Carbenicillin Resistance in *Pseudomonas aeruginosa* from Clinical Material

J. H. DARRELL,* M.B., M.C.PATH., D.C.H.; PAMELA M. WATERWORTH,† F.I.M.L.T.

British Medical Journal, 1969, 3, 141-143

Summary: Strains of *Pseudomonas aeruginosa* resistant to 256 µg. per ml. or more of carbenicillin (Pyopen) were isolated from 17 patients over a period of three months. The infections were not solely due to cross-infection. Low dosage and attempted eradication of the organism from inaccessible sites, such as the bronchi or skin surfaces, by using systemic treatment are two possible causes. Restraint in treating infections of doubtful importance and the use of local applications to appropriate sites with or without systemic treatment is suggested.

Introduction

Carbenicillin (disodium α-carboxybenzyl penicillin; Pyopen) has shown considerable promise in the treatment of infection with *Pseudomonas aeruginosa*. Not only is it effective in urinary tract infections but some success has been reported in the prevention and even the treatment of the much more difficult systemic infections caused by this organism (Brumfitt, Percival, and Leigh, 1967; Van Rooyen, Ross, Bethune, and MacDonald, 1967; Richardson, Spittle, James, and Robinson, 1968; Stratford, 1968). We believe that bacteriological cure of a case of pseudomonas endocarditis has been achieved in this hospital with the drug.

In the past three months we have isolated an increasing number of strains of *Ps. aeruginosa* resistant to carbenicillin. The purpose of this report is to examine the likely reasons for this developing resistance and to suggest possible ways of minimizing it.

Materials and Methods

The strains reported were isolated over a period of three months in routine clinical laboratory work. Initial sensitivity tests were carried out by Stokes's (1968) method, on 5% lysed horse blood agar, a 100-µg. disc being used. A sensitive strain of *Ps. aeruginosa* (minimum inhibitory concentration for carbenicillin 32 µg./ml.) was used as the control organism.

Minimum inhibitory concentrations of the drug for strains showing increased resistance in the disc test were determined by use of a plate dilution technique. A light inoculum (1 in 1,000 dilution of an overnight broth culture) was tested on Oxoid Columbia agar by means of a multiple inoculating device. The minimum inhibitory concentrations of 45 sensitive strains isolated during the same period were also determined. The control strain was included with each batch of tests.

Habituation to carbenicillin of two sensitive strains of *Ps. aeruginosa* (1a and 4a) was investigated in vitro. Serial transfers were performed daily for five days in 5-ml. volumes of tryptone soya broth (Oxoid) containing doubling concentrations of carbenicillin up to 1,024 µg./ml. The inoculum was one standard loopful (0.004 ml.) of the undiluted overnight culture. Minimum inhibitory concentrations for the parent strains and the final cultures were determined as above.

The effect of maintaining organisms by daily subculture for 10 days in a lower concentration of carbenicillin (25 µg./ml.)

was tested, the same strains being used. This concentration was felt to be of the order likely to be attained in the blood as a result of inadequate treatment. This effect was also tested with ampicillin 25 µg./ml. under the same conditions.

Resistant strains were transferred 10 times in broth without antibiotic to determine whether resistance was lost in subculture. After isolation strains were preserved in liquid nitrogen and subcultured as little as possible before minimum inhibitory concentrations were determined.

The organisms were typed by the pyocine-typing method with Gillies and Govan's (1966) set of indicator strains to see whether the observed resistance was due to selection during treatment or whether the resistant strain was a different one acquired by cross-infection.

Results

Table I shows the distribution of minimum inhibitory concentrations of carbenicillin for 45 sensitive strains of *Ps. aeruginosa*. In 34 this was 32-64 µg./ml. A light inoculum was used, as results are known to be considerably influenced by inoculum size (Brumfitt *et al.*, 1967), presumably due to the presence of a minority population of resistant cells. Strains with minimum inhibitory concentrations of 256 µg./ml. or more have been recorded as resistant.

TABLE I.—Activity of Carbenicillin Against Sensitive Strains of *Ps. aeruginosa* (45 Strains)

No. of Strains with Minimum Inhibitory Concentration (µg./ml.) of:						
1,024	512	256	128	64	32	16 or <16
—	—	—	6	15	19*	5

* Including one strain with one or two colonies persisting up to 512 µg./ml.

Data relating to 17 such strains isolated from patients are presented in Table II. The strains were isolated from patients in 10 different wards on more than one occasion in every case. In all but two of the 17 patients an antibiotic of the penicillin group had been given before the resistant organism appeared. In five carbenicillin had been used and resistance was first seen during the course of treatment. In another seven the antibiotic used was ampicillin. In six of these seven patients the first strain of *Pseudomonas* isolated was highly resistant to carbenicillin, as it was in two others, one of whom had received benzylpenicillin with cloxacillin and the other methicillin. Even by the comparatively crude test of pyocine typing the strains were not all the same. Sensitive strains were isolated from six patients before resistance appeared; in four they were of the same pyocine type as the resistant strain isolated subsequently. In the other two cases the resistant strain was of a different type and was isolated from a different site from that first involved. Among the resistant strains appearing de novo, type 10 was relatively common, but at least four other types were represented.

Of the two sensitive strains passaged in increasing concentrations of carbenicillin, the minimum inhibitory concentration for the drug increased at least 16-fold in one case and 32-fold in the other. In both cases it came to exceed 1,024 µg./ml. When the same strains were maintained in a constant concentration

* Senior Lecturer, Department of Bacteriology.
† Senior Demonstrator in Chemotherapy.
Royal Postgraduate Medical School W.12.

of carbenicillin (25 µg./ml.) a fourfold rise in minimum inhibitory concentration occurred in one case and a 16-fold rise in the other. The corresponding increases with ampicillin were twofold and fourfold respectively.

TABLE II.—Strains Isolated During Treatment with Carbenicillin

Case No.	Site of Isolation	Antibiotics Previously given	M.I.C. (µg./ml.) Carbenicillin	Pyocine Type
<i>Strains Isolated during Treatment with Carbenicillin</i>				
1. Mitral valve replacement	Wound swab	Penicillin, streptomycin, kanamycin	64	1/8
	Sputum	Carbenicillin 20 g. daily	1,024	10
2. Double valve replacement	Tracheostomy wound	Penicillin, streptomycin	64	1
		Carbenicillin 10→20 g. daily	1,024	1
3. Intestinal obstruction	Abdominal wound	Nil	32	1/8
		Carbenicillin 12 g. daily	256	1/8
4. Mitral valve replacement	Tracheostomy wound	Penicillin, streptomycin	32	1/8
		Carbenicillin 4 g. daily	256	1/8
5. Carcinoma of colon	Urine	Nil	64	NT
	Abdominal wound	Carbenicillin 4→8 g. daily	1,024	10
<i>Strains from Patients not Treated with Carbenicillin</i>				
6. Respiratory failure	Sputum	Nil	32	1/2
		Ampicillin	256	1/2
7. Carcinoma of bronchus	Sputum	Ampicillin	256	1
8. Urinary infection	Sputum	Ampicillin	1,024	UTa
9. Indwelling catheter	Urine	Ampicillin	1,024	10
10. Chronic bronchitis (#femur)	Sputum	Ampicillin	1,024	10
11. Cushing's syndrome (pituitary implant)	Sputum	Ampicillin, trimethoprim	> 1,024	10
12. Multiple injuries	Tracheostomy suckings	Ampicillin	1,024	10
13. Mitral valve replacement	Tracheostomy suckings	Penicillin, cloxacillin	1,024	10
14. Bronchopleural fistula	Wound swab	Methicillin	1,024	10
15. Rheumatoid arthritis (S.B.E.)	Sacral sore	Penicillin, 15 mega daily	256	UTb
16. Acute renal failure	Urine	Nil	512	NT
17. Carcinoma of bladder	Urine	Nil	512	1

NT = Non-typable. UTa and UTb = Two different strains showing inhibition but not corresponding to a known pyocine type.

The results of subculture of 12 resistant strains in medium free of antibiotic are summarized in Table III. In six of the 12 strains there was a fall in resistance. In only one was resistance completely lost. This loss of resistance was also seen

TABLE III.—Fold Decrease in Resistance to Carbenicillin of 12 Strains of *Ps. aeruginosa* After 10 Transfers in Antibiotic-free Broth

Fold decrease No. of strains	0	-2	-4	-8	-16	-32	-64
	6	2	2	1	0	0	1

in vivo but not in vitro, with the strain from Case 4. The organism was not eradicated by treatment; it became resistant, but reverted to full sensitivity after treatment was stopped. The pyocine type remained the same throughout. In five strains reduction in resistance was moderate (fourfold to 16-fold), the minimum inhibitory concentration remaining at 256 µg./ml. or above in three of these. In the remaining six strains there was no change.

Discussion

The greatest potential use of carbenicillin lies in its activity against *Ps. aeruginosa*. The level of this activity is low compared with that against other Gram-negative rods, the minimum inhibitory concentration for *Ps. aeruginosa* being 32–64 µg./ml.

as against 2–4 µg./ml. for *Escherichia coli* and non-penicillinase-producing strains of *Proteus mirabilis*. The toxicity of other drugs active against *Ps. aeruginosa*, and in the case of the polymyxins their inexplicably poor effect in vivo, makes even this degree of activity (necessitating high doses often requiring intravenous administration) nevertheless useful. That *Ps. aeruginosa* is already showing high levels of resistance to carbenicillin is disturbing.

Increased resistance has been seen during treatment with carbenicillin, but otherwise resistant strains have appeared most commonly after treatment with other antibiotics, usually penicillins. When it occurred during carbenicillin treatment two mechanisms seemed to be operating. Inadequate dosage was not the sole cause, though this is a potent method of selection of resistant mutants, and the necessity for very large doses of this antibiotic for pseudomonal infections other than of the urinary tract does not appear to be widely enough known. In Cases 2, 3, and 4 the resistant organism was of the same pyocine type as the sensitive strain previously isolated, and resistant mutants, existing as a minority population, have probably been selected by low concentrations of the drug. In Case 4 the dose was clearly too low. In Case 3 it was felt to be adequate because of the small size of the patient, but may not have been. After doubling the dose to 20 g. Case 2 had a blood carbenicillin level of 1,125 µg./ml. This would be considered more than adequate for the treatment of systemic infections and to prevent the selection during treatment of resistant mutants, some of which (Cases 3 and 4) show resistance of only a moderate order. Nevertheless, it raises another point—namely, how far are blood levels relevant to surface infection or contamination of skin lesions (for example, surgical wounds or pressure sores), particularly with highly resistant strains with minimum inhibitory concentrations in excess of 1,000 µg./ml.

In the other two patients (Cases 1 and 5) the resistant organism was of a different pyocine type from that originally isolated and was present at a different site. In Case 1 it appeared in the sputum, another site where it may be difficult to achieve high levels of antibiotics in the absence of acute inflammation. In Case 5 successful treatment of a urinary infection with the lower dose regimen was followed by increased resistance of a different strain present in the patient's operation wound. Stratford (1968) reported an exactly similar episode.

The reason for the appearance of carbenicillin-resistant strains of *Pseudomonas* following treatment with other penicillins is not clear. Although we record 10 such cases, the majority following ampicillin, it is not an invariable occurrence. At least as many patients have acquired sensitive strains under the same circumstances. Preliminary experiments indicate that ampicillin is not an efficient agent for increasing resistance to carbenicillin in vitro. We are investigating penicillinase production of strains before and after passage in both carbenicillin and ampicillin. Garrod (1951), however, reported the apparent stimulating effect of penicillin on *Ps. aeruginosa*, and our resistant strains may have followed a relatively non-specific selective action. Half the strains were of one pyocine type (type 10), and the two strains acquired during carbenicillin treatment were also of this type. Though these infections appeared over a short period in widely separated wards, cross-infection may have played a part but was not the sole explanation.

Ps. aeruginosa may infest a site without causing progressive infection. The possibility of eventual blood-stream invasion is worrying, and if not a hazard to the patient the organism constitutes a source of infection for others. The decision to treat or not to treat is a difficult one, and possibly it should more often be the latter. If systemic carbenicillin treatment is chosen nothing less than full dosage (20–30 g. daily) should be used except for urinary infections. Stratford (1968) recommends that if there is any doubt that the infection is not completely localized to the urinary tract the higher dosage schedule should be used here also. Even full doses with or

without the use of probenecid may, however, fail to produce adequate levels at skin surfaces and in the bronchial tree. We have also seen an anuric patient in whom *Pseudomonas* persisted in the bladder despite a blood carbenicillin level of 750 µg./ml.

Perhaps the most fruitful approach to this problem would be the application of suitable topical agents, but not carbenicillin, to any potentially infected accessible sites. Such treatment reduces dissemination from colonized sites and, used in conjunction with systemic treatment, might reduce the emergence and spread of carbenicillin-resistant strains. Though we have been unable to show convincing synergy between carbenicillin and gentamicin, their combined use might be justified to prevent the selection of resistant mutants.

We wish to thank our clinical colleagues under whom the patients referred to were admitted, Miss Audrey Fryer-Kelsey for her technical assistance, and Mrs. Beryl Devenyi for pyocine-typing the strains.

REFERENCES

- Brumfitt, W., Percival, A., and Leigh, D. A. (1967). *Lancet*, 1, 1289.
 Garrod, L. P. (1951). *British Medical Journal*, 1, 205.
 Gillies, R. R., and Govan, J. R. W. (1966). *Journal of Pathology and Bacteriology*, 91, 339.
 Richardson, A. E., Spittle, C. R., James, K. W., and Robinson, O. P. W. (1968). *Postgraduate Medical Journal*, 44, 844.
 Stokes, E. J. (1968). *Clinical Bacteriology*, 3rd ed., p. 179. London: Arnold.
 Stratford, B. C. (1968). *Medical Journal of Australia*, 2, 890.
 Van Rooyen, C. E., Ross, J. F., Bethune, G. W., and MacDonald, A. C. (1967). *Canadian Medical Association Journal*, 97, 1227.

Effects of β -Adrenergic Receptor Blockade on Airway Conductance and Lung Volume in Normal and Asthmatic Subjects

P. S. RICHARDSON,* B.M., B.SC.; G. M. STERLING,† M.B., M.R.C.P.

British Medical Journal, 1969, 3, 143-145

Summary: Ten normal and five asthmatic subjects were given intravenous injections of 10 ml. of 0.9% saline with and without 10 mg. of propranolol, and specific airway conductance was followed in a whole-body plethysmograph. Saline alone had no significant effect on conductance in either group of subjects. Propranolol had no significant effect on conductance in normal subjects, but caused a mean fall of 34.9% in the asthmatics.

Introduction

Blockade of β -adrenergic receptors by propranolol in asthmatic subjects often causes wheezing and a pronounced decrease in vital capacity (V.C.) and forced expiratory volume in one second (F.E.V.₁) (McNeill, 1964; Langer, 1967). The effect of β -blockade on the airways of normal subjects is less certain: Zaid and Beall (1966) found no change in F.E.V.₁ after propranolol, and Marcelle *et al.* (1968) obtained the same result, using the interrupter method to measure airway resistance. On the other hand, McNeill and Ingram (1966) and MacDonald *et al.* (1967), who used the more sensitive method of whole-body plethysmography, reported a significant increase in airway resistance in normal as well as asthmatic subjects after intravenous propranolol. This question is examined further in the present paper, in which the effects of propranolol on airway calibre of normal and asthmatic subjects are compared.

Subjects and Methods

Ten normal adults with no history or signs of chest disease and five asthmatic subjects were studied. The latter were all clinical students or junior medical staff who agreed to take part in the trial after being informed of the possible effects of propranolol on airway resistance in asthmatics. At the time of testing they were all symptom-free, had had no major attack of asthma for at least three months, and had a resting F.E.V.₁ of more than 2 litres.

Airway resistance (Raw) and thoracic gas volume (Vtg) were measured by means of a constant-volume whole-body plethysmograph (DuBois *et al.*, 1956a, 1956b). During the measurements the subjects performed shallow panting at about two breaths per second through a Fleisch pneumotachograph head, and flow and pressure signals were displayed as vector loops, the slopes of which were measured by visual alignment with a rotating Perspex disc mounted in front of the oscilloscope screen (Sterling, 1968). Each value of Raw was derived from the average of five successive short periods of panting after the first two had been discarded. Vtg was measured by the method of DuBois *et al.* (1956a) during attempted gentle respiratory efforts made against a shutter occluding the airway at the mouth. After subtraction of 0.45 cm. H₂O/l./sec. for the resistance of the pneumotachograph head and mouth-piece, Raw was converted to its reciprocal, airway conductance (Gaw), and results are expressed as changes in the ratio:

$$\frac{Gaw}{Vtg} = \text{Specific airway conductance (SGaw)}.$$

Experiments on each subject were done on two days, on one of which 10 mg. of propranolol in 10 ml. of 0.9% saline was injected intravenously, and on the other 10 ml. of saline alone. The order of the injections was varied, 7 out of 10 normal subjects and three out of five asthmatics receiving propranolol first. Raw and Vtg were measured at 15-minute intervals before and after the injections in order to assess spontaneous variability and not to miss any delayed responses. The tests were done "blind" in that the operator reading the vector slopes was not informed of the contents or order of the injections until the end of the study. The subjects were unable to distinguish between saline and propranolol, and were unaware of the order of injections.

* House-physician, Respiratory Department, St. George's Hospital, London S.W.1.

† Research Registrar, Respiratory Department, St. George's Hospital, London S.W.1. Present address: University Laboratory of Physiology, Oxford.

Reprint requests to: Dr. P. S. Richardson, St. George's Hospital, London S.W.1.