In vitro activity of a new 'higher-lactam' antibacterial agent LY 193239

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Summary

***In vitro* activity of the new bicyclic pyrazolidinone LY 193239 (Eli Lilly) was evaluated against 52 clinical isolates of *Haemophilus influenzae* (4 were β-lactamase producers), 32 *Enterococcus faecalis*, 14 *Neisseria gonorrhoeae* (1 β-lactamase-positive) and 19 *Neisseria meningitidis*. Activity was best against *Neisseria* spp. and *H. influenzae*, including penicillinase-producing strains. Results of the time-kill study against a non-enzyme-mediated penicillin resistant strain of *N. meningitidis* indicate that exposure to an antibacterial concentration four times the minimal inhibitory concentration was bactericidal. *E. faecalis* was insensitive.**

S Afr Med J 1991; **79:** 312-313.

A new group of antibacterial agents, the γ-lactams, may now be added to the β-lactams. The three-dimensional structure is nearly superimposable on that of the β-lactams but they contain a five-membered ring instead of the four-membered ring. The action depends on binding to penicillin-binding proteins and there is resistance to degradation by the penicillinases although some cephalosporinases may hydrolyse the γ-lactam ring. The activity of the fully synthetic pyrazolidinone LY 193239 against problem Gram-negative and Gram-positive pathogens was investigated.

In the RSA there are indications that alternative therapy and prophylaxis should be sought against meningococcal infections.[1] *Haemophilus influenzae* is becoming increasingly resistant to semi-synthetic penicillins and *Enterococcus faecalis* is a relatively resistant pathogen. *Neisseria gonorrhoeae* is also displaying burgeoning resistance to penicillin.

Materials and methods

Bacterial strains. Fifty-two clinical isolates of *H. influenzae* (22 were type b) were investigated. Four were resistant to ampicillin (minimal inhibitory concentration (MIC) >32 mg/l) and produced β-lactamase. Apart from 1 cerebrospinal fluid (CSF) isolate the others were all respiratory tract pathogens. Of 32 *E. faecalis* 2 were urinary tract isolates and 1 was from a blood culture while the remainder were isolated from pus. Half of these strains were resistant to ampicillin with ampicillin MICs >512 mg/l. Fourteen *N. gonorrhoeae* strains were all genital isolates and 1 β-lactamase-producing strain showed a penicillin MIC >64 mg/l. Nineteen *N. meningitidis* isolates (7 from CSFs and the remainder from blood cultures) were all fully sensitive to penicillin (MIC <0,05 mg/l), except 1 CSF isolate, which was only inhibited (and killed) by 1 mg/l penicillin.

Antimicrobial agents. Solutions of LY 193239 (supplied by Eli Lilly) and tetracycline were freshly prepared in sterile distilled water. Rifampicin was dissolved in dimethyl sulphoxide and diluted in water. Ampicillin and penicillin were prepared in 0,05M phosphate buffer with final pH 7,0.

MICs. MICs were determined by inoculating appropriate dilutions of log-phase test organisms in serum broth onto freshly prepared chocolate agar media containing appropriate concentrations of individual antibiotics or antibacterial agent with a multipoint inoculator (Mast) so that each tine delivered 5×10^6 colony forming units (CFUs). In the case of *E. faecalis* 2% sheep blood agar plates were used. Plates were incubated for 18 hours at 37°C in air or under 5% CO_2 as required by the organisms investigated. The lowest concentration of antibiotic or antibacterial agent totally inhibiting growth was regarded as the MIC.

Time-kill curve.[2] Data for the time-kill study were obtained after MIC determination. Several 500 ml flasks containing 300 ml Mueller-Hinton broth with appropriate quantities of LY 193239 were prepared. Each flask was inoculated with approximately 3×10^8 CFU/ml. Samples of 100 μl were removed immediately and serial 10-fold dilutions were prepared for colony counts. Cultures were incubated at 37°C and CO_2 was passed through the medium. Further duplicate samples for viable colony estimation were similarly diluted in Mueller-Hinton broth and inoculated by spreading 100 μl on chocolate agar plates at 6 hours and 24 hours.

Results

MICs

Results of agar dilution susceptibility studies are shown in Table I.

Activity of LY 193239 is excellent against the two *Neisseria* species tested. Beta-lactamase-producing strains of *Neisseria* and *Haemophilus* remain sensitive to LY 193239. *E. faecalis* is highly resistant and this resistance appears independent of ampicillin sensitivity.

Time-kill study

The time-kill curves for differing concentrations of LY 193239 plotted against a non-β-lactamase-producing but relatively penicillin-resistant strain of *N. meningitidis* (MIC = 1 mg/l) are shown in Fig. 1. Four times (0,192 mg/l) the LY 193239 MIC (0,048 mg/l) is bactericidal.

Discussion

With the limited resources at their disposal micro-organisms continue to evade the action of antibiotics with remarkable success. In the absence of satisfactory vaccines for meningococcal disease,[3] it is important that novel antibiotics or antibacterial agents be developed and assessed. The prevalence of *N. gonorrhoeae* infections and escalating resistance to first-line

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Accepted 24 Apr 1990.

TABLE I. COMPARATIVE *IN VITRO* ACTIVITY OF LY 193239 AGAINST CLINICAL ISOLATES

Strain (No.)	Antibiotic/ antimicrobial	MIC mg/l for strains 50%	90%	MIC range (mg/l)
***H. influenzae* (48)**	**LY 193293**	**⩽ 1,56**	**⩽ 3,125**	**0,78 - 12,5**
Non-β-lactamase	**Ampicillin**	**⩽ 0,5**	**⩽ 0,5**	**0,125 - 2,0**
β-lactamase (4)	**LY 193293**	**3,125**	**3,125**	**0,78 - 3,125**
	Ampicillin	**64,0**	**128,0**	**32,0 - 128,0**
***E. faecalis* (32)**	**LY 193293**	**> 100**	**> 100**	**25 - >100**
	Ampicillin	**0,5**	**> 512**	**0,5 - >512**
***N. gonorrhoeae* (14)**	**LY 193293**	**0,195**	**0,195**	**0,195 - 0,195**
	Penicillin	**0,03**	**0,125**	**< 0,01 - 256***
	Tetracycline	**1,0**	**1,0**	**0,5 - 2,0**
***N. meningitidis* (19)**	**LY 193239**	**0,048**	**0,048**	**0,048 - 0,048**
	Penicillin	**0,125**	**0,125**	**0,03 - 1,0**
	Rifampicin	**0,06**	**0,125**	**0,01 - 0,25**

* Penicillinase-producing

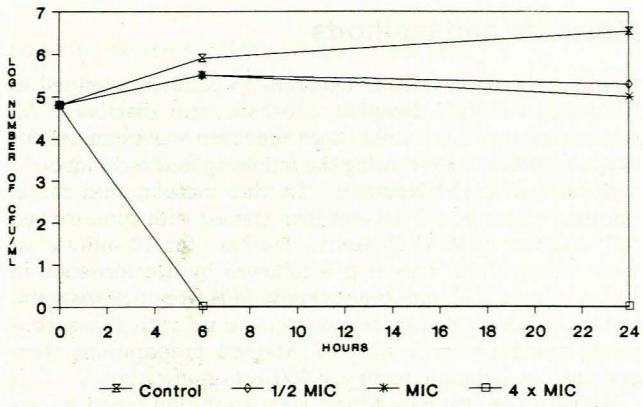

Fig. 1. Activity at differing concentrations of LY 193239 on a relatively penicillin-resistant but non-β-lactamase-producing strain of *N. meningitidis* isolated from CSF (penicillin MIC = 1 mg/l; LY 193239 MIC = 0,48 mg/l).

drugs also demand attention. Community-acquired *Haemophilus* infections lower school attendance and productivity. Consideration of inherently difficult to treat endogenous enterococcal infections is important.

In this study the bicyclic pyrazolidinone LY 193239 displayed excellent activity against *Neisseria* spp. and against *H. influenzae*, particularly against β-lactamase-producing strains. Enterococci emerged unaffected by this drug.

The chemical stability of LY 193239 makes it an attractive prospect (J. Ternansky, Eli Lilly Research Division — personal communication).

In the CSF levels of penicillin approach 0,8 mg/l, which is not bactericidal for certain local strains examined in this and another study.[1]

Rifampicin is widely used in antituberculosis therapy so that other organisms present in the community (*N. meningitidis* in the carrier state) may develop resistance to it. Conversely, rifampicin should probably be reserved for antituberculosis therapy in this country for similar reasons of resistance occurring in *Mycobacterium tuberculosis* exposed to rifampicin used for other purposes, such as contacts of meningococcal disease patients. In this context LY 193239 may also have a place in the clinical setting.

It may be concluded that future clinical prescription of LY 193239 will prove valuable against *Neisseria* and *Haemophilus* infections and the indications are that penicillinase production by these organisms does not influence its antibacterial acitivity.

We wish to thank Eli Lilly for the opportunity to investigate the activity of LY 193239 on clinical isolates from the local environment.

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