"PENBRITIN"—A NEW BROAD-SPECTRUM ANTIBIOTIC

PRELIMINARY PHARMACOLOGY AND CHEMOTHERAPY

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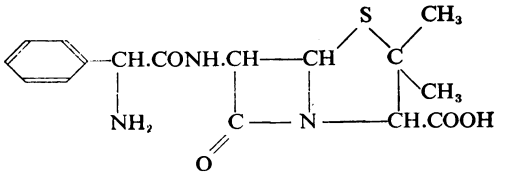
None of the present family of synthetic or natural penicillins possess appreciable activity against Gram-negative bacilli. Those penicillins which are absorbed orally are inactive in this respect. Since the isolation of 6-aminopenicillanic acid (Batchelor *et al.*, 1959), one of the principal objectives has been the development of a new penicillin which is absorbed orally and is effective against Gram-negative bacilli. Doyle and his colleagues have now synthesized a new penicillin, "penbritin" (6[D(−)-α-aminophenylacetamido]penicillanic acid), which, from the biological studies carried out, has been found to possess these properties.

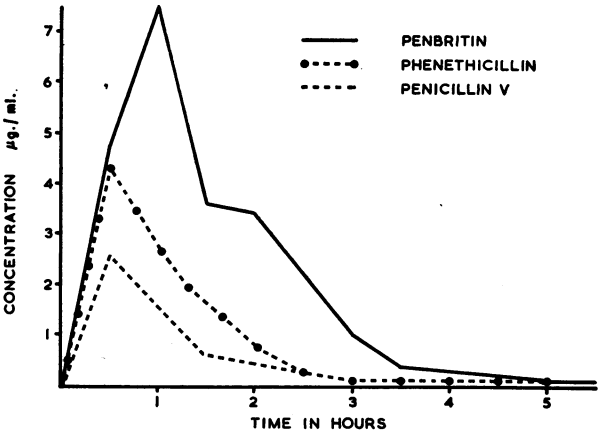


Penbritin; 6[D(−)-α-aminophenylacetamido]penicillanic acid.

Pharmacology

Penbritin (B.R.L. 1341) is non-toxic to mice, rats, dogs, and cats. Single doses up to 5 g./kg. have been administered orally and subcutaneously to mice and rats without observable toxic effects. Intravenously the amount given has been limited by solubility, but as much as 2 g./kg. has been administered to mice without lethal effects, although muscle tremor, slow respiration, and mild clonic convulsions have been noted. Similarly, in cats no adverse effects on the blood-pressure and respiration were seen with doses up to 100 mg./kg. intravenously. A total of 3 g./kg. was administered in divided doses over a period of one hour without toxic effects.

When administered orally to groups of 12 rats over a period of 12 weeks at doses of 500 and 100 mg./kg. no effect on the growth was observed; neither were any biochemical, haematological, or histological abnormalities seen. Penbritin, when administered orally to three dogs, 250 mg./kg. twice daily over a period of four weeks, likewise produced no toxic symptoms, apart from slight loosening of the stools. It gives good blood-levels after oral administration. In dogs, maximum blood-levels of 7.2 μg./ml. have been obtained after 20 mg./kg., the peak occurring one hour after dosing. On the other hand, after 20 mg. phenethicillin and penicillin V per kg. the peak levels which occurred in 30 minutes were 4 and 2.6 μg./ml. respectively (see Chart). In addition, the blood-levels were more persistent with penbritin, this effect having also been noted in humans (Knudsen *et al.*, 1961). After absorption the antibiotic is evenly distributed throughout the body tissues apart from kidney and liver, where higher concentrations than in the serum are found. The antibiotic which is excreted in urine and bile is concentrated considerably in these



Mean serum concentrations in groups of five dogs after oral administration of 20 mg. penbritin, phenethicillin, and penicillin V per kg.

fluids. The concentration in the bile is 300 times and in the urine 800 times that found in the blood. Penbritin is eliminated by the kidney by renal tubular secretion and glomerular filtration in a similar manner to penicillin G and methicillin (Acred *et al.*, 1961).

Chemotherapy

Penbritin has been tested in mice infected with *Staphylococcus aureus* (Smith), *Streptococcus pyogenes* (group A), *Salmonella typhi-murium*, and *Klebsiella pneumoniae*, and compared with penicillin V, phenethicillin, tetracycline, and chloramphenicol. The activity was assessed by determining the dose which cured 50% of a group of 10 mice infected with one of the above organisms. Penbritin, penicillin V, and phenethicillin all had the same order of activity against *Staph. aureus* by the oral route, the CD50 values in each case being 0.3 mg./kg., but tetracycline was fully 10 times less effective, having a CD50 of 5.2 mg./kg.; chloramphenicol was ineffective orally and subcutaneously. Penbritin, penicillin V, and phenethicillin were highly active against *Str. pyogenes*, giving an oral CD50 of 0.1 mg./kg.; tetracycline gave a CD50 of 0.5 mg/kg., while in this instance chloramphenicol was active with a CD50 of 3.2 mg./kg.

Against the Gram-negative infections penbritin was surprisingly effective when compared with tetracycline and chloramphenicol. The CD50 values against *Salm. typhi-murium* were, respectively, 19, 125, and 310 mg./kg., and against *Kleb. pneumoniae* 12, >400, and 165 mg./kg. Penicillin V and phenethicillin were completely inactive.

Conclusions

Penbritin is an effective non-toxic oral penicillin possessing a wide spectrum of activity. Animal studies indicate that it is better absorbed and gives more prolonged blood levels than penicillin V and phenethicillin. Its effectiveness against infections due to staphylococci and streptococci is equal to that of the existing oral penipenicillins. In this respect there is agreement with the *in vitro* activities (Rolinson and Stevens, 1961). On the other hand, the activity of penbritin *in vivo* against infections produced by Gram-negative organisms is considered to

be greater than that found with tetracycline and chloramphenicol. This is in marked contrast to the *in vitro* titres, where only small differences are found.

We thank Mr. F. P. Doyle and his colleagues for the preparation of penbritin.

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ABSORPTION AND EXCRETION OF "PENBRITIN"

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"Penbritin" (6[D(−)-α-aminophenylacetamido]penicillanic acid; B.R.L. 1341) is a new penicillin derived from the penicillin nucleus, 6-aminopenicillanic acid, with a broad spectrum of activity against both Gram-positive and Gram-negative organisms (for full microbiological details, see Rolinson and Stevens, 1961).

This paper describes the investigations undertaken to determine a suitable dosage of penbritin for preliminary clinical trials.

Since penbritin is highly stable in acid medium it could be well absorbed orally. Experiments were therefore designed to investigate the serum concentrations and urinary excretions obtained with various oral doses of the new penicillin.

Methods

Seventeen subjects (10 male and 7 female) aged 19–29 years (mean 25 years), weight 50–91 kg. (mean 65 kg.), took part in this study. Each subject was designated a letter of the alphabet and each retained the same letter throughout the investigations. Except where otherwise stated, all doses were given in the fasting state, the subjects having refrained from taking breakfast until after the two-hour specimen of blood had been withdrawn from the antecubital vein. All specimens were taken with Bayer "O" "venules," and assays were carried out on the freshly separated serum on the same day as the test was performed.

Assay Procedure

Penbritin concentrations were determined by the cup-plate biological assay method using *Sarcina lutea* ATCC 9341 as test organism. Nutrient agar ("oxoid" No. 2) was seeded with 5 ml. of an overnight broth culture per 500 ml. of agar and poured into large assay plates to give an agar depth of approximately 4 mm. Plugs of agar were removed to give holes of 7 mm. diameter, which were filled with the solutions to be assayed. The plates were incubated at 30° C. overnight. The ring diameters were then measured and the concentrations read off in the usual way from the standard line.

Standard solutions of penbritin of 1, 0.5, 0.2, 0.1, 0.05, and 0.02 μg./ml. were used on each assay plate. Standards and unknowns were so arranged on plates as to compensate both for any variations in agar thickness and for the time factor in filling the plate.

The samples to be assayed were diluted to give a concentration of approximately 0.2 μg./ml. The diluent for the unknown samples and for the standard solutions was a 4% solution of bovine plasma albumin fraction V (Armour Pharmaceutical Co.) in M/20 phosphate buffer, *p*H 7. This solution had been found by experiment to have the same effect as human serum on the assays of this penicillin by this method.

Tetracycline concentrations were determined by the cup-plate biological assay method using a strain of *Bacillus mycoides* as test organism. Standard solutions of tetracycline of 20, 10, 5, 2, 1, and 0.5 μg./ml. were used on each assay plate.

The samples to be assayed were plated neat and the diluent for the standard solutions was human serum.

Experiment 1.—A 250-mg. capsule of penbritin was given to 10 subjects; blood samples were taken at ½, 1, 2, 4, and 6 hours after dosing, and the serum was assayed for the concentration of penbritin in terms of μg./ml. The 0–6-hour urine fractions were also collected and assayed for penbritin. Table I sets out the individual and mean results obtained in this experiment.

Experiment 2.—Two 250-mg. capsules were given to seven subjects and a similar procedure was adopted to that in experiment 1. Table II sets out the results obtained in this experiment.

Experiment 3.—Three 250-mg. capsules were given to seven subjects, and the investigation was carried out as in experiments 1 and 2. The results are set out in Table III.

Experiment 4.—Finally, four 250-mg. capsules were given to 10 subjects and a similar procedure was adopted as in the previous experiments. Table IV sets out the results obtained.

Fig. 1 shows graphically the means obtained in the four experiments, and Fig. 2 shows a dose-response curve for the two-hour levels.

TABLE I.—*Serum Concentrations and Urinary Excretion of Penbritin After a Single 250-mg. Dose*

Subject	Dose mg.	Serum Concentrations (μg./ml.)					Urine mg. in 6 hr.
		½ hr.	1 hr.	2 hr.	4 hr.	6 hr.	
A	250	0·3	2·6	2·7	0·5	0·1	83
B	250	1·4	3·5	2·1	0·3	0·08	92
C	250	0·2	2·6	2·3	0·3	0·1	111
D	250	0·7	2·2	2·4	0·5	0·2	51
G	250	1·4	1·8	1·7	0·6	0·3	59
H	250	1·4	2·7	2·0	0·5	0·2	125
K	250	1·0	1·3	1·2	0·7	0·3	108
M	250	1·1	2·8	2·4	0·4	0·2	57
N	250	0·2	0·8	1·3	0·5	0·1	64
P	250	0·4	1·6	1·3	0·3	0·1	71
Mean	..	0·81	2·19	1·94	0·46	0·17	82 (33%)

TABLE II.—*Serum Concentrations and Urinary Excretion of Penbritin After a Single 500-mg. Dose*

Subject	Dose mg.	Serum Concentrations (μg./ml.)					Urine mg. in 6 hr.
		½ hr.	1 hr.	2 hr.	4 hr.	6 hr.	
A	500	1·0	3·1	3·6	0·9	0·2	169
G	500	0·5	2·4	2·8	1·0	0·4	105
L	500	0·02	0·5	3·9	1·0	0·3	63
N	500	0·5	0·9	3·6	1·0	0·2	119
P	500	2·5	4·9	3·2	0·45	0·1	135
R	500	0·18	2·8	4·7	1·0	0·2	135
Z	500	0·5	4·6	4·7	0·8	0·2	158
Mean	..	0·74	2·7	3·8	0·88	0·2	126 (25%)