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Activity of Selected Beta-Lactam Antibiotics Against *Mycobacterium leprae*[1]

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The beta-lactam antibiotics (penicillins, cephalosporins, and cephamycins) have a similar mechanism of action, i.e., the inhibition of transpeptidases, the enzymes that cross-link peptidoglycans into the rigid, skeletal structure of the bacterial cell wall. Members of this group of antibiotics are active against a wide range of bacteria, both gram-positive and gram-negative. Their *in vitro* activity against mycobacteria has been described by Misiek, *et al.* ([5]), who found that cephalosporins with pyridyl or aminomethylphenyl moieties in the 7-position were active against *Mycobacterium tuberculosis.* This conclusion was confirmed by Sanders, *et al.* ([6]) who tested a series of cultures of *M. tuberculosis.* The compounds they studied were (listed from greatest to least activity): ceforanide, cephapirin, cephalothin, cephazolin, cephalexin, cephaloglycin, and cephaloridine. Sanders, *et al.* ([6]) also tested activity against *M. kansasii* and *M. intracellulare* and found approximately the same ordering of activity among the compounds, although the minimal inhibitory concentrations (MIC) were higher for these two *Mycobacterium* species than for *M. tuberculosis.*

Activity of cephaloridine against *M. leprae* in mice was reported by Gaugas ([3]) who treated animals from the day of infection with 300 mg/kg of the drug daily. We studied cephaloridine by the kinetic method, a procedure that allows the differentiation of bacteriostasis from a bactericidal-type activity, and found it to have bactericidal-type activity in a similar dosage (10 mg/day) ([10]). Cephaloglycin (0.1% in the diet) also produced bactericidal-type activity in mice, but cephalexin at the same dosage was inactive.

Leprosy has been treated largely with dapsone as single-drug therapy. Recently, however, an increase in primary and secondary dapsone resistance has been observed ([1]). In response, a World Health Organization study group has recommended multidrug regimens including dapsone, rifampin, and clofazimine ([2]). Although dapsone is still useful in untreated cases because primary resistance, when present, is usually at a low level, the loss of the effectiveness of this important drug to leprosy control, even in limited areas, would be a most serious development. An additional effective drug is urgently needed.

MATERIALS AND METHODS

The kinetic method for determining the activity of drugs against *M. leprae* was used in this study. This method has recently been described in detail ([9]). In brief, CFW female mice were infected in a hindfoot pad on day 0 with 5000 *M. leprae* cells (Strain N2409) from mouse passage (Subpassage C-35). Drug treatment (6 days per week) with 10 mg/kg by subcutaneous injection into the nuchal area was started on day 70 and continued until day 124 except that cephalexin and cephadrine were mixed into the diet at a level of 0.1%. To monitor the growth curve of the leprosy bacilli, acid-fast bacteria in the pooled foot pad tissues of four mice from a single treatment group were counted at regular intervals, and the amount of growth-delay was judged graphically under the assumption that the generation time in the logarithmic phase was 12.5 days. The probability (p) values state the estimated prob-

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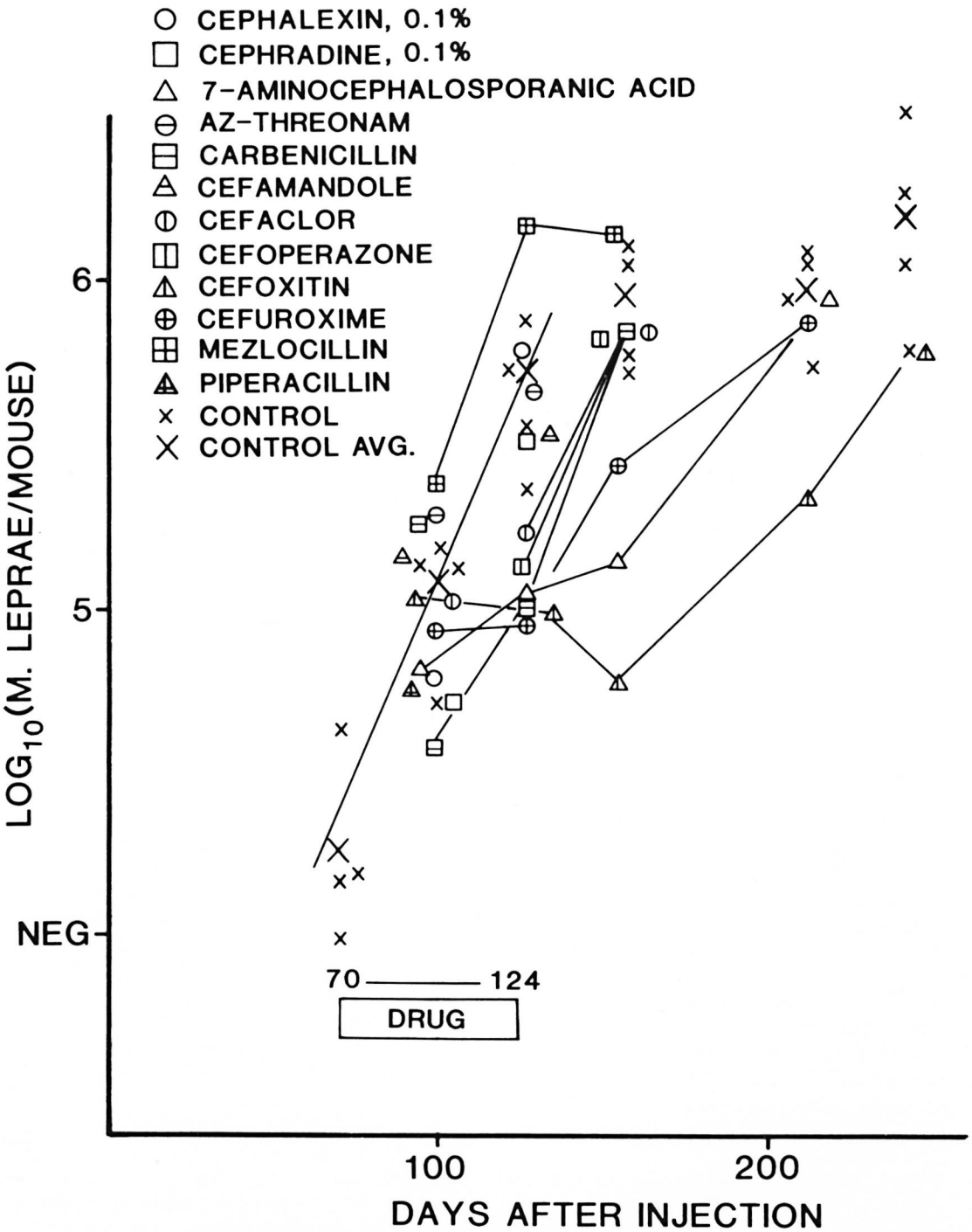


FIG. 1. Activity of 12 beta-lactam compounds for *M. leprae* as measured by the kinetic method in mice. Mice were infected on day 0 and treated with drugs for the time indicated. Counts of AFB in the pooled foot-pad tissues of four mice from each group were carried out on the days indicated. Values for all drug-treated groups are shown only on the day 98. Thereafter, for simplification the only groups shown are those with counts less than any control group and they are shown only until they exceed any one of the control groups.

THE TABLE. *Activity of 12 beta-lactam antibiotics against* M. leprae.

Drug[a]	Growth delay (days)	Probability value (p) vs controls
Cephalexin (0.1%)	0	NS[b]
Cephradine (0.1%)	0	NS
7-Aminocephalosporanic acid	52	<0.04
Az-threonam	0	NS
Carbenicillin	23	NS
Cefamandole	0	NS
Cefaclor	21	NS
Cefoperazone	17	NS
Cefoxitin	102	<0.008
Cefuroxime	39	<0.04
Mezlocillin	−14	<0.04
Piperacillin	0	NS

[a] The dosage was 10 mg/day subcutaneously 6 days a week from day 70 through day 124 (through day 119 for 7-aminocephalosporanic acid) or 0.1% in the diet for cephalexin and cephadrine.

[b] NS = not statistically significant.

ability ([9]) that the difference between growth curves is caused by chance alone.

Az-threonam and cephradine were contributed by E. R. Squibb and Sons; carbenicillin by Roerig (Pfizer); cefamandole, cefaclor, and cephalexin by Lilly Research Laboratories; cefoperazone by Pfizer; cefoxitin by Merck Sharp and Dohme Research Laboratories; cefuroxime by Glaxo; mezlocillin by Miles Pharmaceuticals; and piperacillin by Lederle Laboratories. 7-Aminocephalosporanic acid was purchased from Sigma Chemical Company.

RESULTS

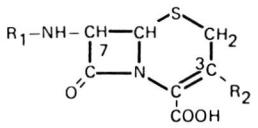
Significant ($p < 0.05$) inhibition of the growth of *M. leprae* was obtained by treatment with 7-aminocephalosporanic acid, cefoxitin, and cefuroxime (Fig. 1 and The Table). Apparent growth promotion was seen with mezlocillin.

There were three somewhat unusual features in this experiment. First, the growth of *M. leprae* in the controls was about 4 weeks early (usually it is just emerging into the countable range on day 98). Second, the inhibitory drugs appeared to be slow in exerting their effect. Consequently the differences between the control and the effectively treated groups did not become distinct until day 126, 56 days after the start of treatment. We used the interval starting at day 126 to estimate the p values. Third, as stated, mezlocillin appeared to increase the growth rate of *M. leprae*. This phenomenon is not unique since growth promotion of *M. tuberculosis* by cloxacillin was observed earlier ([4]).

DISCUSSION

In the kinetic method ([7]) for testing drugs for their activity against *M. leprae*, the drug is administered for a limited period, e.g., from day 70 to day 126. A drug that exerts pure bacteriostasis will cause a growth-delay equivalent to the period that the drug is present in the foot-pad tissue in effective concentration. A drug that is bactericidal will, of course, prevent growth completely. One that kills only a part of the cells will produce a growth delay that exceeds the period that the drug is present by an amount that is a function of the fraction of *M. leprae* killed. With the schedules used here, for most drugs the number of days that the drug is present in effective concentration is approximately equal to the number of days of its administration. When repository activity is present, as is the case with clofazimine ([11]), the effective period of the drug may extend beyond the period of administration. An additional complicating factor is the possible presence of bacteriopause ([12]), which is bacteriostasis that persists for a time after the drug has disappeared. Two explanations are given for this type of activity. One, the drug binds to the bacterium and is only slowly released. Two, the drug induces a defect that the bacterium needs time to repair.

Because of the possibility of bacteriopause, we have used the term "bactericidal-type" for those non-repository drugs that cause growth-delay in excess of the period of drug administration. The list of drugs that cause bactericidal-type growth-delay is quite short ([8]), and contains only: a) the drugs known to be useful in the therapy of leprosy patients (i.e., dapsone, rifampin, clofazimine); b) a few drugs for which a question of safety remains for the dosage indicated from the mouse data (ethionamide, prothionamide, thiacetazone); c) compounds related to those listed, e.g., certain sulfon-



COMPOUND	R1	R2	GROWTH DELAY (DAYS)
7-AMINOCEPHALOSPORANIC ACID	H–	$-CH_2-O-C(=O)-CH_3$	52 (49)[c]
CEPHALOGLYCIN	$-NH-CH_2-C(=O)-$	$-CH_2-O-C(=O)-CH_3$	81 (63)[b]
CEFOXITIN *	$-CH_2-C(=O)-$	$-CH_2-O-C(=O)-NH_2$	102 (54)[c]
CEFUROXIME	$-C(=O)-C(=N-OCH_3)-$	$-CH_2-O-C(=O)-NH_2$	39 (54)[c]
CEPHALORIDINE	$-CH_2-C(=O)-$	$-CH_2-N^+$	179 (75)[a]
CEPHALEXIN	$-CH(NH_2)-C(=O)-$	$-CH_3$	0 (63)[b] 0 (54)[c]
CEPHRADINE	$-CH(NH_2)-C(=O)-$	$-CH_3$	0 (54)[c]
CEFAMANDOLE	$-CH(OH)-C(=O)-$	$-CH_2-S-$	0 (54)[c]
CEFACLOR	$-CH(NH_2)-C(=O)-$	–Cl	21 (54)[c]
CEFOPERAZONE	$C_2H_5-N-C(=O)-NH-CH(OH)-C(=O)-$	$-CH_2-S-$	17 (54)[c]

FIG. 2. Structural formulas and observed activity for *M. leprae* of the beta-lactam antibiotics studied. Numbers in parentheses following the days of growth-delay refer to total number of days mice received treatment. Az-threonam is not shown because its structure is unique. The footnotes a, b, and c refer, respectively, to a published experiment ([10]), an unpublished experiment, and the present experiment. *Cefoxitin, a cephamycin, is further characterized by insertion of a methoxy residue ($-OCH_3$) at position 7 of the beta-lactam ring.

amides and rifamycins; and d) certain cephalosporins.

Most of the drugs on this list are active orally and are effective when administered mixed in the diet of the mouse, thereby providing a relatively constant intake and plasma level throughout the day. With the beta-lactam compounds, however, only a few are active against other organisms when administered by mouth, and of these only cephaloglycin (a drug no longer produced) has been found active against *M. leprae.* Most beta-lactam drugs must be administered by injection. Because all of those we tested have short plasma half-lives, the tissue concentrations would not have remained long at inhibitory levels after the single daily injection.

In the present study we tested 12 beta-lactams—3 penicillins, 8 cephalosporins, and 1 cephamycin. None of the penicillins caused growth-delays, but the apparent growth-promotion caused by mezlocillin may have been caused by interference with cross-linking in the cell envelope, thereby rendering it more permeable to nutrients.

Figure 2 gives the structural formulas and activities against *M. leprae* of the single cephamycin and 9 of the 10 cephalosporins tested so far (az-threonam is not included because of its unique structure). Two cephalosporins (cephaloridine and cephaloglycin) have shown bactericidal-type activity, and two more (7-aminocephalosporanic acid and cefuroxime) have shown lesser but significant activity. The single cephamycin tested (cefoxitin) had bactericidal-type activity. Among the active compounds, 7-aminocephalosporanic acid and cephaloglycin have the same R_2 substituent ($-CH_2OCOCH_3$). Cefoxitin and cefuroxime share the R_2 substituent also ($-CH_2O-CONH_2$). The two most active compounds, cefoxitin and cephaloridine, have the same R_1 group. Among the inactive compounds there were also shared moieties. There were no shared R_1 or R_2 substituents between the active and inactive compounds. A relationship between activity and plasma half-life is not apparent. Thus, the study provides the first clues as to the molecular requirements for activity against *M. leprae.* Not much relationship to the requirement for *in vitro* activity against *M. tuberculosis, M. kansasii,* or *M. intracellulare* ([5, 6]) is suggested. Of course, one would wish for a much higher degree of activity for leprosy, preferably by oral administration. Nevertheless, even if more active compounds are not found, it is possible that some beta-lactam compounds, because they interfere with cell-wall synthesis, will be useful in combination with other active compounds.

SUMMARY

Twelve beta-lactam antibiotics were tested for activity against *Mycobacterium leprae* growing in the foot pads of mice. Two cephalosporins (7-aminocephalosporanic acid and cefuroxine) and one cephamycin (cefoxitin) showed significant activity against *M. leprae,* and one penicillin (mezlocillin) exerted possible growth-promoting activity. These results suggest that particular molecular structures may be required for activity against *M. leprae.*

RESUMEN

Se probó la actividad de 12 antibióticos (beta-lactamas) sobre el *Mycobacterium leprae* cultivado en la almohadilla plantar del ratón. Dos cefalosporinas (ácido 7-aminocefalosporánico y cefuroxina) y una cefamicina (cefoxitina) mostraron significante actividad contra el *M. leprae.* Una penicilina (mezlocilina) ejerció una aparente actividad promotora del crecimiento. Estos resultados sugieren que se requieren ciertas estructuras moleculares particulares para ejercer una actividad determinada sobre el *M. leprae.*

RÉSUMÉ

On a étudié douze antibiotiques de la classe des bétalactames, quant à leur activité contre *Mycobacterium leprae* dans les coussinets plantaires de la souris. Deux céphalosporines (l'acide 7-aminocéphalosporanique et la céfuroxine), de même qu'une céphamycine (la céfoxitine) ont montré une activité significative contre *M. leprae.* Une pénicilline (la mézlocilline) présentait une certaine activité pour favoriser la croissance. Ces résultats suggèrent que des structures moléculaires particulières sont peut-être nécessaires pour l'activité contre *M. leprae.*

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