ABSORPTION AND EXCRETION OF A NEW ANTIBIOTIC (BRL 1241)

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BRL 1241 (" celbenin ") is a new antibiotic derived from the penicillin nucleus (6-aminopenicillanic acid) isolated and characterized in the Beecham Research Laboratories (Batchelor *et al.*, 1959), and chemically it is sodium 6-(2,6 dimethoxybenzamido)penicillanate monohydrate. BRL 1241 has a spectrum similar to that of penicillin G, but in addition to activity against penicillin-sensitive staphylococci it is active against all penicillin-resistant staphylococci at a concentration of 1.25–2.5 μg./ml. (Rolinson *et al.*, 1960). BRL 1241 is both stable and active in the presence of staphylococcal penicillinase.

It has been shown to be non-toxic in animal studies, and single doses of up to 2.5 g./kg. have been given intravenously to male albino mice with complete absence of side-effects (Brown and Acred, 1960).

BRL 1241 is unstable in acid medium and cannot be given orally; it must therefore be administered by the intramuscular or intravenous route.

Before the value of a new antibiotic can be assessed a suitable dosage scheme must be established. This paper describes the investigation carried out to determine this.

Methods

Assay of BRL 1241 in Body Fluids

Antibiotic 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 BRL 1241 of 10, 5, 2.5, 1, 0.5, and 0.25 μg./ml. were used on each assay plate. Standards and unknowns were so arranged on the 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 2.5 μg./ml. The diluent for the unknown samples and for the standard solutions was a 2% 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.

Experiment I

Before any human studies were undertaken the drug had been shown to be non-toxic in animals, and one of us received a test dose of 20 mg. of BRL 1241 in 1 ml. of distilled water by intramuscular injection. No untoward reactions were observed. The dose was gradually increased to 60 mg. in other subjects without complaint. It was therefore decided to investigate the serum concentrations obtained from a single 100-mg. injection (1 ml.) of the sodium salt. The serum levels obtained in three subjects are shown in Table I. It will be seen that the blood levels obtained were too low to be of therapeutic value in view of the sensitivity of the resistant staphylococcus to BRL 1241 (1.25–2.5 μg./ml.).

TABLE I

Subject	Penicillin	Dose	Serum Concentrations (μg./ml.)				
			0 hr.	¼ hr.	½ hr.	1 hr.	2 hr.
1	BRL 1241	100 mg.	0	1·3	2·7	2·6	0·7
2	BRL 1241	100 ,,	0	0·5	1·2	1·5	0·6
3	BRL 1241	100 ,,	0	1·2	3·0	1·3	0·3

Experiment II

The investigation was repeated in three subjects, each receiving 100 mg. of BRL 1241 every two hours for three doses, and in one subject, who received an initial dose of 200 mg. of BRL 1241 and then 100 mg. two-hourly for a further two doses. The object of this experiment was to determine whether repeated doses of 100 mg. of BRL 1241 were still free from side-effects and that an initial dose of 200 mg. could be tolerated.

Urine was collected in two subjects (A and D) over the six-hour period. The results are shown in Table II.

TABLE II

Subject	Penicillin	Route	Time Given	Dose (mg.)	Serum Concentration (μg./ml.)				Urine mg. 0–6 hr.
					¼ hr.	½ hr.	1 hr.	2 hr.	
A	BRL 1241	I.M.	10 a.m.	100	1·4	2·2	1·4	0·3	
			12 noon	100	2·2	2·7	2·0	0·4	
			2 p.m.	100	2·0	2·5	2·2	0·3	186
B	BRL 1241	I.M.	10 a.m.	100	1·1	2·7	1·5	0·3	
			12 noon	100	2·8	3·0	2·0	0·4	
			2 p.m.	100	2·5	3·1	3·6	0·8	
C	BRL 1241	I.M.	10 a.m.	100	1·7	2·4	1·2	0·3	
			12 noon	100	1·7	2·3	1·8	0·5	
			2 p.m.	100	1·0	2·2	1·4	0·3	
D	BRL 1241	I.M.	10 a.m.	200	1·7	3·1	4·1	1·5	
			12 noon	100	2·1	2·7	2·8	0·8	
			2 p.m.	100	2·0	1·9	2·5	0·8	245

In subjects A and D the urinary excretion of BRL 1241 over the six-hour period was 186 mg. and 245 mg., equivalent to 62% and 61% of the respective total doses. Side-effects were not noted, and the antibiotic appeared to be excreted in a manner similar to penicillin G.

It will be seen from the Table that the serum concentrations obtained would be inadequate for treatment purposes.

It was decided to attempt a longer-term study, preferably in a patient suffering from resistant staphylococcal infection, and to obtain serum concentration and urinary excretion data together with bacteriological study of the infecting organism. At this stage sufficient material was available for a 24-hour treatment only, but it was felt that the information obtained would be invaluable for future clinical studies.

Clinical Study I

A man aged 28, weighing 85 kg., had a secondary penicillin-resistant staphylococcal infection of the right hallux after surgical treatment. He was treated with BRL 1241. The prime object of this study was to

ascertain at what dosage adequate blood levels could be obtained and over what period of time these levels were likely to be present. Of secondary importance was the assessment of the success of the treatment, as the patient was treated for only about 24 hours. The dosage

TABLE III

Time	Blood	Urine
Zero	Sample 1 (control)	Empty Bladder, Discard Specimen
Intramuscular injection of 300 mg. of BRL 1241 (1·5 ml.)		
10 min.	Sample 2	
20 ,,	,, 3	
30 ,,	,, 4	
40 ,,	,, 5	
50 ,,	,, 6	
60 ,,	,, 7	
70 ,,	,, 8	
80 ,,	,, 9	
90 ,,	,, 10	
100 ,,	,, 11	
110 ,,	,, 12	
120 ,,	,, 13	
Intramuscular injection of 400 mg. BRL 1241 (2 ml.)		
2½ hr.	Sample 14	
2¾ ,,	,, 15	
2¾ ,,	,, 16	
3 ,,	,, 17	
3¼ ,,	,, 18	
3½ ,,	,, 19	
4 ,,	,, 20	0–4 hr. urine collected
Intramuscular injection of 400 mg. BRL 1241 (2 ml.)		
4½ hr.	Sample 21	
5 ,,	,, 22	
6 ,,	,, 23	
Intramuscular injection of 300 mg. BRL 1241 (some doubt about whether actually given—see curve, Fig. 1)		
7 hr.	Sample 24	
8 ,,	,, 25	4–8 hr. urine collected
Intramuscular injection of 300 mg. BRL 1241 (2ml.)		
9 hr.	Sample 26	
Midnight		8–12 hr. urine collected
Intramuscular injection of 300 mg. BRL 1241		
4 a.m. Intramuscular injection of 300 mg. BRL 1241		
8 ,,		12–20 hr. urine collected
Intramuscular injection of 300 mg. BRL 1241		

scheme, with times of collection of blood and urine samples, is shown in Table III and Fig. 1.

It will be seen that a total of 2.6 g. was administered over a 20-hour period (there is some doubt about whether the six-hour dose of 300 mg. was in fact given, as this dose is in no way reflected in the blood-level curve—Fig. 1).

The total amount excreted in the urine was 905 mg. This amount was excreted over a period of 20 hours (not including the excretion of the final dose). For purposes of calculation a total of 2 g. was administered and 905 mg. excreted—that is, 45% of the dose. The maximum serum concentration was 9.5 μg./ml. on this scheme of dosage. It will be seen from the serum concentration curve that two-hourly injections tended to result in a "build up" of BRL 1241 in the serum.

Swabs taken from the wound before treatment showed the presence of resistant staphylococci. Swabs taken 24 hours after the cessation of treatment with BRL 1241 showed the absence of staphylococci, and the wound appeared cleaner. After treatment no abnormalities were noted in the blood count or in liver- or renal-function tests.

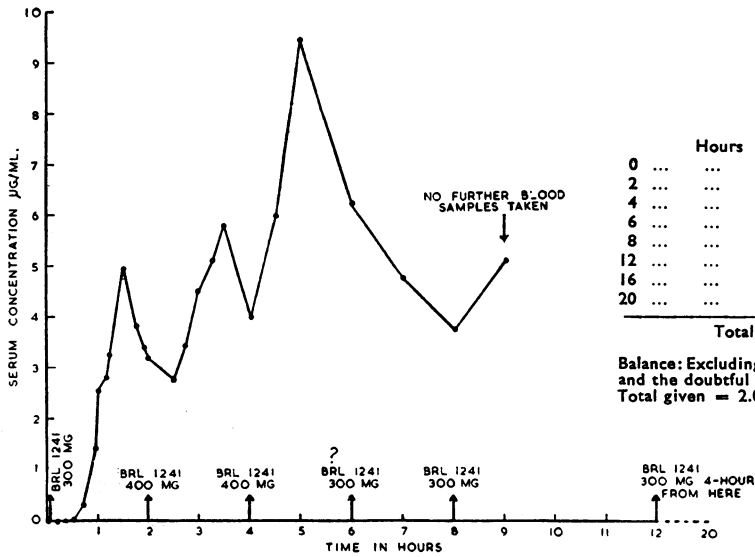
It was concluded that treatment at this dosage would be inadequate, since the serum levels obtained were not particularly high and the frequency of the injections would, in any case, be prohibitive.

Clinical Study II

A girl aged 17, weighing 50 kg., developed pneumonia at the base of the right lung with a pleural effusion after repair of atrial septal defect. Chest *x*-ray examination confirmed the pulmonary condition, and a specimen of the pleural fluid yielded a profuse growth of *Staph. pyogenes* (coagulase-positive). The organism was resistant to penicillin, sulphonamides, and streptomycin, and slightly sensitive to chloramphenicol, erythromycin. and achromycin.

The patient failed to respond to chloramphenicol and erythromycin, and treatment was changed to ristocetin intravenously. On this treatment her total white-cell count fell from 16,000 to 4,900, of which only 49% were polymorphonuclear leucocytes. Her temperature remained at 102° F. (38.9° C.), and her condition was deteriorating.

It was decided to treat her with BRL 1241. The first dose of 450 mg. (1.5 ml.) was given intramuscularly, followed two hours later by 600 mg. intramuscularly; then, as she was receiving intravenous fluids, 750 mg. of BRL 1241 in 50 ml. of glucose-saline, run in over 30 minutes, was given three-hourly for two days. Later the rate of infusing BRL 1241 was raised and 1 g. was given over a period of 15 minutes every three hours. As intravenous treatment was subsequently discontinued,



Hours	BRL 1241 mg.	BRL 1241 mg. in Urine
0	300	—
2	400	—
4	400	80
6	?300	—
8	300	210
12	300	105
16	300	—
20	300	510
Total	2,600	905

Balance: Excluding the 20-hr. injection, which could not have been excreted, and the doubtful 6-hr. injection, then:
Total given = 2.0 g. Total excreted = 0.905 g. = 45% of total dose.

FIG. 1.—Dosage scheme with times of collection of blood and urine samples.

she was given 1 g. of BRL 1241 every four hours by intramuscular injection. The latter solutions contained BRL 1241 at a concentration of 500 mg./ml. The serum concentrations and urinary excretions obtained from this study are shown in Table IV.

Thirty-six hours after the administration of BRL 1241 her temperature fell to normal, and an *x*-ray film of the chest taken two days after the beginning of treatment showed partial clearing of the basal pneumonia. The patient continued on BRL 1241 for a further 14 days, receiving a total of 100 g. altogether. No toxic side-effects were observed and no local reactions occurred at the site of the injection. If given slowly by deep intramuscular injection, BRL 1241 was no more painful than penicillin G.

TABLE IV

Time	Dose (mg.)	Route	Serum Concentration (μg./ml.)	Urine
Day 1:				
4.00 p.m.			0	Day 1: 4–9.30 p.m., 0·9 g. excreted out of 1·8 g. administered
4·20 ,,	450	I.M.	—	
4.40 ,,			22 0	
5.10 ,,			17·0	
6.10 ,,			14·5	
6.20 ,,	600	I.M.		
7.00 ,,			18·0	
7.20 ,,			17·0	
7.50 ,,			14·5	
8.00 ,,	750	I.V. over 30 min.		
9.00 ,,			46 0	
9.30 ,,			42 0	
11.00 ,,	750	,,		
Day 2:				
2.00 a.m.	750	,,		Day 1–2: 9.30 p.m.–5 a.m., 2·25 g. excreted out of 2·25 g. administered
5.00 ,,	750	,,		
8.00 ,,	750	,,		
10.55 ,,			8·7	Day 2: 5 a.m.–1.30 p.m. 0·9 g. excreted out of 1·5 g. administered
11.00 ,,	750	,,		
12.00 noon			30.0	
12.30 p.m.			17 0	
1.30 ,,			10·0	
2.00 ,,	750	,,	No further blood samples taken	Day 2–3: 2 p.m.–8 a.m. 1·6 g. excreted out of 4·5 g. administered. (Last dose at 8 a.m., excluded from calculation)
5.00 ,,	750	,,		
8.00 ,,	750	,,		
11.00 ,,	750	,,		
Day 3:				
2.00 a.m.	750	,,		
5.00 ,,	750	,,		
8.00 ,,	750	,,		

Total BRL 1241 given, 10·05 g. Total BRL 1241 excreted, 5·65 g. (56%).

Fourteen days after the cessation of treatment with BRL 1241 the patient died in cardiac failure associated with heart block. Post-mortem findings showed an atrial septal defect to be present, with vegetations and pus over the suture site. Swabs taken at the time of the post-mortem examination showed no evidence of a recurrence of the staphylococcal infection, but coliforms were isolated from most organs.

It appeared from these initial studies that a dose of 1 g. four-hourly would probably provide adequate serum concentrations for therapeutic trials, and, to confirm this, 12 subjects were given 1 g. of BRL 1241 (2 ml.) intramuscularly. The individual and mean serum concentrations obtained are shown in Table V and Fig. 2.

TABLE V.—*Serum Concentration in 12 Subjects After Injection of 1 g. BRL 1241 intramuscularly*

Subject	Serum Concentration (μg./ml.)						Urine mg.
	0 hr.	½ hr.	1 hr.	2 hr.	4 hr.	6 hr.	0–6 hr.
A	0	16·0	16·5	4·7	0·7	0·2	735
B	0	9·7	16·8	7·1	1·8	0·2	
C	0	7·9	11·4	7·6	2·2	0·2	
D	0	21·8	12·8	4·8	0·5	0·2	
E	0	19·8	14·5	3·4	0·2	0·2	625
F	0	15·1	11·5	2·4	0·5	0·2	
G	0	27·3	16·3	4·4	0·3	0·2	
H	0	16·1	11·3	4·8	0·4	0·2	702
J	0	26·5	25·8	6·8	0·8	0·2	
K	0	16·9	20·3	6·8	1·4	0·2	
L	0	19·5	15·5	7·0	1·3	0·3	
M	0	13·8	10·5	3·8	0·6	0·2	
Average	0	17·6	15·3	5·3	0·9	0·2	68%

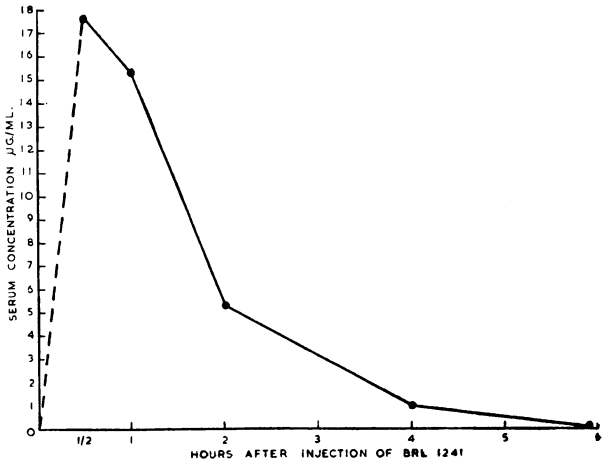


FIG. 2.—Mean serum concentrations in 12 subjects after intramuscular injection of 1 g. of BRL 1241.

Clinical Study III

A man aged 58, who for many weeks had suffered from multiple boils from which resistant staphylococci were isolated, was treated for three days with 1 g. of BRL 1241 (2 ml.) four hourly, followed by a further three days' treatment of 1 g. of BRL 1241 six-hourly. Previous treatment with chloramphenicol and erythromycin had been unsuccessful. Blood and urine samples were taken during the course of treatment, and Table VI shows the serum concentrations of BRL 1241 obtained 46% of the dose administered was excreted in the urine over a 24-hour period.

TABLE VI.—*Serum Concentrations Over a Six-hour Period and Amount of Dose Excreted Over a 24-Hour Period During Treatment with BRL 1241 by Intramuscular Injection of 1 g. Six-hourly*

Subject	Serum Concentration (μg./ml.)						Urine
	½ hr.	1 hr.	2 hr.	4 hr.	5 hr.	6 hr.	
X	20·3	13·4	4·4	1·6	0·5	0·3	

Urine

(1) 3 p.m.	Discard
(2) 3–9 p.m.	364 mg.
(3) 9 p.m.–3 a.m.	1,050 ,,
(4) 3 a.m.–3 p.m.	450 ,,
Total excreted over 24-hour period, (approximately 46·5%) ..	1,864 ,,

At the end of the six-day period there was complete resolution of all the boils, and the patient was discharged from hospital ; there has been no recurrence.

Summary and Conclusions

Investigations were carried out on a new antibiotic (BRL 1241) to determine a suitable scheme of dosage for future therapeutic studies.

The results of the investigations on the absorption and excretion of BRL 1241 showed that a dosage of 1 g. (2 ml.) of the sodium salt, four- to six-hourly by intramuscular injection, would be satisfactory for future therapeutic trials in adults. The antibiotic was well absorbed and was excreted in the urine in a similar manner to penicillin G. No toxic side-effects were noted and no local reactions were observed at the site of injection. If given slowly by deep intramuscular injection BRL 1241 was no more painful than penicillin G.

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"Celbenin" is a registered trade name of Beecham Research Laboratories Limited.

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REPORT ON CLINICAL USE OF BRL 1241 IN CHILDREN WITH STAPHYLOCOCCAL AND STREPTOCOCCAL INFECTIONS

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As in most hospitals, we have experienced in recent years a number of severe infections due to *Staphylococcus aureus*, some of which have been virtually intractable. In such infections the outcome depends to a large extent upon the coincidence of whether or not the organism is sensitive to benzylpenicillin; we would agree with our colleagues at Guy's Hospital (Douthwaite and Trafford, 1960) that some of the newer antibiotics are of limited use in this field.

We therefore followed with interest the isolation of synthetic derivatives of 6-aminopenicillanic acid by Batchelor *et al.* (1959). When sodium 6-(2,6 dimethoxybenzamido)penicillanate monohydrate (BRL 1241; "celbenin") was presented to us, together with some preliminary information, by Drs. Knudsen and Rolinson, we conducted a number of tests *in vitro* upon pathogenic organisms isolated in this hospital (Stewart, 1960). The results of these tests, particularly the activity of the compound against penicillinase-forming staphylococci, convinced us that the new development was of therapeutic importance, and we therefore embarked upon a clinical trial, reported here, in children with staphylococcal and streptococcal infections.

Methods

BRL 1241 was supplied to us, for the clinical evaluation, as a sterile solution containing 500 mg. base per ml. On the basis of a few preliminary results, we adopted as standard dosage a regime of 100 mg./kg./day, given intramuscularly in four equal six-hourly doses, for five days at least. In one child (Case 1) with septicaemia we gave 150 mg./kg./day in six four-hourly doses during seven days of a 30-day course. All injections were given in dry-sterilized, silicone-coated syringes and, in this trial, no diluents or additives were included in the injections. No other systemic antibacterial drugs were given along with BRL 1241, and, in all except four cases (indicated below), no local antibacterial substances were used. Between injections, the solution of drug was kept at 4° C. or, where practicable, frozen solid.

To ensure uniformity of procedure, the detail of organization of this trial was left in the hands of one person, who consulted with his colleagues regarding the eligibility and assessment of each individual case. Our aim was to design a trial in which the efficacy (or otherwise) of BRL 1241 could be assessed by elimination of the pathogen and parallel clinical improvement in cases showing, so far as was possible, the minimum of complicating factors. We excluded from our series cases in which infection may have been passive, and indeterminate cases with mixed infections, though, in fact, several such cases were treated.

With these reservations during the trial, there was no departure from individual practice with regard to diet, hydration, etc. The seven cases with infection due to *Staph. aureus* type 80, and a few others, were nursed throughout in isolation cubicles.

In each of our cases the responsible pathogen was isolated before treatment and assayed for sensitivity to BRL 1241, among other substances. Further specimens and swabs were taken during and after treatment, as often as practicable. Leucocyte counts, E.S.R.s, haemoglobin estimations, and urine examinations were performed as usual. The special laboratory methods used in the assessment of sensitivity of organisms *in vitro* to BRL 1241 and in the assay of the drug in body fluids are described by Stewart, 1960.

Staphylococcal Infections

Seventeen children with various forms of infection due to *Staph. aureus* were treated with BRL 1241. In each case the organism was isolated on one or more occasions before treatment and was assayed for sensitivity to BRL 1241 as well as to our standard range of antibiotics. In each instance, as described by Stewart (1960), the organism was sensitive to therapeutic levels of BRL 1241 which had a strong bactericidal effect in concentrations of 2–5 μg./ml. Thirteen of the 17 infecting strains were resistant to benzylpenicillin (10 μg./ml. or more), and seven belonged to phage type 80. Twelve of the patients had received treatment with one or more antibiotics prior to receiving BRL 1241.

The results (Table I) showed that there was clinical improvement, of varying degree, with elimination of the