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Penetration of piperacillin into bronchial mucosa and sputum

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ABSTRACT Bronchial mucosal biopsies were obtained during fibreoptic bronchoscopy in 12 patients receiving a new semisynthetic penicillin, piperacillin. The piperacillin levels estimated in bronchial mucosa exceeded those required to eradicate organisms associated with acute bronchitis, *Haemophilus influenzae* and *Streptococcus pneumoniae*, and compared favourably with those required for activity against a wide variety of anaerobic and Gram-negative organisms including *Pseudomonas aeruginosa*. Sputum and serum piperacillin levels were obtained from eight patients with bronchial disease receiving a five to seven day course (8 to 16 g/day). Sputum/serum level ratios were constant for the two dosages (10·7% for 8 g/day; 14·3% for 16 g/day) suggesting a diffusion transfer process, although the presence of pus in the sputum appeared to facilitate penetration. Seven patients achieved sputum levels exceeding those required for activity against *Haemophilus influenzae* and *Streptococcus influenzae*, and four for *Pseudomonas aeruginosa*. This study provides pharmacokinetic support of the use of piperacillin in bronchopulmonary infection.

Piperacillin sodium is a new semisynthetic penicillin which possesses a wide range of antibacterial activity against aerobic Gram-positive and Gram-negative bacteria, particularly *Pseudomonas aeruginosa*, and also against anaerobic organisms.[1-5] Piperacillin is active against many *Klebsiella pneumoniae* and Gram-negative organisms resistant to carbenicillin and ticarcillin and possesses greatest activity against those organisms susceptible to all three antibiotics.[6-9] Pharmacokinetic studies indicate that piperacillin has an average half-life of one hour in healthy subjects and a high renal clearance because of tubular excretion which may become saturated at high serum concentrations.[10 11] Piperacillin has been shown to provide effective therapy for serious infections caused by susceptible organisms, although the rate of emergence of resistant organisms during treatment requires investigation.[12]

The purpose of this study was to evaluate the penetration of piperacillin into bronchial mucosa and sputum and to compare the piperacillin concentrations achieved with the in vitro antibacterial activity of the antibiotic.

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Methods

STUDY ONE

Twelve patients who were having fibreoptic bronchoscopy for investigation of pulmonary disease were selected for this study. Informed, written consent was obtained. Their clinical details are shown in table 1. Six patients received 2 g doses and six, 4 g doses of piperacillin, by intravenous injection over five minutes at six-hourly intervals. Fibreoptic bronchoscopy was performed 30 to 45 min after the fourth dose in 11 patients and after the eighth dose in one patient, using a Fujinon FB-SBF bronchoscope under local anaesthesia (10 ml of viscous xylocaine as a mouth gargle; 5 ml of 4% topical xylocaine as a cricothyroid membrane injection). Bronchial secretions were aspirated and five to eight bronchial mucosal biopsies were taken from lobar or segmental bronchi. Bronchial secretions were collected in a trap specimen plastic container. It was sometimes necessary to increase the volume by adding normal saline via the bronchoscope, when the dilutional factor was calculated. The character of the bronchial secretions was recorded—that is, mucoid, purulent, blood-stained. A venous blood sample was taken at the time of the bronchial biopsies and all specimens were assayed for piperacillin.

Table 1 *Age, sex, weight, underlying lung disease, and clinical indication for fibreoptic bronchoscopy or pleural aspiration for the 13 patients*

Patient	*Age (yr)*	*Sex*	*Weight (kg)*	*Underlying lung disease*	*Indication for bronchoscopy or pleural aspiration*
1	71	M	82	Chronic bronchitis	Unresolved pneumonia
2	71	M	63	Chronic bronchitis, idiopathic pulmonary fibrosis	Unresolved pneumonia
3	63	M	43	Chronic bronchitis	Unresolved pneumonia
4	68	M	55	Chronic bronchitis, bronchial carcinoma	Pathology of peripheral mass
5	61	M	65	Bronchial carcinoma	Diagnosis of pleural effusion
6	67	M	65	Bronchiectasis	Haemoptysis
7	54	M	65	Chronic bronchitis, bronchial carcinoma	Diagnosis of hilar mass
8	88	M	56	Chronic bronchitis, bronchial carcinoma	Unresolved pneumonia
9	80	M	62	Chronic bronchitis, tuberculosis	Unresolved pneumonia
10	59	M	60	Chronic bronchitis	Haemoptysis
11	65	M	65	Chronic bronchitis	Unresolved pneumonia
12	67	M	65	Chronic bronchitis	Unresolved pneumonia
13	82	M	70	Chronic bronchitis, left heart failure	Diagnosis of pleural effusion

If pathology requiring bronchial brushings or biopsy was found or if further bronchial secretions were required for microbiological or cytological examination, the bronchoscope was reinserted and the procedure continued.

Two patients required aspiration of pleural fluid for diagnosis and this procedure was performed 30 to 45 minutes after piperacillin dose with their informed, written consent and the clinical details of these patients (5, 13) are shown in table 1. The pleural fluid and corresponding serum sample were assayed for piperacillin.

STUDY TWO

Eight patients, who on admission to hospital with a history consistent with acute bronchial infection were considered to require antibiotic therapy, were selected to receive a five to seven day course of piperacillin. Informed, written consent was obtained. The clinical details of these patients are shown in table 2. All patients were producing ample quantities of sputum throughout the day and none had received antibiotics or mucolytic agents during the previous five days. The patients received either 8 or 16g/day of piperacillin in four equally divided doses at six-hourly intervals by intravenous injection over five minutes. During the study other necessary treatment was continued as indicated.

Venous blood samples were taken at 0·5, 2, and 4 hours after the morning drug administration on day 1 (first full treatment day of four doses) and on at least two other days between day 2 and the completion of treatment (days 5 to 7). Renal and hepatic function were assessed before and after treatment. Sputum was collected in plastic sterile containers and for each period the degree of purulence was classified according to the following scheme[13]: mucopurulent—75% pus or more; 50% pus; 25% pus; trace of pus, mucoid—no pus. Sputum specimens were obtained before the start of piperacillin and during the following periods: 0 to 0·5, 1·5 to 2, and 3·5 to 4 hours after the morning dose on day 1 and the days corresponding to the blood sampling. Each sputum sample was assayed for piperacillin. Sputum was also collected before and after treatment for culture on appropriate media for common respiratory pathogens.

Table 2 *Age, sex, weight, underlying disease, and sputum purulence before and after treatment with piperacillin (8 g/day—1 to 4; 16 g/day—5 to 8) for the eight patients*

Patient	*Age (yr)*	*Sex*	*Weight (kg)*	*Underlying lung disease*	*Sputum purulence*	
					Before treatment	*After treatment*
1	71	M	51	Chronic bronchitis	mucoid	mucoid
2	72	M	58	Chronic bronchitis	50% pus	mucoid
3	63	M	56	Bronchiectasis	75% pus	mucoid
4	59	M	95	Chronic bronchitis, pneumonia	mucoid	mucoid
5	74	F	54	Bronchiectasis	75% pus	75% pus
6	78	F	56	Bronchiectasis	75% pus	75% pus
7	74	M	55	Chronic bronchitis	mucoid	trace of pus
8	63	M	54	Chronic bronchitis	mucoid	mucoid

PREPARATION OF SPECIMENS

Serum and expectorated sputum samples were stored immediately after collection at 4°C. Sputum was homogenised in Sputolysin (Calbichem—Behring Corp, La Jolla, California), a concentrated dithiothreitol solution, to extract piperacillin. This procedure was performed immediately before assay and involved a 1:1 dilution of the sputum. When necessary, the bronchial secretions obtained at bronchoscopy were also homogenised in this manner. Control studies indicated that Sputolysin did not interfere with the piperacillin assay. The volume of bronchial secretions was measured to determine the dilution factor if there had been the addition of normal saline. Bronchial biopsies were weighed as soon as possible after they had been taken in order to avoid drying of the bronchial tissue. The mean weight of specimens from each patient was 9·0 mg (range 2·7 to 14·6 mg) and as each specimen contained five to eight biopsies, each biopsy weighed less than 2 mg. All specimens were then kept at 4°C until 10 min before assay, when the tissue was ground in a measured volume of 1% phosphate buffer (0·1-0·2 ml) to extract the antibiotic. The tissue was not washed because of its small size as it was considered that this procedure might remove antibiotic from the tissue. All assays were performed on the day of collection.

PIPERACILLIN ASSAY

Piperacillin concentrations were determined using a microbiological agar disc diffusion assay, employing *Sarcina lutea* ATCC 9341 as the test organism. Assays were performed using 23 cm square Nunc-Bio assay plates (A/S Nunc, Roskilde, Denmark) containing 100 ml of Antibiotic Medium No 1, pH 6·5 (Oxoid Ltd, London), seeded with *Sarcina lutea* at a concentration of 2×10^6 organisms per ml. Standard 12·7 mm diameter assay discs (Schleicher and Schuell Inc, Keene, NH) were used. Standard concentrations of sodium piperacillin (Cyanamid Pty Ltd, Gosport, England) in the range 0·63 to 40·0 μg/ml were prepared for each assay from aliquots of a stock solution (10^4 μg/ml) stored at −20°C. The standards were prepared in antibiotic-free pooled human serum for assaying serum piperacillin levels, and in 1% phosphate buffer (pH 6·0) for assaying levels in expectorated sputum, bronchial secretions, and bronchial biopsies. All samples were assayed in duplicate, and zone sizes were measured to the nearest 0·1 mm using Vernier calipers. Regression analysis statistics were used to calculate the unknown piperacillin concentrations. The correlation coefficients of the regression lines varied from r = 0·9955 to r = 0·9995 in serum and from r = 0·9957 to r = 0·9995 in buffer. The sensitivity of the assay was limited to 0·3 μg/ml.

Results

STUDY ONE

The individual and mean ± SE serum, bronchial

Table 3 *Dosage of piperacillin (g/dose), number of doses at six-hourly intervals before bronchoscopy or pleural aspiration and the individual and mean ± SE piperacillin concentrations in serum, bronchial secretions, bronchial mucosal biopsies, and pleural fluid*

Patient	*Dose (g/dose)*	*Number of doses*	*Serum*	*Piperacillin concentration (μg/ml)*		
				Bronchial secretions	*Bronchial mucosa*	*Pleural fluid*
1	2	4	54·3	6·2	5·7	
2	2	4	108·4	2·0	12·4	
3	2	4	83·7	10·2	24·1	
4	2	4	73·9	2·7	57·2	
5	2	8	89·0	4·0	28·3	
6	2	4	102·0	9·8	24·6	
Mean			85·2	5·8	25·4	
SE			8·0	1·5	7·3	
7	4	4	119·2	76·7*	17·1	
8	4	4	266·2	7·8	45·4	
9	4	4	296·6	6·3	24·7	
10	4	4	234·3	23·0*	75·4	
11	4	4	126·1	52·3*	70·6*	
12	4	4	135·6	22·3	97·7*	
Mean			196·3	31·4	55·2	
SE			32·1	11·3	12·8	
5	2	4	110·0			10·0
	2	28	77·7			20·4
13	2	8	162·3			77·2

*Bloodstaining.

Table 4 *Serum piperacillin concentrations after the morning dose in eight patients receiving either 8 or 16 g/day piperacillin (2 or 4 g six-hourly)*

Time (h) Day	*Number of patients*	*Serum piperacillin concentration (μg/ml)*		
		0·5	*2·0*	*4·0*
		Dosage: 8 g/day		
1	3	Mean 129·5	40·1	11·3
		SE 31·7	8·7	2·4
		Range 97·4-193·0	29·5- 57·4	5·6-16·7
2	3	Mean 108·3	53·0	16·8
		SE 24·2	5·2	5·0
		Range 60·0-132·9	43·6- 61·7	7·0-23·3
3	1	Mean 72·8	40·2	20·5
5	3	Mean 76·5	28·0	11·6
		SE 13·3	5·0	4·2
		Range 51·9- 97·7	18·3- 34·7	3·6-17·7
6	2	Mean 80·5	30·5	10·1
		SE 18·8	1·9	3·3
		Range 61·7- 99·3	28·6- 32·4	6·8-13·3
		Dosage: 16 g/day		
1	4	Mean 347·9	97·7	30·4
		SE 59·1	16·2	6·7
		Range 174·1-429·9	75·7-145·5	18·0-49·4
2	4	Mean 277·7	105·8	38·9
		SE 22·9	29·2	16·8
		Range 233·3-342·0	48·0-187·0	12·0-87·5
6	2	Mean 310·5	130·5	50·1
		SE 119·4	60·1	26·5
		Range 191·1-429·9	70·4-190·6	23·6-76·5
7	2	Mean 330·0	83·3	19·5
		SE 73·0	7·3	1·5
		Range 257·0-403·0	76·0- 90·6	18·0-21·0

Table 5 *Sputum piperacillin concentrations after the morning dose in eight patients receiving either 8 or 16 g/day piperacillin (2 or 4 g six-hourly)*

Time (h) Day	*Number of patients*	*Sputum piperacillin concentration (μg/ml)*		
		0·5	*2·0*	*4·0*
		Dosage: 8 g/day		
1	4	Mean 2·7	3·9	2·0
		SE 1·2	1·1	0·9
		Range 0·3- 5·8	0·7- 5·4	0·3- 4·4
2	3	Mean 3·7	6·9	5·0
		SE 1·3	3·1	1·3
		Range 1·2- 5·2	1·2-11·9	2·8- 7·4
3	1	Mean 1·1	0·4	0
5	3	Mean 2·5	3·5	1·6
		SE 1·5	2·6	1·6
		Range 0 - 5·1	0 - 8·6	0 - 4·7
6	2	Mean 0·5	1·0	0·8
		SE 0·5	0·6	0·8
		Range 0 - 1·0	0·4- 1·5	0 - 1·5
		Dosage: 16 g/day		
1	4	Mean 13·0	8·9	8·9
		SE 6·7	4·1	4·0
		Range 0 -31·7	0 -20·0	0 -19·2
2	4	Mean 11·2	12·9	8·4
		SE 4·7	4·9	4·6
		Range 1·0-19·7	0 -20·6	0·3-20·6
6	2	Mean 6·1	12·1	15·4
		SE 0·6	1·1	5·5
		Range 5·5- 6·6	11·0-13·1	9·9-20·8
7	2	Mean 2·9	5·2	3·9
		SE 2·9	5·2	2·9
		Range 0 - 5·8	0 -10·4	1·0- 6·7

secretion and bronchial mucosal piperacillin concentrations for the 12 patients are shown in table 3. Bronchial secretions were mucoid in all patients except in three in whom secretions were blood-stained and there were two patients in whom some of the bronchial biopsies appeared slightly blood-stained (see table 3). Both serum ($p < 0.01$) and bronchial secretion ($p < 0.05$) but not bronchial mucosal levels were significantly higher with the 4 g than with the 2 g dose. The pleural fluid and serum piperacillin levels in the two patients are shown in table 3.

STUDY TWO

The mean ± SE serum and sputum piperacillin levels for the eight patients are shown in tables 4 and 5 respectively. The degree of sputum purulence is indicated in table 2. *Haemophilus influenzae* was cultured from sputum before and after treatment in patient 3 (table 2). Sputum piperacillin levels were compared with the degree of sputum purulence and with both doses there was a positive correlation ($r = 0.47$, $p < 0.001$), although when the doses were considered separately, there was a positive correlation only with the 16 g/day dose (8 g/day: $r = 0.27$, $p > 0.10$; 16 g/day: $r = 0.43$, $p < 0.01$). To assess the concentration of piperacillin in the sputum and serum over the total period of measurement, the areas under the curves (AUC) from 0·5 to 4 hours were calculated for each patient on each study day and the mean ± SE results are shown in table 6. The ratios of AUC sputum/AUC serum were calculated as an assessment of sputum penetration of piperacillin and the mean ± SE results are shown in table 6. There was a significant increase in sputum ($p < 0.01$) and serum AUCs ($p < 0.001$) but not in sputum AUC/serum AUC ratios with the 16 g/day compared with the 8 g/day dose. There was a significant positive correlation between sputum AUC/serum AUC ratio and sputum purulence ($r = 0.54$, $p < 0.01$). There were no significant changes in renal or hepatic biochemical function or any reported unwanted effects with the piperacillin treatment.

Table 6 *Mean ± SE areas (AUC) under the serum concentration/time (0·5 to 4·0 h) curves, areas under the sputum concentration/time curves and the ratios, sputum AUC/serum AUC for the eight patients receiving either 8 or 16 g/day piperacillin (2 or 4 g six-hourly)*

	AUC Sputum	*AUC Serum*	*AUC Sputum/ AUC Serum*
Piperacillin dosage: 8 g/day			
Mean	12·9	112·2	10·7
SE	3·5	11·1	2·7
Range	0-38·3	46·7-180·1	0-27
Piperacillin dosage: 16 g/day			
Mean	42·0	313·1	14·3
SE	9·0	40·5	3·5
Range	0-92·5	163·5-581·8	0-38

Discussion

Serum, bronchial mucosa, and sputum piperacillin levels were measured in this study to assess the potential of piperacillin for the treatment of bronchopulmonary infection. Bronchial tissue antibiotic concentration will be relevant for the treatment of aspiration lung abscess which starts as a local suppurative infection in bronchi or bronchioles and spreads into the lung and in bronchopneumonia. Antibiotic concentration is likely to be higher in alveolar tissue than in bronchi because of the enormous surface area contact between alveolar walls and capillaries and in lobar pneumonia, an acinar disease primarily, serum antibiotic levels may be more relevant.

Bronchial infection may coexist with pneumonia in patients with chronic bronchitis and emphysema and the most common infecting organisms are *Haemophilus influenzae* and *Streptococcus pneumoniae*.[13] The bronchial mucosa is the site of infection in acute exacerbations of chronic bronchitis and the eradication of infection will depend upon bronchial mucosal antibiotic concentration. It is assumed that a gradient of concentration of antibiotic exists from blood through bronchial mucosa to bronchial secretions. Bronchial secretions act as a reservoir for bacterial growth which may produce reinfection if not suppressed adequately by appropriate sputum antibiotic levels.

There have been few studies which have examined antibiotic concentrations in vivo in human lung and bronchus. Surgically resected lung tissue may be used by dosing the patient before surgery. Liss and Norman,[14] using a fluorescence technique, showed a lower doxycycline concentration in alveolar tissue than in bronchiolar epithelium but this did not correlate with the assayed concentration. Gartmann[15] found that lung but not bronchial doxycycline levels were higher than in serum, whereas Kroening[16] found the ratio of whole blood to lung tobramycin concentration to be 2:1. Both investigators comment concerning the need to remove blood from the specimen which must inevitably be incomplete and may result also in loss of tissue antibiotic. Kroening[16] determined the haemoglobin concentration of the supernatant fluid after centrifugation of the lung tissue and applied a mathematical formula to assess lung concentration without blood admixture.

This study demonstrates a new method for obtaining bronchial mucosa for antibiotic level estimation by biopsy during fibreoptic bronchoscopy. The advantage of this procedure is that the biopsies are

relatively atraumatic, although in two patients slight bloodstaining was apparent in some biopsies and this blood would have contributed to the piperacillin concentration achieved. In future studies such biopsy specimens should be discarded. The similar range of bronchial mucosal levels with the two doses suggests some saturation of the transfer process of antibiotic from blood into bronchial mucosa. Microbiological studies of piperacillin sensitivity have indicated that 100% of *Streptococcus pneumoniae* and *Haemophilus influenzae* are susceptible at concentrations less than 0·1 and 0·25 μg/ml respectively,[2 5] 75 to 100% of penicillin-sensitive Staphylococci between 8 and 25 μg/ml,[2 5] 88 to 100% of anaerobic organisms at 20 μg/ml,[4] 40 to 90% of *Klebsiella pneumoniae* and other Gram-negative organisms between 8 and 32 μg/ml and 88-100% of *Pseudomonas aeruginosa* between 8 and 25 μg/ml.[1 2 4 10 17] Thus, in this study bronchial mucosal piperacillin levels far exceeded those required for organisms associated with acute bronchitis and should produce effective chemotherapy for the majority of organisms in pulmonary infection.

We consider antibiotic levels in bronchial secretions collected at bronchoscopy to be unreliable for various reasons. Bronchial secretions may be contaminated by blood because of pulmonary pathology or after biopsy procedures. If saline is added when the volume is low, the dilutional factor cannot always be determined accurately. Although the local anaesthetic agent disappears rapidly into peripheral bronchi, this may contaminate bronchial secretions and inhibit subsequent bacterial growth during the antibiotic assay. Piperacillin levels in pleural fluid were variable and of similar order of magnitude to those in bronchial mucosa. No bacterial growth was obtained from the pleural fluid in the two patients and piperacillin levels in empyema require further investigation.

There was a wide variation in sputum piperacillin levels. A significant dose-response was obtained for the two doses, although sputum AUC/serum AUC ratios remained constant suggesting simple diffusion to be the likely process of transfer. The presence of pus in the sputum appeared to facilitate the transfer of piperacillin, such as occurs with ampicillin,[18] amoxycillin,[19] and erythromycin.[20] Sputum AUC/serum AUC ratios remained constant during the course of treatment in contrast to tetracycline[21] and doxycycline[22] levels which tend to rise as treatment progresses. Piperacillin sputum levels were above those required for *Haemophilus influenzae* and *Streptococcus pneumoniae* on the first two days in seven of the eight patients. In four patients, levels greater than 8 μg/ml were achieved during the course of treatment, indicating efficacy against *Pseudomonas aeruginosa* which may be a pathogen bronchiectasis and cystic fibrosis. Sputum penetration of piperacillin appears similar to carbenicillin which has been studied in patients with cystic fibrosis by Marks *et al*[23] who found sputum/serum carbenicillin level ratios to be always less than 20%.

The efficacy of an antibiotic in the treatment of infection can only be assessed by the clinical response of the patient. However, the measurement of serum, bronchial mucosa, and sputum levels of antibiotic may together provide a guide to the potential value of an antibiotic in bronchopulmonary infection. These results indicate that piperacillin may provide effective chemotherapy against a wide variety of infecting organisms in bronchopulmonary infection and that clinical studies with this antibiotic are warranted because of the increasing emergence of resistance among organisms to currently available antibiotics.

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