Pseudomonas cepacia Resistance to Antibacterials

R. M. E. RICHARDS [x] **and JOAN M. RICHARDS**

Received April 16, 1979, from the *Department of Pharmaceutics, University of Strathclyde, Glasgow G1 1XW, Scotland.* Accepted for publication June 4, 1979.

Abstract □ Reproducible growth rates of *Pseudomonas cepacia* were obtained. *P. cepacia* had a markedly different resistance pattern to single and combined antibacterials from that characteristic of *Pseudomonas aeruginosa*. Benzalkonium and chlorhexidine were more active against log phase *P. cepacia* than against log phase *P. aeruginosa*, but polymyxin B sulfate was inactive against log phase *P. cepacia* at all concentrations tested (≤16 units/ml). Antagonism of antibacterial activity between edetate disodium–benzalkonium and edetate disodium–chlorhexidine combinations was marked with 16-hr *P. cepacia* and with 16-hr *Staphylococcus aureus*. Phenylethanol–benzalkonium and phenylethanol–chlorhexidine combinations had no more than additive activity against log phase *P. cepacia*. These results have relevance to hospital disinfection and preservation of pharmaceutical solutions.

Keyphrases □ *Pseudomonas cepacia*—resistance to antibacterial agents, benzalkonium chloride, chlorhexidine, phenylethanol □ Antibacterial agents—*Pseudomonas cepacia*, resistance to benzalkonium chloride, chlorhexidine, phenylethanol

Pseudomonas cepacia is one of a number of unusual *Pseudomonas* species that have increased in clinical significance since the 1960's (1).

In 1950 (2), Burkholder described *P. cepacia*. Subsequently, other workers described the same species but did not recognize it as *P. cepacia* (3–6). The designations "eugonic oxidisers group 1" (EO-1) (4), *Pseudomonas kingii* (5), and *Pseudomonas multivorans* (6) were given before it was established that all were *P. cepacia* (7, 8).

Soil, vegetation, and water are natural environmental sources (2, 3, 6, 9), but the hospital environment also has proved to be a source of *P. cepacia;* several clinical infections have occurred (10–14). The infecting organism has been shown to survive common concentrations of chlorhexidine (11, 15), a cetrimide–chlorhexidine combination (12), benzalkonium chloride (13), and a dimethylbenzylammonium chloride–phenoxypolyethoxyethanol combination (14, 16).

P. cepacia is resistant also to a wide range of antibiotics, and its antibiotic-resistance spectrum is different from that of *Pseudomonas aeruginosa* (1, 17).

Table I—Doubling Times of Replicate Log Phase *P. cepacia* Cultures

Culture	Mean Doubling Time, min	95% Confidence Limits
A	34.5	33.6–35.6
B	34.3	33.0–35.7
C	34.7	33.3–36.1
D	34.3	33.1–35.6
E	34.4	33.1–35.8
F	35.4	34.3–36.5

Table II—Effect of Benzalkonium Chloride on the Doubling Times of Log Phase *P. cepacia* Cultures

Benzalkonium, μg/ml	Mean Doubling Time, min	95% Confidence Limits
None	35.2	34.1–36.4
1	41.1	39.6–42.7
2	45.8	44.5–47.3
4	70.0	62.5–79.5

Table III—Effect of Chlorhexidine Gluconate on the Doubling Times of Log Phase *P. cepacia* Cultures

Chlorhexidine, μg/ml	Mean Doubling Time, min	95% Confidence Limits
None	36.4	35.3–37.6
1	54.8	47.2–65.4
2	81.5	62.2–103.6

Table IV—Effect of Polymyxin B Sulfate on the Doubling Time of Log Phase *P. cepacia* Cultures

Polymyxin, units/ml	Mean Doubling Time, min	95% Confidence Limits
None	34.3	33.4–35.3
1	33.8	32.3–35.4
2	34.4	33.2–35.9
4	34.5	33.3–35.7
8	34.8	33.8–35.9
16	35.6	34.2–37.2

The present investigation was undertaken to evaluate the resistance of *P. cepacia* to chemical antibacterials including combinations previously shown to be active against *P. aeruginosa* (18–23).

EXPERIMENTAL

Materials—*P. cepacia*[1] and *Staphylococcus aureus*[2] were the test organisms. Nutrient broth No. 2[3] and nutrient agar[3] were the liquid and solid culture media, respectively. Chlorhexidine gluconate[4], benzalkonium chloride[5] (alkyldimethylbenzylammonium chloride: C_{14}, 50%; C_{12}, 40%; and C_{16}, 10%), polymyxin B sulfate[6], phenylethanol[7], and edetate disodium[7] were used as supplied.

Methods—Growth rate determinations and estimates of killing times of one or two chemical antibacterial systems were carried out. Viable counts were determined by the pour plate method, and cultures were maintained as described previously (24). Incubation was at 37°.

Estimation of Growth Rates—Log phase cultures were prepared as follows. A separate *P. cepacia* agar stab culture was used as the source of inoculum for each determination to prepare 10 ml of 24-hr broth culture. A loopful of this culture was used to inoculate 25 ml of broth in a 250-ml conical flask shaking at 100 throws/min. After 16 hr, this culture was diluted 1:50 with prewarmed broth and shaken at 150 throws/min for 10 min to disperse clumps. Aliquots, 5 ml, were added separately to 95-ml quantities of prewarmed broth in 250-ml conical flasks shaking at 100 throws/min. Growth was followed for each culture as was already described for *P. aeruginosa* (19). Typical growth rates (Table I) were calculated as described previously (25).

The effect of chemicals on the growth rate was determined for single chemicals and for combinations as for *P. aeruginosa* (19, 25). Tables II–IV give the growth rates of *P. cepacia* grown in broth and in broth plus varying concentrations of benzalkonium chloride, chlorhexidine gluconate, or polymyxin B sulfate. Tables V and VI show the effect of a combination of benzalkonium chloride and phenylethanol and a combination of chlorhexidine gluconate and phenylethanol, respectively.

[1] NCTC 10661, National Collection of Type Cultures, Colindale, London, England.
[2] NCTC 6751, National Collection of Type Cultures, Colindale, London, England.
[3] Oxoid, Oxo Ltd., London, England.
[4] I.C.I., Alderly Park, Macclesfield, Cheshire, England.
[5] Rohm & Haas (UK) Ltd., Croydon, England.
[6] Burroughs and Wellcome Ltd., Dartford, England.
[7] B. D. H., Poole, Dorset, England.

0022-3549/79/1100-1436$01.00/0

Table V—Effect of a Combination of Benzalkonium Chloride and Phenylethanol against Log Phase *P. cepacia* Cultures

Chemical	Mean Doubling Time, min	95% Confidence Limits
None	34.5	33.2–35.9
Phenylethanol, 0.05%	57.6	54.1–61.4
Phenylethanol, 0.1%	107.8	96.8–121.6
Phenylethanol, 0.05%, + benzalkonium chloride, 1 μg/ml	66.5	61.2–72.9
Benzalkonium chloride, 1 μg/ml	48.4	45.6–51.5
Benzalkonium chloride, 2 μg/ml	62.4	59.1–66.1

Table VI—Effect of a Combination of Chlorhexidine Gluconate and Phenylethanol against Log Phase *P. cepacia* Cultures

Chemical	Mean Doubling Time, min	95% Confidence Limits
None	35.6	34.6–36.8
Phenylethanol, 0.05%	52.6	51.1–54.3
Phenylethanol, 0.1%	112.6	102.3–125.2
Phenylethanol, 0.05%, + chlorhexidine gluconate, 0.5 μg/ml	72.7	70.5–75.1
Chlorhexidine gluconate, 0.5 μg/ml	44.9	43.0–46.9
Chlorhexidine gluconate, 1.0 μg/ml	59.0	55.5–63.0

Estimation of Killing Times—Determinations for *P. cepacia* and *S. aureus* were carried out separately as described previously (26) (Tables VII and VIII).

RESULTS AND DISCUSSION

Table I shows that the growth rates of replicate log phase cultures prepared as described gave reproducible results. Benzalkonium and chlorhexidine showed greater activity against exponentially growing cultures of *P. cepacia* than against log phase *P. aeruginosa* cultures (27).

Phenylethanol plus either benzalkonium or chlorhexidine had an additive effect against log phase *P. cepacia* cultures (Tables V and VI), but a synergistic effect was demonstrated with these combinations against *P. aeruginosa* under similar conditions (18, 20, 25).

Polymyxin B sulfate, ≤16 units/ml, showed no activity against *P. cepacia*. This finding is in marked contrast to results obtained with *P. aeruginosa*, where 1 unit/ml reduced the growth rate (21). The growth rates of five *P. cepacia* cultures plus five graded concentrations of polymyxin were indistinguishable from the growth rates in broth containing no added chemical (Table IV compared with Table I).

Study of polysorbate 80 on the activity of benzalkonium and chlorhexidine against log phase *P. cepacia* indicated that polysorbate 80 antagonized their antibacterial action[8]. This result also differs from that seen with *P. aeruginosa*, where synergism was observed (27).

The killing time results (Tables VII and VIII) show a difference, with respect to edetate disodium resistance, between *P. cepacia* and *P. aeruginosa*. In fact, *P. cepacia* had similar resistance to *S. aureus* toward the edetate disodium combinations with benzalkonium or chlorhexidine. This finding has considerable application to the formulation of ophthalmic solutions and contact lens solutions, which often contain edetate disodium–antibacterial agent combinations as the preservative system. Edetate disodium plus benzalkonium in ophthalmic solutions had increased activity against *P. aeruginosa* (28), but edetate disodium with either benzalkonium or chlorhexidine had less activity against *P. cepacia* and *S. aureus* than either benzalkonium or chlorhexidine solutions used singly. That is, edetate disodium antagonized the action of both chemicals against these two organisms.

Phenylethanol plus benzalkonium or chlorhexidine had shorter killing times against 16-hr *P. cepacia* cultures at the concentrations tested than any of the chemicals alone. These results in conjunction with the *P. cepacia* growth rate results indicate that the kill of *P. cepacia* cells obtained with the phenylethanol plus either benzalkonium or chlorhexidine is likely the result of each chemical exerting its individual action independently of the second member of the combination. The overall effect appears to be additive.

[8] R. M. E. Richards and J. M. Richards, unpublished results.

Table VII—Killing Times at 22° for the Test Chemicals Singly and in Combination against ~2 × 10^6 Organisms (16-hr *P. cepacia*)/ml

Chemical	Concentration, %	Killing Time, min
Benzalkonium chloride	0.01	120–150
Phenylethanol	0.4	>360
Edetate disodium	0.1	>360
Benzalkonium + phenylethanol	0.01 0.4	60–90
Benzalkonium + edetate disodium	0.01 0.1	>360
Chlorhexidine gluconate	0.01	60–90
Chlorhexidine gluconate + phenylethanol	0.01 0.4	<15
Chlorhexidine gluconate + edetate disodium	0.01 0.1	>360

Table VIII—Killing Times at 22° for the Test Chemicals Singly and in Combination against ~2 × 10^6 Organisms (16-hr *S. aureus*)/ml

Chemical	Concentration, %	Killing Time, min
Benzalkonium chloride	0.004	45–60
Phenylethanol	0.4	>360
Edetate disodium	0.1	>360
Benzalkonium + phenylethanol	0.004 0.4	45–60
Benzalkonium chloride + edetate disodium	0.004 0.1	180–240
Chlorhexidine gluconate	0.004	120–150
Chlorhexidine gluconate + phenylethanol	0.004 0.4	45–60
Chlorhexidine gluconate + edetate disodium	0.004 0.1	>360

The different resistance pattern of *P. cepacia* to *P. aeruginosa* is important in the hospital situation where a breakdown in disinfectant procedure can have serious consequences for the compromised patient.

The antagonism of benzalkonium and chlorhexidine by edetate disodium against *S. aureus* is probably most significant for chlorhexidine, which is less active against *S. aureus* than benzalkonium.

Significant differences in the structure of the cell envelope of the two pseudomonads are implicated by the marked difference in sensitivity to polymyxin alone or to edetate disodium–benzalkonium, edetate disodium–chlorhexidine, phenylethanol–benzalkonium, or phenylethanol–chlorhexidine as combinations (29).

REFERENCES

(1) A. von Graevenitz, *Prog. Clin. Pathol.*, **5**, 185 (1973).

(2) W. H. Burkholder, *Phytopathology*, **40**, 115 (1950).

(3) M. B. Morris and J. B. Roberts, *Nature (London)*, **183**, 1538 (1959).

(4) E. O. King, National Communicable Disease Center, Atlanta, Ga., 1964, rev. 1967; through Ref. 1.

(5) V. Jonsson, *Int. J. Syst. Bacteriol.*, **20**, 255 (1970).

(6) R. Y. Stanier, N. J. Palleroni, and M. Doudoroff, *J. Gen. Microbiol.*, **43**, 159 (1966).

(7) R. W. Ballard, M. J. Palleroni, M. Doudoroff, and M. Mandel, *ibid.*, **60**, 199 (1970).

(8) D. C. Sands, M. N. Schroth, and D. C. Hildebrand, *J. Bacteriol.*, **101**, 9 (1970).

(9) D. Taplin, D. C. J. Bassett, and P. M. Mertz, *Lancet*, **2**, 568 (1971).

(10) R. G. Mitchell and A. C. Hayward, *ibid.*, **1**, 793 (1966).

(11) D. W. Burdon and J. L. Whitby, *Br. Med. J.*, **2**, 153 (1967).

(12) D. C. J. Bassett, J. J. Stokes, and W. R. G. Thomas, *Lancet*, **1**, 1188 (1970).

(13) G. L. Gilardi, *Appl. Microbiol.*, **20**, 521 (1970).

(14) I. Phillips, S. Eykyn, M. A. Curtis, and J. J. S. Snell, *Lancet*, **1**, 375 (1971).

(15) D. C. E. Speller, M. E. Stephens, and A. C. Viant, *ibid.*, **1**, 798 (1971).

(16) P. C. Hardy, G. M. Ederer, and J. M. Matsen, *N. Engl. J. Med.*,

282, 33 (1970).
(17) J. J. Rahal, M. S. Simberkoff, and P. J. Hyams, *J. Infect. Dis., Suppl.*, 128, 762 (1973).
(18) R. M. E. Richards and R. J. McBride, *J. Pharm. Sci.*, 61, 1075 (1972).
(19) *Ibid.*, 62, 585 (1973).
(20) *Ibid.*, 62, 2035 (1973).
(21) *Ibid.*, 63, 54 (1974).
(22) R. M. E. Richards and R. H. Cavill, *J. Pharm. Sci.*, 65, 76 (1976).
(23) R. M. E. Richards and R. H. Cavill, *J. Pharm. Pharmacol.*, 28, 935 (1976).
(24) M. R. W. Brown and R. M. E. Richards, *ibid.*, 16, 41T (1964).
(25) R. M. E. Richards and R. J. McBride, *ibid.*, 23, 141S (1971).
(26) R. M. E. Richards and L. M. Mizrahi, *J. Pharm. Sci.*, 67, 380 (1978).
(27) M. R. W. Brown and R. M. E. Richards, *J. Pharm. Pharmacol.*, 16, 51T (1964).
(28) R. M. E. Richards, *ibid.*, 23, 136S (1971).
(29) *Ibid.*, 30, 14P (1978).

Urinary Iodine Excretion Rates following Intrathecal Injections of Iodinated Organic Carbonates

A. E. STAUBUS *x, B. N. NEWTON ‡, L. C. KLEIN ‡, A. B. WEINRIB *, and A. L. KUNZ ‡

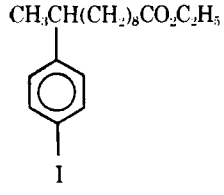
Received May 17, 1978, from the **College of Pharmacy, Ohio State University, Columbus, OH 43210,* and the ‡*Research and Development Department, Lafayette Pharmacal, Division of Alcon Laboratories, Inc., Lafayette, IN 47902.* Accepted for publication June 4, 1979.

Abstract □ Oily iodinated organic carbonates were investigated for use as myelographic media. The urinary excretion of total iodine was used to monitor the apparent elimination rate of these compounds from the subarachnoid space. Within the chain length series of C_2–C_6, the decrease of elimination rates and disposition rate constants with increasing chain length was demonstrated. This observation is consistent with a dissolution rate-limited elimination model. Such a model was derived and successfully NONLIN computer fitted to the observed elimination data. The model-derived parameter of clearance from the cerebrospinal fluid through the lipid "blood–brain barrier" correlated well with the compound's water solubilities and projected octanol–water partition coefficients. Additional compounds need to be tested to evaluate the postulated model system.

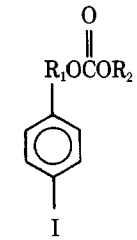
Keyphrases □ Myelographic agents—oily iodinated organic carbonates, biological elimination □ Pharmacokinetics—myelographic agents, oily iodinated organic carbonates, biological elimination □ Contrast media—oily iodinated organic carbonates, biological elimination

Positive-contrast myelography is the X-ray visualization of the subarachnoid space using a radiopaque substance. It is used to evaluate trauma to the spinal column and to delineate tumors and other canal obstructions. The only approved myelographic agent in the United States is iophendylate [ethyl 10-(*p*-iodophenyl)undecylate[1]] (I).

Iophendylate has been used in the United States in several million examinations since the early 1940's. It is considered to be very safe; however, it is removed at the conclusion of the examination because its biological elimination has been reported to be 1 ml/year (1). Water-soluble contrast agents have been used in Europe for over 30 years, but they have not been approved for use in the United States due to their higher incidence of adverse reactions.



I



II

$R_1 = -(CH_2)-_n$, n = 0–4
$R_2 = -C_nH_{2n+1}$, n = 1–10

Drawbacks with the current agents necessitated a search for a readily absorbable, nontoxic, oily myelographic medium. Oily iodinated organic carbonates of the general Structure II (2) were investigated. To understand quantitatively the biological elimination of these compounds, four representative carbonates were selected for study.

EXPERIMENTAL

The urinary excretion of total iodine was used to monitor the apparent elimination rate of the four carbonates from the subarachnoid space. In preliminary studies, the rate-limiting step for the overall elimination of compounds from the body appeared to be absorption from the subarachnoid space.

In an additional study, a series of rats was sacrificed at 24, 48, and 72 hr and on Day 5 following suboccipital injection. Other than expected high brain tissue levels, the only detectable iodine concentrations were in trace amounts (~0.3 μmole/g) found in skin samples at 24, 48, and 72 hr. Heart, kidney, liver, leg muscle, lung, and intestine samples on any of the 4 days and Day 5 skin samples all showed no detectable iodine. Consequently, the urinary excretion of the iodinated metabolites should reflect the relative absorption rates. For statistical purposes, each of the four compounds was tested in six rats. This method quantitatively confirmed the radiographic disappearance observed in these animals.

Thirty-six adult Sprague–Dawley female rats, 197–225 g, were divided into six equal groups. Each group was dosed with a different test compound. The purity of each test compound (Table I) was determined by GLC and NMR and agreed with analytically pure samples (~99% pure).

[1] Pantopaque.

0022-3549/79/1100-1438$01.00/0