opposed to a high concentration of a β-lactam compound kills at the faster rate. Against strain M261 at 50 μg/ml, MDL 19,592 and cephalexin killed at essentially an identical rate over 24 hours, a rate that was faster than that observed with cephradine (Fig. 2c). Of the strains tested, all three compounds at 50 μg/ml killed cells of strain M249 at the fastest rate, with all compounds killing at the same rate. An initial population of 3×10^5 cfu/ml was reduced to about 10^5 cfu/ml at 6 hours and about 10^8 cfu/ml at 24 hours. Thus, MDL 19,592 was as effective or more effective than cephalexin and cephradine in killing strains of resistant *S. aureus*. While cephradine was occasionally less effective than the other two compounds, it is not known whether this is significant.

As expected, MDL 19,592 was found to be quite resistant to *S. aureus* penicillinases. When the rate of hydrolysis of benzylpenicillin by penicillinases from four strains was assigned the value of 2,000,

the means of the four relative hydrolytic rates for MDL 19,592, cephradine, and cephalexin were 3, 4, and 10, respectively.

Studies *In Vivo*

As compared to cephalexin and cephradine, MDL 19,592 was the superior drug against *S. aureus* infections caused by benzylpenicillin-resistant and sensitive strains, being generally two to three times more effective than the other compounds (Table 6). In a second series of studies that only included the resistant strains, MDL 19,592 also was found to be superior to the newer orals, as well (Table 6). While cefadroxil was similar to cephalexin and cephradine in comparisons with MDL 19,592, cefaclor was clearly the least active of the drugs tested with cefroxadine being the next least active. Under our conditions, the *in vivo* results best reflect the results obtained *in vitro* using dense inocula (Tables 2 and 3), as mentioned previously.

Amongst the parenteral cephalosporins, cephalothin and cefazolin are commonly used against *S. aureus* infections in man. When administered orally or subcutaneously, MDL 19,592 was significantly superior to either compound against these infections in mice caused by resistant strains. It was more effective than cephalothin against one of the two infections caused by sensitive strains and equally effective as cefazolin against both of the sensitive strains (Table 7). The poor potency of cephalothin for strain M202 cannot be explained; similar results were obtained in other tests. As can be seen, the MIC data for these compounds were not predictive of *in vivo* efficacy. In these studies, this probably partially reflects differences in serum-binding, MDL 19,592 being minimally so (shown later); and in the case of cephalothin, its metabolism by the mouse is extensive[11,12].

Certain penicillins are often described as being the "drugs of first choice" for infections caused by benzylpenicillin-resistant *S. aureus*. When compared with these compounds *in vivo*, MDL 19,592 administered orally or subcutaneously was superior to nafcillin, dicloxacillin, oxacillin, and methicillin administered subcutaneously and/or orally (Table 8). The *in vitro* potency of nafcillin and the isoxazole penicillins was not predictive of *in vivo* efficacy in these studies either. This could, at least in part, reflect the high serum-binding of these β-lactam antibiotics.

With respect to other model infections, MDL 19,592 was superior to cephalexin *in vivo* against *S. pyogenes* and marginally superior against one of two *S. pneumoniae* model infections (Table 9). And,

Table 7. Comparative activity of MDL 19,592, cefazolin, and cephalothin against experimental *S. aureus* infections in mice.

Strain	Challenge (cfu)	ED_{50} (mg/kg/dose)[a]				MIC (μg/ml)[b]		
		MDL 19,592		Cefazolin	Cephalothin			
		po[c]	sc[c]	sc	sc	MDL 19,592	Cefazolin	Cephalothin
M238[d]	6×10^7	7.6	5.8	45	66	2	0.8	0.4
M258[d]	1×10^8	14.6	16.9	104	50	4	0.8	0.4
M240[e]	4×10^8	0.18	0.38	0.33	0.22	1	0.2	0.2
M202[e]	4×10^7	0.51	0.26	0.53	21	0.5	0.1	0.1

[a] Compounds were dissolved or suspended in water and administered 1 and 4 hours after infection.
[b] Determined with an inoculum of 2×10^5 cfu.
[c] po: Administered *per os*, sc: administered subcutaneously.
[d] Benzylpenicillin-resistant.
[e] Benzylpenicillin-sensitive.

Table 8. Comparative activity of MDL 19,592 and penicillinase-resistant penicillins against experimental *S. aureus* infections in mice.

Strain	Challenge (cfu)	ED_{50} (mg/kg/dose)[a]						
		MDL 19,592		Nafcillin		Dicloxacillin	Oxacillin	Methicillin
		po[c]	sc[c]	po	sc	po	sc	sc
M238[d]	6×10^7	5.5	5.8	63	31	>200	32	13.3
M258[d]	1×10^8	11	11.6	87	22	237	35	21
M240[e]	4×10^8	0.7	0.25	NT	NT	>40	3.5	2.1

Strain	MIC (μg/ml)[b]				
	MDL 19,592	Nafcillin	Dicloxacillin	Oxacillin	Methicillin
M238[d]	2	0.4	0.2	0.4	1.6
M258[d]	4	0.4	0.1	0.4	1.6
M240[e]	1	0.2	0.05	0.1	0.8

[a,b,c,d,e] See Table 7.

Table 9. Comparative activity of MDL 19,592 and cephalexin against experimental streptococcal infections in mice.

Organism	Challenge (cfu)	Compound	ED_{50} (mg/kg/dose)[a]				MIC (μg/ml)
			po[b]		sc[b]		
			Test		Test		
			1	2	1	2	
S. pyogenes St139	2×10^4	MDL 19,592	1.3	1.1	1.3	1.1	0.4
		Cephalexin	3.1	3.9	2.4	3.3	0.6
S. pyogenes St141	4×10^3	MDL 19,592	1.6	1.9	1.6	1.8	0.3
		Cephalexin	5.7	4.1	5.2	5.9	0.4
S. pneumoniae D137	2×10^2	MDL 19,592	20	43	25	34	1.6
		Cephalexin	28	39	38	40	2.0
S. pneumoniae D815	5×10^3	MDL 19,592	7	15	10	13	0.7
		Cephalexin	12	21	19	24	0.8

[a] See Table 7.
[b] See footnote [c] Table 7.

Table 10. Comparative oral activity of MDL 19,592 and cephalexin against experimental Gram-negative bacterial infections in mice.

Organism	Challenge (cfu)	Compound	ED_{50} (mg/kg/dose)[a]
Escherichia coli Es59	1×10^3	MDL 19,592	25[b]
		Cephalexin	5.1[b]
Klebsiella pneumoniae K39	5×10^3	MDL 19,592	34
		Cephalexin	8.5
Salmonella schottmuelleri Sa27	1×10^7	MDL 19,592	50[b]
		Cephalexin	9.5[b]
Proteus mirabilis P6	1×10^3	MDL 19,592	>200
		Cephalexin	62

[a] Compounds were dissolved or suspended in 5% $NaHCO_3$ and administered 1 and 4 hours after infection.
[b] Average of two tests.

as expected, cephalexin was superior to MDL 19,592 against Gram-negative bacterial infections (Table 10). Only limited testing against Gram-negative bacteria *in vivo* was warranted based upon results obtained *in vitro*.

Concentrations of MDL 19,592 and cephalexin attained in blood serum of mice after oral administration at 40 mg/kg are shown in Fig. 3. Both drugs were rapidly absorbed, with the highest blood levels being observed at 15 minutes; these were 53 and 43 μg/ml for MDL 19,592 and cephalexin, respectively. As shown, the blood levels of the two antibiotics declined at a similar rate. A fairly linear dose response was obtained when MDL 19,592 was given orally at levels from 5 to 80 mg/kg. For example, at 80 mg/kg, the peak blood level (at 10 to 15 minutes) was about 85 μg/ml. As determined by an ultrafiltration method, serum from mice dosed with MDL 19,592 or cephalexin bound the antibiotics only 11 and 4%, respectively. Similar results were obtained with spiked serum. Urine collected from mice at 60 minutes after an 80 mg/kg oral dose of MDL 19,592 contained the antibiotic as the only bioactive entity as determined by chromatography coupled with bioautography.

In summary, the very favorable results obtained with MDL 19,592 as compared to other β-lactam compounds against benzylpenicillin-resistant *S. aureus in vivo* as well as the good results obtained against streptococcal mouse infections indicated that MDL 19,592 had clinical potential for treating otitis media and infections of the respiratory tract, skin, skin structures, bone and urinary tract caused by susceptible organisms. It also was felt that its lower potency and narrower spectrum of activity for genera of the Enterobacteriaceae as compared to other oral cephalosporins could be viewed as a plus if one considered that its primary potential use would be against Gram-positive coccal infections. Thus, as mentioned in the introduction, single and multiple-dose pharmacokinetic and safety studies in humans were conducted[3,4].

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