PRELIMINARY MICROBIOLOGICAL AND PHARMACOLOGICAL EVALUATION OF 6-(R-α-AMINO-3-THIENYLACETAMIDO) PENICILLANIC ACID (BL-P 875)

K. E. PRICE, J. A. BACH*, D. R. CHISHOLM, M. MISIEK and A. GOUREVITCH

Research Division, Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York, U.S.A.

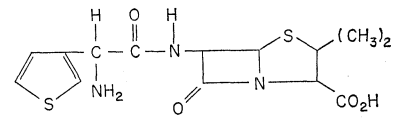
(Received for publication November 18, 1968)

BL-P 875, a new semisynthetic penicillin, was found to possess broad-spectrum antibacterial activity. Its inhibitory potency for both gram-negative and gram-positive organisms was comparable in all respects to that of ampicillin. Despite this, BL-P 875 proved to be significantly more active than ampicillin when administered by the oral route in a number of experimental bacterial infections of the mouse. Median curative doses (CD_{50}) of BL-P 875 in infections produced by *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and 1 of 2 *Escherichia coli* strains were less than one-half those of ampicillin. On the other hand, comparable doses were required for curing mice challenged with *Staphylococcus aureus*, *Diplococcus pneumoniae*, or *Salmonella enteritidis*.

Since no differences in acid stability, bactericidal effect, or serum binding were found for the 2 compounds, the probable explanation for BL-P 875's superior therapeutic activity is its higher degree of oral absorbability. BL-P 875 concentrations in mouse blood after oral administration were significantly higher (about 2-fold) than those of ampicillin at both one-half and 1 hour post-administration. Greater absorbability was also found in rats, where about twice as much BL-P 875 as ampicillin was recovered from urine after administration of a single oral dose. No differences in mouse blood levels or urine recovery values could be demonstrated for the parenterally-administered antibiotics.

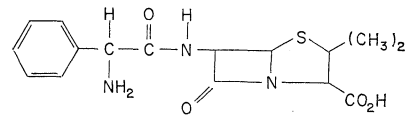
BL-P 875, 6-(R-α-amino-3-thienylacetamido) penicillanic acid, is a semisynthetic derivative of 6-aminopenicillanic acid (6-APA) that was selected as an interesting candidate from a screening program designed to identify compounds with broad spectrum antimicrobial properties and/or good absorbability from the gastrointestinal tract. Although its level of intrinsic activity and breadth of antibacterial spectrum in *in vitro* tests appear comparable to that of ampicillin, 6-(R-α-

Fig. 1. Chemical structures of BL-P 875 and ampicillin. Nomenclature from CAHN[2].



BL-P 875

6-(R-α-Amino-3-thienylacetamido) penicillanic acid



Ampicillin

6-(R-α-Amino-phenylacetamido) penicillanic acid

* Present address: Microbiology Control Department, The Upjohn Company, Kalamazoo, Michigan, U.S.A.

amino-phenylacetamido) penicillanic acid, BL-P 875 appears to be a more effective oral therapeutic agent in several experimental mouse infections. The present report describes experiments which suggest that the greater efficacy of the compound can be attributed to the fact that it is more readily absorbed from the gastrointestinal tract. Structures of the two penicillins are shown in Fig. 1.

Materials and Methods

Antibiotics. The epimer of 6-(α-amino-3-thienylacetamido) penicillanic acid, which was prepared by acylation of 6-APA with the R amino acid, has a higher level of intrinsic activity than the epimer prepared from the S amino acid. The sodium salts and the acid trihydrate of the more active form (BL-P 875) were synthesized by the Organic Chemistry Research Department of Bristol Laboratories[3]. Although the acid trihydrate form of the penicillin was used in some tests (all MIC and certain blood level and CD_{50} studies), and the sodium salt of BL-P 875 in others, it is probable that all tests can be considered to have been carried out with the compound in the form of the sodium salt. This suggestion is based on the fact that the acid trihydrate was always solubilized in 5 % $NaHCO_3$ solution.

Ampicillin was utilized as a control compound in all studies. The dosage form (acid trihydrate or sodium salt) of ampicillin employed in a given experiment was usually the same as that of BL-P 875. Penicillins were bioassayed by an agar diffusion method[6] using *Staphylococcus aureus* ATCC 6538P as the test organism.

Measurement of *in vitro* antibacterial activity. Minimum inhibitory concentration (MIC) values were determined either by the standard 2-fold broth dilution technique[4] utilizing Brain Heart Infusion Broth (BBL)+1 percent Supplement C (Difco) and an inoculum concentration of $1\sim5\times10^5$ cells/ml or by agar dilution methods. In the latter procedure, antibiotics were incorporated into Nutrient Agar (Difco) or Trypticase Agar (BBL) with 2 % defibrinated sheep blood. After plates solidified, the surface was inoculated with undiluted to 10^2 dilutions of overnight broth cultures by means of the Steers Multiple Inoculator apparatus[9]. This instrument permitted the simultaneous inoculation of up to 30 different bacterial strains on a single plate. The endpoints (MIC) in both procedures were determined after overnight incubation at 37°C. These were considered to be the lowest concentrations at which an appreciable diminution in bacterial growth occurred. Most of the bacterial strains employed were clinical isolates.

Measurement of bactericidal effects. The relative rate at which sodium salts of BL-P 875 and ampicillin kill cells of *Klebsiella pneumoniae* (A9977) was determined. Five$\times10^6$ cells/ml from an 18~hr Heart Infusion Broth (HIB) culture were exposed at 37°C to several concentrations of each antibiotic for various periods of time. The number of viable cells remaining in the HIB cultures at various times after addition of the compounds was determined as follows: Samples were removed at the desired time and where indicated, subjected to *Staphylococcus aureus* M2 penicillinase[5] in sufficient quantity to destroy all remaining antibiotic within one minute after addition. All samples were then plated out on Heart Infusion Agar (HIA) by spreading 0.1 ml over the agar surface. After overnight incubation at 37°C, the number of colony-formers was determined.

Acid stability determination. The half-life of the sodium salts of BL-P 875 and ampicillin in 0.002 M citric acid-HCl buffer solution, pH 2.0, was determined. Solutions containing the compounds at a concentration of 100 μg/ml were incubated at 37°C and sampled at 0, 3, 5, and 24 hr. The half-life or length of exposure causing a 50 % decrease in antimicrobial activity (measured by bioassay after neutralization) was then calculated.

Serum binding estimation. The extent to which the two penicillins are bound to human serum protein was measured by estimating "free" antibiotic in serum. This was

accomplished by assaying serum samples that contained various compound concentrations against standards prepared in 0.1 M pH 7.0 phosphate buffer by an agar diffusion technique[8]. The percentage sodium BL-P 875 and sodium ampicillin bound over a concentration range of 0.3~1.0 μg/ml was determined.

Mouse protection tests. The median curative doses of BL-P 875 and ampicillin were determined for a variety of experimental gram-positive and gram-negative bacterial infections in 18~22 g, male, Swiss-Webster mice. In each case, mice were infected by intraperitoneal (IP) administration of sufficient bacterial cells to kill all non-treated mice within 48 hr (20~5,000 LD_{50}). Challenge doses of *Proteus mirabilis*, *Escherichia coli*, *Salmonella enteritidis*, and *Staphylococcus aureus* were given as suspensions in 5 % mucin. No mucin was utilized in the other infections. The antibiotics were administered by intramuscular (IM) or oral routes either at the time of challenge, 4 hr after challenge, or both at the time of challenge and 4 hr later. The number of mice employed at each dosage level ranged from 5 to 20 depending upon the particular experiment. At the termination of the experiment, the number of surviving mice were recorded and the CD_{50} value (the total dose in mg/kg required to cure 50 % of the infected mice) estimated by means of a log-probit plot. When suitable, data were analyzed statistically by the method of LITCHFIELD and WILCOXON[7].

Antibiotic absorption studies in rodents. All laboratory blood level studies employed male, Swiss-Webster mice ranging in weight from 18~22 g. Mice utilized in oral absorption experiments were fasted for 18 hr prior to administration of drug by means of a hypodermic syringe fitted with a blunt 18-gauge needle. Food and water *ad libitum* were available to mice used in studies where the penicillins were administered by intramuscular or intravenous (IV) routes. In all cases, blood samples were collected from the orbital sinuses into heparinized capillary tubes (Clay-Adams) as described by WICK and BONIECE[10]. Each one of the mice was bled at each of the predesignated sampling times. Assay of blood antibiotic concentrations was conducted as follows: The tip of the blood-filled capillary tube was touched immediately after collection to the surface of a 1/4" (6.35 mm) diameter filter-paper disc (Schleicher and Schuell #740E) until saturation occurred. Each disc was then placed on the surface of a Seed Agar plate previously inoculated with *S. aureus* ATCC 6538P. Diameters of inhibition zones were determined after incubating the plates at 37°C for 18 hr. Antibiotc potency of the samples was estimated by use of a dose-response curve that had been prepared by supplementing normal heparinized mouse blood with known amounts of the penicillins. Differences in average blood levels were analyzed by means of STUDENT's "T" test.

The cumulative rate of urinary excretion of the sodium salts of BL-P 875 and ampicillin over a 24-hr period was determined after IM and oral administration of 20 mg/kg of the penicillins to fasted rats. After each antibiotic was administered by IM or oral route to 8 Sprague-Dawley rats, urine samples were collected over dry-ice during intervals of 0~3, 3~6, and 6~24 hr post-treatment. Samples from each time period were pooled and the pool assayed against a standard line prepared by spiking normal urine with known quantities of drug. Urine volumes for each collection period were recorded and the figures used to calculate total urinary excretion.

Acute toxicity test. The drug dosage producing 50 % mortality (LD_{50}) in normal male, Swiss-Webster mice was investigated for orally administered BL-P 875 acid trihydrate. Survivors on the 10 th day after administration of a single dose were noted and the LD_{50} calculated[7].

Injection pain and irritation studies. Sprague-Dawley, 175~200 g, male rats were used to determine whether pain upon injection and/or local tissue irritation occurred following injection of BL-P 875 and ampicillin into the plantar side of the hind paw. Aqueous suspensions as concentrated as 3.1 % were injected (0.1 ml volume) into one hind paw of each of 10 rats/dose level. The other hind paw always received a similar quantity

of vehicle alone. Pain upon injection was estimated subjectively, while the degree of irritation or paw edema was determined by the volume displacement method[1]. Measurements were made at 1, 2, 4, 24, and 48 hr. A compound causing a mean increase in paw volume of 0.25 ml above the control paw volume, maintained for at least 4 hr, was considered to be irritating.

Results

In vitro Studies

The *in vitro* antibacterial spectra of BL-P 875 and ampicillin have been compared in Tables 1~3.

Both compounds had excellent activity against all of the gram-positive species tested (Table 1), except for β-lactamase-producing staphylococci. In no instance was there an appreciable difference in the response of the organisms to the two antibiotics.

MIC values of the penicillins were also found to be of the same order for each

Table 1. Relative *in vitro* activity* of BL-P 875 and ampicillin against gram-positive organisms

Organisms	No. of strains	Inoculum (Dil. of 18-hour culture)	MIC** range		MIC (Geometric mean)	
			BL-P 875	Ampicillin	BL-P 875	Ampicillin
Staphylococcus aureus (penase −)	16	10^2	0.04 ~0.16	0.04 ~0.08	0.66	0.60
Staphylococcus aureus (penase +)	3	10^2	16~32	16~32	23	23
Diplococcus pneumoniae	4	10^1	0.004~0.016	0.008~0.032	0.008	0.016
Streptococcus pyogenes	4	10^1	0.004~0.016	0.004~0.016	0.007	0.008
Streptococcus faecalis	4	10^1	1~2	1~2	1.7	1.5
Bacillus anthracis	1	10^1	1.004~0.016	0.008~0.016	0.008	0.012
Clostridium perfringens	2	Undil.	0.125~0.25	0.125~0.25	0.18	0.18
Listeria monocytogenes	2	10^1~10^2	0.5 ~2	0.5 ~2	1	1

* Activity of antibiotics determined on Trypticase Soy Agar (BBL)+2 % sheep blood. Inocula added by means of the Steers Multiple Inoculator apparatus[9].
** Minimum inhibitory concentration in μg/ml.

Table 2. Relative *in vitro* activity* of BL-P 875 and ampicillin against strains of *Enterobacteriaceae*

Organism	No. of strains	Medium***	MIC** range		MIC (Geometric mean)	
			BL-P 875	Ampicillin	BL-P 875	Ampicillin
Escherichia coli	3	NA	0.5 ~ 4	0.5 ~ 2	2	1
Escherichia coli	1	NA	500	500	500	500
Enterobacter aerogenes	3	TSA	32~250	16~250	63	63
Enterobacter cloacae	1	TSA	8~ 16	16~ 32	11.5	22
Klebsiella pneumoniae	1	NA	0.5	0.5 ~ 1	0.5	0.7
Klebsiella pneumoniae	2	NA	63~125	63~125	88	88
Proteus mirabilis	2	NA	0.13~0.25	0.13~0.5	0.2	0.28
Proteus sp. (Indol +)	2	NA	0.25	0.25~0.5	0.25	0.33
Proteus sp. (Indol +)	2	NA	4~ 16	4~ 16	7	8
Proteus sp. (Indol +)	1	NA	125	63~125	125	88
Salmonella sp.	2	NA	0.13~0.5	0.13~0.25	0.21	0.16
Shigella sonnei	1	NA	0.25	0.25	0.25	0.25
Shigella flexneri	1	NA	500	500	500	500
Serratia marcescens	1	TSA	125	125	125	125

* Inocula (10^2 dilution of 18 hour culture) added to agar surface by means of the Steers Multiple Inoculator apparatus[9].
** Minimum inhibitory concentration in μg/ml.
*** NA: Nutrient Agar (Difco). TSA: Trypticase Soy Agar (BBL)+2 % sheep blood.

Table 3. Relative *in vitro* activity* of BL-P 875 and ampicillin against miscellaneous gram-negative organisms

Organism	No. of strains	Medium***	Inoculum (Dil. of 18-hour culture)	MIC** range		MIC (Geometric means)	
				BL-P 875	Ampicillin	BL-P 875	Ampicillin
Haemophilus influenzae	3	BHI	10^4	0.25 ~0.5	0.125~0.5	0.33	0.16
Brucella bronchiseptica	1	TSA	10^2	32	32	32	32
Neisseria meningitidis	2	BHI	10^2	0.008~0.04	0.016~0.08	0.018	0.036
Veillonella parvula	1	TSA	Undil.	≤0.125	≤0.125	≤0.125	≤0.125
Vibrio comma	1	TSA	10^1	1	1	1	1
Mima polymorpha	1	TSA	10^2	16	16	16	16
Herellea sp.	1	TSA	10^2	32	63	32	63
Pseudomonas aeruginosa	3	NA	10^2	>500	>500	>500	>500
Alkaligenes faecalis	1	TSA	10^2	8	8	8	8
Sphaerophorus necrophorus	1	TSA	Undil.	63	63	63	63
Sphaerophorus pseudonecrophorus	1	TSA	Undil.	63	32	63	32

* Inocula added to agar surface by means of the Steers Multiple Inoculator apparatus[9] unless otherwise indicated.
** Minimum inhibitory concentration in μg/ml.
*** Brain Heart Infusion Broth (BBL)+1 % Supplement C (Difco) used in 2-fold dilution test. TSA: Trypticase Soy Agar (BBL)+2 % sheep blood. NA: Nutrient Agar (Difco).

of the strains of *Enterobacteriaceae* studied (Table 2). The one *Serratia* strain (pigmented) tested was found to be very resistant to the compounds, while both *Salmonella* species were quite sensitive. All the other genera studied were represented by both sensitive and resistant strains.

The final group of MIC values (Table 3) were obtained in tests that included a widely diverse group of gram-negative bacteria. Members of the genera *Haemophilus*, *Neisseria*, *Veillonella*, and *Vibrio* proved to be quite sensitive to the penicillins while all others showed some degree of resistance. As was found with the previously discussed groups of microorganisms, there was no difference in the overall antibacterial activity of BL-P 875 and ampicillin.

The relative bactericidal action of the two penicillins was determined for a strain of *Klebsiella pneumoniae* (A9977). Semi-logarithmic plots of viable cell numbers present after exposure of the bacteria to several different antibiotic concentrations for various time periods are presented in Fig. 2.

Fig. 2. The numbers of viable *Klebsiella pneumoniae* (A9977) cells present in Heart Infusion Broth cultures containing various concentrations of BL-P 875 or ampicillin are shown. The cultures, which had an initial count of 5×10^6 cells/ml, were incubated at 37°C and sampled for viable cell enumeration at the indicated times.

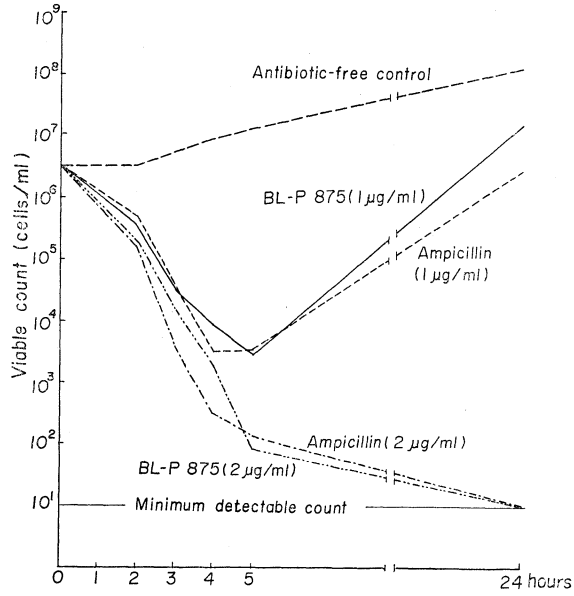


Fig. 3. The relative stability of sodium BL-P 875 and sodium ampicillin incubated at 37°C in pH 2.0 citric acid-HCl buffer. The initial antibiotic concentration was 100 μg/ml.

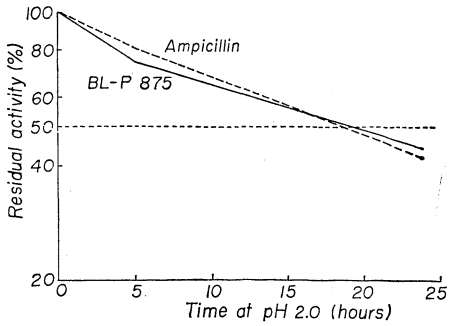


Table 4. The extent of binding* of sodium salts of BL-P 875 and ampicillin to human serum proteins

Concentration (μg/ml)	Percent Bound	
	BL-P 875	Ampicillin
1.0	22	24
0.5	28	30
0.3	32	37

* Estimated by the relative agar diffusion assay potency of antibiotic solutions in pH 7.0 phosphate buffer and 100 percent human serum.

It can be seen that both BL-P 875 and ampicillin caused rapid and apparently complete kill of the *K. pneumoniae* cells at a concentration of 2 μg/ml. At one-half this dosage, a great deal of kill took place initially, but the number of survivors was obviously sufficient to permit resumption of culture growth at some point between 5 and 24 hr. Growth was just visible at 24 hr, but readily apparent after 48 hr incubation, whereas no viable cells could be detected at this time in samples from the tubes originally containing 2 μg/ml of the antibiotics. Thus, these data show that bactericidal concentrations of BL-P 875 and ampicillin for *K. pneumoniae* (A9977) were only 2-fold higher than the concentrations permitting visible growth within 24 hr.

The stability of BL-P 875 to low pH has been studied in the laboratory. Fig. 3 compares the residual bioactivity of this compound and ampicillin after their exposure to pH 2.0 buffer for various time periods.

It is apparent that the compounds lose activity very slowly under this condition since the half-lives of both were in excess of 15 hr.

Also studied was the extent to which BL-P 875 and ampicillin are bound to human serum. Data are shown in Table 4.

Although the percentages of BL-P 875 and ampicillin bound to serum were low in all instances, binding of both tended to increase slightly as their concentration decreased from 1.0 μg/ml to 0.3 μg/ml. These results indicate that there are no significant differences in the behavior of the two antibiotics in human serum samples.

In vivo Studies

The strains of bacteria employed in experimental mouse infections had shown comparable *in vitro* susceptability to BL-P 875 and ampicillin. The relative therapeutic efficacy of these antibiotics in mouse infections was determined for 4 different strains of gram-positive bacteria (Table 5).

Staphylococcus aureus SMITH, a non-penicillinase producer, responded to low doses of both penicillins. Oral CD_{50} values obtained with the two compounds were slightly lower than the IM values, possibly due to the greater number of LD_{50} doses used to challenge the IM-treated mice. Regardless of the treatment route, however, no differences between the compounds could be demonstrated.

Table 5. Relative effectiveness of BL-P 875 and ampicillin in experimental gram-positive infections* of mice

Challenge organism	No. of LD_{50}'s	No. of mice per dose level	Treatment		CD_{50} (total mg/kg)	
			Route	Time (hours post-infection)	BL-P 875	Ampicillin
Staphylococcus aureus Smith (non-penicillinase producer)	2,000	5	IM	0	1.6	1.6
	1,000	5	PO	0	0.4	0.5
	1,200	20	PO	0+4	0.5**	0.4
Staphylococcus aureus 1633-2 (penicillinase-producer)	50	5	IM	0	>500	>500
Diplococcus pneumoniae	150	20	PO	0+4	0.9	0.8
Streptococcus pyogenes Digonnet	5,000	10	PO	0+4	1.3	2.1
	1,000	10	PO	0+4	1.0***	1.8

* Mice were infected by the IP route.
** Underlined CD_{50} values were obtained with acid trihydrates; all others with sodium salts.
*** CD_{50} significantly lower than that of ampicillin ($P<0.05$).

Table 6. Relative effectiveness of BL-P 875 and ampicillin in experimental gram-negative infections* of mice

Challenge organism	No. of LD_{50}'s	No. of mice per dose level	Treatment		CD_{50} (total mg/kg)	
			Route	Time (hours post-infection)	BL-P 875	Ampicillin
Klebsiella pneumoniae	500	5	IM	0	45	40
	300	5	PO	0	30	52
	50	5	PO	0	15**	35
	90	20	PO	0+4	12***	20
Proteus mirabilis	2,000	10	PO	0+4	17.4***	44.8
	5,000	10	PO	0+4	15.0***	40
Escherichia coli (Juhl)	2,000	5	PO	0	60	62
	1,000	20	PO	0+4	50	60
Escherichia coli (A9660)	1,500	10	PO	0+4	7	14
	900	10	PO	0+4	5	9
Salmonella enteritidis	200	5	PO	0	8	11
	20	20	PO	0+4	7	8

* Mice were infected by the IP route.
** Underlined CD_{50} values were obtained with acid trihydrates; all others with sodium salts.
*** CD_{50} significantly lower than that of ampicillin ($P<0.05$).

The drugs also had comparable ability to control the infection produced by the *Diplococcus pneumoniae* strain, but were without effect at doses up to 500 mg/kg in the *Staphylococcus aureus* 1633-2 (penicillinase-producer) infection.

The *Streptococcus pyogenes* infection also responded well to both compounds, but BL-P 875 showed superiority to ampicillin. The differences in CD_{50} values, although small, are probably meaningful since a P value of <0.05 was obtained in one test and a value of <0.10 in another.

BL-P 875 also gave lower oral CD_{50} values than ampicillin in some of the infections produced by gram-negative bacteria. Data from these experiments are summarized in Table 6.

It is apparent from the results obtained in the experiments with *Klebsiella pneumoniae* that, although the protective effects of the penicillins were comparable when given by the IM route, BL-P 875 was about twice as effective when treatment was

per os. An analysis of data in the last *K. pneumoniae* expreiment indicated that the CD_{50} of BL-P 875 was significantly lower ($P<0.05$) than that of ampicillin.

Orally-administered BL-P 875 also had greater effectiveness than ampicillin in the *Proteus mirabilis* and possibly in the *Escherichia coli* (A9660) infections, but had a similar level of activity in the *E. coli* Juhl and *Salmonella enteritidis* tests.

CD_{50} values for a given compound appear to be similar regardless of whether the acid trihydrate or the sodium salt was utilized.

Table 7 shows that, although the treatment regimen employed for the *K. pneumoniae* (A9977) infection had a dramatic effect on the total quantity of penicillins required for cure, the superiority of BL-P 875 over ampicillin was maintained under all test conditions.

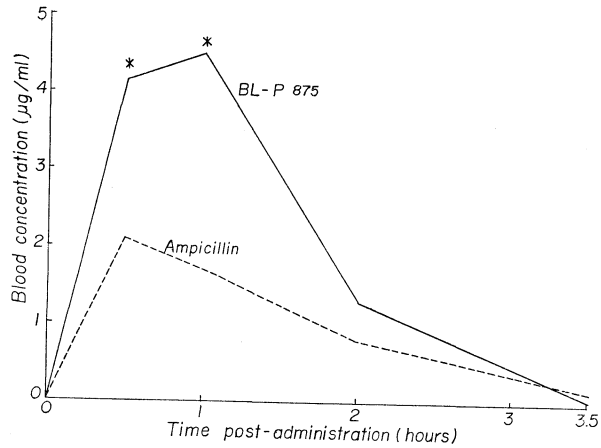
Table 7. Effect of treatment regimen on the therapeutic efficacy of the acid trihydrates of BL-P 875 and ampicillin

No. of mice* per dose level	Treatment		CD_{50} (total mg/kg)	
	Route	Time (hours post-infection)	BL-P 875	Ampicillin
10	PO	0+4	14**	26
10	PO	0	56**	86
10	PO	4	102	146

* All mice were challenged by the IP route with 25 LD_{50} of *Klebsiella pneumoniae* (A9977)

** CD_{50} significantly lower than that of ampicillin ($P<0.05$).

Fig. 4. Average blood concentrations of the sodium salts of BL-P 875 and ampicillin following oral administration of a 20 mg/kg dose. Twenty-four mice were employed for each of the penicillins. The values marked with an asterisk are significantly higher ($P<0.5$) than the corresponding ampicillin values.



The fact that BL-P 875 gave IM CD_{50} values comparable to those of ampicillin (Tables 5 and 6), but was more effective upon oral administration (despite having identical MIC values for the challenge organisms), suggested that the former compound may be more readily absorbed from the gastrointestinal tract.

Several experiments were carried out to investigate the relative oral absorbability of BL-P 875 and ampicillin in the mouse. In the first study, the sodium salts of BL-P 875 and ampicillin were each administered to 24 mice at a dosage of 20 mg/kg. Average blood level concentrations are shown in Fig. 4.

It can be seen that the sodium salt of BL-P 875 gave considerably higher blood level values than the sodium salt of ampicillin at both one-half and one hr post-administration.

In the second study, the acid trihydrates of the compounds were administered as aqueous suspensions. Blood levels obtained when each of the compounds was given orally to 12 mice at a dosage of 30 mg/kg are presented in Fig. 5.

As was found in the preceding experiment, blood concentrations of BL-P 875

Fig. 5. Average blood concentrations of the acid trihydrates of BL-P 875 and ampicillin following oral administration of a 30 mg/kg dose. Twelve mice were employed for each of the compounds. The values marked with an asterisk are significantly higher ($P<0.5$) than the corresponding ampicillin values.

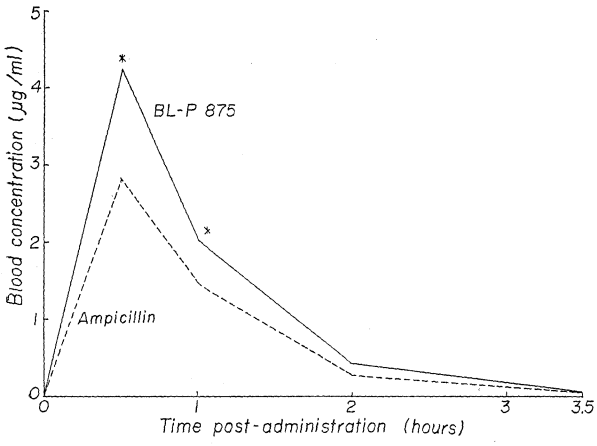


Fig. 6. Average blood concentrations of the sodium salts of BL-875 and ampicillin following intravenous administration of 5.0 and 2.5 mg/kg doses. Twelve mice were employed for each of the compounds at each dose level.

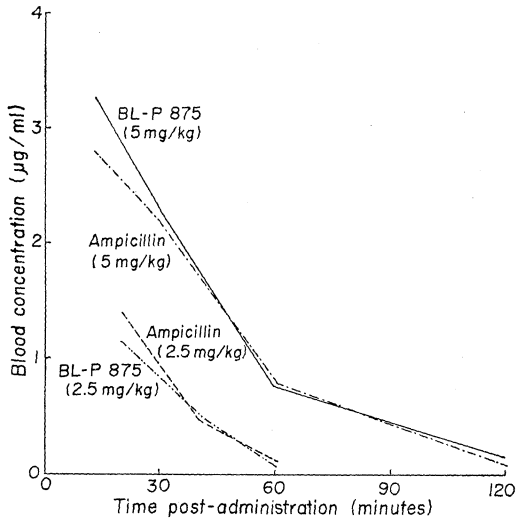


Fig. 7. Average blood concentrations of the sodium salts of BL-P 875 and ampicillin following intramuscular administration of a 5 mg/kg dose. Sixteen mice were employed for each of the compounds.

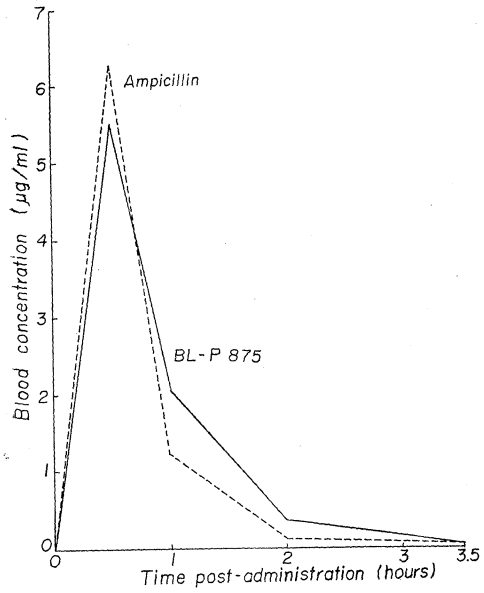
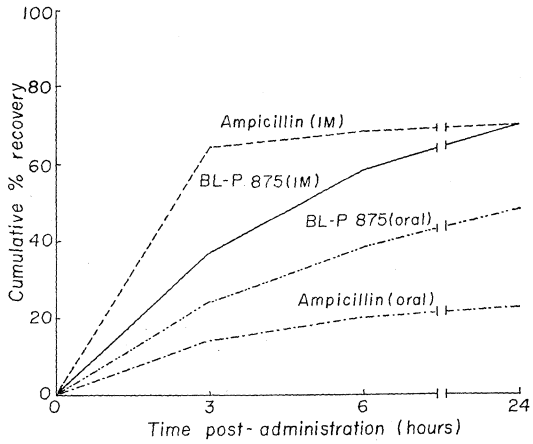


Fig. 8. The cumulative percentage recovery of BL-P 875 and ampicillin from the urine of rats after intramuscular and oral administration of a 20 mg/kg dose. Eight rats were employed for each of the compounds for each route of administration.



were significantly higher than those of ampicillin at both one-half and one hr post-administration, but were only equivalent after this time.

BL-P 875, like most penicillins, proved to have very little toxicity when admini-

stered by the oral route. No gross evidence of toxicity was observed in normal mice even after administration of a 7,500 mg/kg dose.

The relative absorbability of the two penicillins after their parenteral administration to mice was also studied. Fig. 6 shows blood concentrations after IV administration of the sodium salts of the compounds, while Fig. 7 compares levels obtained with the same preparations after their IM administration to mice.

In contrast to results obtained in oral absorption studies, no significant differences in blood concentrations of BL-P 875 or ampicillin could be shown following their IV or IM administration to mice. Evidence of pain on injection or local irritation due to the antibiotics was not noted in these studies or in one where doses as high as 20 mg/kg were injected into the highly sensitive footpad of the rat.

The final experiment to be reported was undertaken to measure, in rats, the relative extent to which BL-P 875 and ampicillin are eliminated via the urinary tract. The cumulative recovery of each of the antibiotics from urine samples collected over a 24-hr period after their IM and oral administration is shown in Fig. 8.

Antibiotic recovery values, particularly those obtained after IM injection, suggest that BL-P 875 may be eliminated at a somewhat slower rate than ampicillin. The total quantity of antibiotic excreted within 24 hr, however, was identical for the two compounds when administration was by the IM route and about twice as high for BL-P 875 as ampicillin after oral administration.

Discussion

BL-P 875, differing chemically from ampicillin only in the nature of its side-chain ring structure, possesses comparable biopotency and has an identical spectrum of antibacterial activity in *in vitro* tests. However, the finding that this thiophene analogue of ampicillin has greater *in vivo* therapeutic efficacy following oral administration suggests that there are significant pharmacological differences between the two penicillins. The maximum extent of the superiority displayed by BL-P 875 in experimental mouse infections is about 2-fold and is not readily apparent with all challenge organisms. Further testing with a greater number of animals at each dosage level might permit differences in responses of such infections to be demonstrated, or it may be that the pharmacological differences between the two antibiotics have no bearing on the response of these particular infections to therapy.

Examination of the absorption properties of the two penicillins shows unequivocally that BL-P 875 gives higher oral mouse blood and dog serum (Bristol Laboratories, unpublished data) concentrations than ampicillin. The finding that the parenterally-administered compounds give similar mouse blood levels and do not differ in their *in vivo* therapeutic effectiveness, demonstrates that, for a given route of administration, there is a good correlation between degree of absorbability and efficacy in experimental mouse infections.

The relative quantities of the two antibiotics recovered from rat urine within a 24-hr period after injection are completely consistent with the differences in peak blood levels produced in the mouse. Yet to be confirmed, however, is the suggestion stemming from the urine antibiotic recovery study, that BL-P 875 is eliminated at a slower rate than ampicillin. This finding could not have been predicted from results of the mouse blood level studies which show that, relative to ampicillin, there is no significant prolongation of BL-P 875 concentrations after IM or oral administration. Furthermore, the antibiotic decay curves after IV administration of the two compounds are essentially superimposable.

If the greater oral absorbability of BL-P 875 that occurs in laboratory animals can also be demonstrated in humans, it would seem desirable to subject this compund to full scale clinical investigation. Any new penicillin having potency equal to, and absorption characteristics superior to those of ampicillin, one of the most widely used and possibly the most versatile of all antibiotics, deserves this consideration.

Acknowledgements

The authors acknowledge the capable technical assistance of ROBERT BUCK, JAMES QUIGLEY, and GRACE WRIGHT. They also wish to extend their appreciation to Dr. V. KADAR for conducting the mouse toxicity study as well as the rat urinary excretion experiments and to Dr. A. W. PIRCIO for carrying out the rat footpad irritation study.

Literature Cited

1) BUTTLE, G.A.H.; P. F. D'ARCY, E. M. HOWARD & D. N. KELLETT: Plethysmometric measurement of swelling in the feet of small laboratory animals. Nature 179 : 629, 1957
2) CAHN, R. S.: An introduction to the sequence rule—a system for the specification of absolute configuration. J. Chem. Education 41 : 116~125, 1964
3) CHENEY, L. C. & Y. G. PERRON: Antibacterial compositions comprising alpha-aminothenylpenicillins. U. S. Patent 3,342,677, 1967
4) GOUREVITCH, A.; G. A. HUNT, J. R. LUTTINGER, C. C. CARMACK & J. LEIN: Activity of α-phenoxyalkyl penicillins against sensitive and resistant staphylococci. Proc. Soc. Exptl. Biol. & Med. 107 : 455~458, 1961
5) GOUREVITCH, A.; T. A. PURSIANO & J. LEIN: Inactivation of staphylococcal penicillinase by reaction with resistant penicillins. Nature 195 : 496~497, 1962
6) GROVE, D. C. & W. A. RANDALL: Assay methods of antibiotics. A laboratory manual. Medical Encyclopedia, Inc. New York, N. Y., 1955
7) LITCHFIELD, J. T., Jr. & F. WILCOXON: A simplified method of evaluating dose~effect experiments. J. Pharm. Exptl. Therap. 96 : 99~113, 1949
8) SCHOLTAN, W. & J. SCHMID: Die Bindung der Penicilline an die Eiweißkörper des Serums und des Gewebes. Arzneimittel-Forsch. 12 : 741-750, 1962
9) STEERS, E.; E. L. FOLTZ & B. S. GRAVES: An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. & Chemoth. 9 : 307~311, 1959
10) WICK, W. E. & W. S. BONIECE: *In vitro* and *in vivo* laboratory evaluation of cephaloglycin and cephaloridine. Appl. Microbiol. 13 : 248~253, 1965