ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1985, p. 912–915
0066-4804/85/060912-04$02.00/0

Vol. 27, No. 6

Amoxicillin-Clavulanic Acid in the Treatment of Lower Respiratory Tract Infections Caused by β-Lactamase-Positive *Haemophilus influenzae* and *Branhamella catarrhalis*

RICHARD J. WALLACE, JR.,[1]* LORRAINE C. STEELE,[1] DANDREA L. BROOKS,[1] J. ILEICE LUMAN,[2] REBECCA W. WILSON,[2] AND JERRY W. McLARTY[3]

Departments of Microbiology,[1] Pathology,[2] and Epidemiology/Biomathematics,[3] The University of Texas Health Center at Tyler, Tyler, Texas 75710

Received 26 December 1984/Accepted 26 March 1985

Twenty-one adult patients hospitalized with lower respiratory tract infections due to *Branhamella catarrhalis* or *Haemophilus influenzae* or both were treated with the combination of oral amoxicillin and potassium clavulanate (Augmentin) in an open, noncomparative clinical trial. Diseases included pneumonia, empyema, and exacerbations of bronchiectasis and chronic lung disease. Thirteen of 16 *B. catarrhalis* and six of nine *H. influenzae* isolates were β-lactamase positive. The patients with *B. catarrhalis* were treated for a mean of 5.3 days, and those with *H. influenzae* were treated for a mean of 7.0 days. The overall response to therapy was excellent, with 18 of 19 β-lactamase-producing strains eradicated on therapy. One patient secondarily infected with *Pseudomonas aeruginosa* was a clinical failure, and two patients with *H. influenzae* who became culture positive again after therapy were considered microbiologic failures. Gastrointestinal side effects (especially nausea) were common, although all patients completed a course of therapy. Sputum levels of amoxicillin were surprisingly low (<0.05 to 0.54 μg/ml), a finding which may explain the high relapse rate (22%) seen with *H. influenzae*, as these are below the usual MICs of amoxicillin for this organism. The combination of amoxicillin plus potassium clavulanate appears to be an excellent drug for treatment of β-lactamase-producing strains of these two species, although mild gastrointestinal side effects are common.

Branhamella (formerly *Neisseriae*) *catarrhalis* is an aerobic, nonpigmented, gram-negative diplococcus which clinically has been grouped with the nonpathogenic *Neisseriae* spp. Recent studies have demonstrated its potential for producing disease in children with otitis media (13) or sinusitis (18) and in adults with chronic lung disease (10, 14, 15). The organism has been recovered from blood cultures, although rarely (15), and has been cultured and demonstrated in the lung histologically in patients who have died with pneumonia (10, 14). Although both malignancy and immune deficiency (4, 10) have been stressed as major predisposing diseases, recent studies suggest that chronic lung disease is the usual predisposing factor (14). An important consideration in determining therapy is the fact that about 75% of pathogenic isolates produce β-lactamase and are resistant to penicillin and ampicillin (13).

Recent studies in our hospital, which follow a large number of patients with severe chronic lung disease, have shown *B. catarrhalis* to be our third most common potential pathogen in sputa. Our most common pathogen (as with most patients with chronic lung disease) is *Haemophilus influenzae*, of which about 10 to 15% are β-lactamase positive (R. J. Wallace, Jr., unpublished observations).

Because of the excellent in vitro activity of potassium clavulanate and amoxicillin (Augmentin) against β-lactamase-producing strains of *B. catarrhalis* (6) and *H. influenzae* (20), we studied the efficacy of this drug combination in an open, noncomparative clinical trial with 21 patients hospitalized with lower respiratory tract disease due to either of these potential pathogens.

* Corresponding author.

MATERIALS AND METHODS

Study design. Adult patients who were admitted to the University of Texas Health Center at Tyler with signs and symptoms of lower respiratory tract infection including a history of cough and purulent sputum, with or without fever and chest pain, and who had a sputum Gram stain or culture suggestive of *B. catarrhalis* or *H. influenzae* were candidates for the study. Diagnosis of pneumonia was based on a chest X-ray examination which revealed development of a new pulmonary infiltrate. Patients with a history of chronic lung disease who had the above symptoms but lacked development of a pulmonary infiltrate upon X-ray examination were considered to have exacerbation of chronic lung disease.

Written informed consent was obtained from all patients in accordance with The University of Texas Health Center at Tyler Human Subjects Investigation Committee. Patients were excluded from the study if they had a history of allergy to penicillin or cephalosporins, if they had a creatinine level in serum greater than 2.5 mg/100 ml, if they were pregnant, or if their condition or other circumstances created a situation whereby therapy would be unevaluable.

Amoxicillin plus potassium clavulanate was administered by mouth every 8 h with a dose of 500 mg of amoxicillin and 125 mg of clavulanic acid (potassium clavulanate). The duration of treatment depended upon which organism had been isolated from culture. Those patients with sputum containing *B. catarrhalis* were treated for 5 days, and those with sputum containing *H. influenzae* were treated for 7 to 10 days.

Response to therapy was evaluated bacteriologically by sputum cultures obtained during and after therapy. Clinical laboratory studies were repeated after therapy to monitor for

TABLE 1. Clinical and laboratory characteristics of 21 patients treated with amoxicillin plus potassium clavulanate for lower respiratory tract infections

Clinical variable	No. or mean (±SD)
Male/female	14/7
Age	66.6 (±10) yr
CLD[a]	18
Smokers	20
Pack yr smoking	48 (±12.6)
Home oxygen	16
Alcohol abuse	1
Clinical disease	
Pneumonia	12
Exacerbation of CLD	9
Admission leukocyte count	13,695 (±4,199) per mm^3
Admission pO_2[b]	60.4 (±12.2)
Admission pCO_2	41.7 (±8.6)

[a] CLD, Chronic lung disease.
[b] Many patients were on supplemental O_2 at the time admission blood gases were drawn.

the presence of adverse reactions. Patients were observed daily for side effects and reactions as well as for evidence of clinical response. Patients were considered to clinically respond to therapy if their temperature (if elevated) returned to normal, their sputum lessened in amount and became thinner and less purulent, their abnormal laboratory values returned to base line, and they looked and felt better.

Laboratory. Admission laboratory data included blood cultures and sputum for Gram stain and culture. Patients were considered for inclusion in the study only if their sputum sample contained >25 leukocytes and fewer than 10 squamous cells per low-power field (7, 19) and showed a predominance of either gram-negative coccobacilli or gram-negative diplococci or both on Gram stain (17). Organisms were quantitated on the Gram stain as rare (0 to 2), few (2 to 10), and moderate-to-loaded (>10) organisms per high-power field.

Additional laboratory tests included the following: complete urinalysis; analysis of serum levels of creatinine, blood urea nitrogen, alkaline phosphatase, glutamic oxaloacetic transaminase, albumin, bilirubin, sodium, potassium, and total protein; hemoglobin and hematocrit; total and differential leukocyte count; and platelet count.

Bacterial pathogens were isolated and identified by standard laboratory methods. *H. influenzae* grew only on chocolate agar under 10% CO_2 and required both NAD and hemin to grow. Biotyping of selected strains was performed by using the Minitek system (1). *B. catarrhalis* had a typical Gram stain, grew as nonpigmented colonies on 5% sheep blood agar or chocolate agar, failed to ferment glucose, maltose, sucrose, or lactose, and produced DNase. β-Lactamase was detected by using the chromogenic cephalosporin (Cefinase). Susceptibility testing for both *B. catarrhalis* and *H. influenzae* was performed by the disk diffusion method, using criteria of the National Committee for Clinical Laboratory Studies except that the zone size criteria for *Staphylococcus aureus* were used to determine susceptibility to ampicillin and penicillin for *B. catarrhalis*. (*B. catarrhalis* was tested on Mueller-Hinton agar and *H. influenzae* was tested on chocolate agar).

Blood for peak levels in serum (2 h after administration) and random sputum levels were obtained from patients on days 2 to 4 of therapy. Drug assays for clavulanic acid were performed by a microbiologic assay as previously described (3) and specifically detailed for us by Beecham Laboratories. *Klebsiella pneumoniae* ATCC 29668 was used as the test organism. Amoxicillin was also assayed microbiologically by using *Sarcina lutea* ATCC 9341 as the test species. Sputa were diluted 1:2 in 10% acetylcysteine to reduce viscosity. Because of the instability of clavulanic acid, sputa and sera were frozen at −70°C and assayed within 5 days. All drug controls were made up fresh the day of the assay.

Statistics. Changes in blood counts and chemistries were tested statistically by paired *t* tests and Wilcoxon matched-pairs tests.

RESULTS

Twenty-one patients were entered in the study (Table 1). There were 14 males and 7 females, with a mean age of 67 years. All patients had underlying lung disease, and all but one were smokers. The severity of the lung disease is reflected by the fact that 16 patients were on home oxygen.

Most patients were mildly to moderately ill. Ten patients had pneumonia, one patient had atelectasis with pneumonia, one patient had an infected bronchopleural fistulae, and nine patients had exacerbation of chronic lung disease, including bronchiectasis.

All patients had either *B. catarrhalis* (13 patients) or *H. influenzae* (5 patients) or both (3 patients) in their sputa (Table 2). In 14 patients, one of these two organisms was the only pathogen recovered. No patients had positive blood cultures. Six additional potential pathogens were cultured from sputa: *Streptococcus pneumoniae* (three isolates), *Klebsiella* spp. (two isolates), and *Acinetobacter* sp. (one isolate). Of these latter organisms, only the *Streptococcus*

TABLE 2. Aerobic bacterial pathogens recovered from the pretreatment sputa of 21 patients with lower respiratory tract disease treated with amoxicillin plus potassium clavulanate

Pathogen	Total no. of isolates	No. of isolates with the following characteristic:		
		β-Lactamase positive (%)	Isolated in pure culture	>10 organisms per HPF on sputum Gram stain[a]
B. catarrhalis	16	13 (81)	10	14
H. influenzae	9[b]	6 (67)	4	5
S. pneumoniae	3	NT[c]	0	2
Gram-negative bacilli[d]	3	NT	0	0

[a] All sputum samples contained >25 leukocytes and 0 to 9 squamous cells per low-power field (magnification, ×100) and thus were considered quality samples (20). HPF, High-power (oil immersion) field (magnification, ×1,000).
[b] One patient had two strains of *H. influenzae*, and three patients had both *H. influenzae* and *B. catarrhalis*. All 21 patients had *H. influenzae* or *B. catarrhalis* or both as a pathogen.
[c] NT, Not tested.
[d] Includes two isolates of *Klebsiella* and one of *Acinetobacter* sp.

pneumoniae were present in large numbers on the admission sputum Gram stain even in retrospect. Thirteen of 16 *B. catarrhalis* (81%) and 6 of 9 *H. influenzae* (67%) isolates were β-lactamase positive. All isolates of *H. influenzae* produced disk zone diameters of >20 mm to the 30-μg disk of amoxicillin plus potassium clavulanate (mean, 25 mm), while all isolates of *B. catarrhalis* produced zone diameters of >30 mm with a mean of 36 mm for β-lactamase-positive strains and 40 mm for the three β-lactamase-negative strains.

Bacteriologic response. The microbiologic response in these patients was excellent. Of the 16 patients with *B. catarrhalis*, only 3 had positive cultures within 48 h of institution of therapy, and all were culture negative after therapy. The 13 patients with *B. catarrhalis* without *H. influenzae* were treated for a mean of 5.3 (±0.6) days. Of the eight patients with *H. influenzae*, four patients were negative on all subsequent cultures, two patients were culture positive during therapy but were negative after therapy, and another two patients were negative on therapy but became positive again after therapy was stopped. These eight patients were treated for a mean of 7.0 (±1.8) days. The two isolates of *H. influenzae* obtained posttherapy had the same phenotype as the original infecting strain (one was nontypable, biotype II, β-lactamase negative, and the other was nontypable, biotype II, β-lactamase positive). Thus, 18 of 19 β-lactamase-producing isolates of *H. influenzae* and *B. catarrhalis* were successfully eradicated on therapy with amoxicillin plus potassium clavulanate.

Clinical response. The clinical response in these patients was also excellent in both patients with pneumonia and exacerbations of chronic lung disease. None of the patients died, and all were successfully discharged from the hospital. One patient was a clinical failure and required a course of other antibiotics. This was an elderly lady with bilateral bronchiectasis and chronic respiratory failure. Her initial sputum sample contained β-lactamase-positive *B. catarrhalis* which quickly cleared on culture with therapy. Sputa, however, remained grossly purulent and copious in amount, and the patient remained moderately ill. Repeat sputum Gram stain and culture revealed large numbers of *Pseudomonas aeruginosa*. Improvement followed therapy with tobramycin and ticarcillin and continued postural drainage.

Side effects. The mean leukocyte counts and bilirubin levels were lower after therapy than before, but the differences were not statistically significant; creatinine levels were significantly lower ($P < 0.01$). No abnormalities in blood chemistries or blood counts were noted after therapy. Side effects with the drug were mild but common. Six patients (28.6%) had nausea. Two of these six patients also had vomiting (10%), and two of these patients and two additional patients without nausea or vomiting (19%) had diarrhea. In only patient was the drug treatment stopped (because of diarrhea), and this patient had completed an adequate course of therapy.

Drug levels. Ten samples of sputum had amoxicillin levels of <0.5 μg/ml. An additional 17 samples had levels with a range of 0.11 to 0.54 μg/ml with a mean of 0.22 (±0.12) μg/ml. The levels in the two patients with only β-lactamase-negative organisms (one had no detectable levels and the other had a level of 0.22 μg/ml) were similar to the levels in the 19 patients with β-lactamase-positive organisms. The median for all 27 samples was 0.11 μg/ml. In contrast, peak amoxicillin levels in serum were excellent, with a mean of nine patients of 8.7 (±6.0) μg/ml.

The levels of clavulanic acid in both sputa and sera were low. In 20 patients, levels in sputa were <0.1 μg/ml, and only one value was 0.3 μg/ml. The mean level in serum was 0.82 μg/ml.

DISCUSSION

The β-lactamases of *B. catarrhalis* and *H. influenzae* (TEM-1) are highly susceptible to clavulanic acid, with as little as 0.08 μg/ml producing 50% inhibition of both enzymes in an in vitro assay (6). In MIC determinations, 0.5 to 1.0 μg of clavulanic acid per ml is enough to reduce the MICs of ampicillin or amoxicillin for β-lactamase-producing strains of *H. influenzae* or *B. catarrhalis* to those of non-enzyme-producing strains (20). These studies strongly suggested that clinical studies in which this drug concentration was used would be successful against these two pathogens. Successful clinical trials of amoxicillin plus potassium clavulanate in the treatment of lower respiratory tract infections in the United States have been reported, but these have not included β-lactamase-producing strains (12, 16). In the current study, 18 of 19 β-lactamase-positive isolates of *H. influenzae* and *B. catarrhalis* were eradicated by the combination of amoxicillin plus potassium clavulanate, demonstrating the in vivo efficacy of this combination. Similar results with a smaller number of isolates have been reported in studies of treatment of bronchitis from England, where this drug has been marketed for several years (2, 5, 8, 11).

The levels of amoxicillin in sputa were surprisingly low, although similar studies by May and Delves (9) with high-dose oral ampicillin and by Havard et al. (8) with amoxicillin at the same dose as in the present study showed that sputum levels rarely exceed 0.5 μg/ml and most average only 0.2 to 0.3 μg/ml. Levels in serum, however, were within the expected range. Given these low levels and the usual MIC for *H. influenzae* of 0.5 μg/ml for ampicillin, it is somewhat surprising that the drug is effective in eradicating this organism from sputa. The importance of sputum levels in treatment of bronchitis has not been established, however, and clearly, ampicillin and amoxicillin are very effective despite their apparent low therapeutic margin. The levels of clavulanic acid were also low (most were undetectable or <0.1 μg/ml). In a previous study with the same dose of amoxicillin plus potassium clavulanate, sputum levels of clavulanic acid were somewhat higher, with a mean of 0.23 μg/ml, and were comparable to amoxicillin levels (8). Because levels of clavulanic acid in both sera and sputa were less than expected, and given the instability of clavulanic acid, technical problems may explain in part our low levels. Again, despite these low levels, the drug was clearly present in adequate concentrations to block β-lactamase and allow for amoxicillin activity.

B. catarrhalis was isolated from 16 patients. Four of these patients had pneumonia, with *B. catarrhalis* being the only recovered pathogen in three. We believe that this organism is the most frequently overlooked lower respiratory pathogen in patients with chronic lung disease and was the cause of pneumonia in most of the above cases. Development of bactericidal antibodies after lower respiratory infections due to *B. catarrhalis* in patients similar to these has been described (3a), suggesting that isolation of these organisms in this setting may have pathologic significance.

The duration of therapy necessary for the treatment of various lower respiratory pathogens is not well established. Five days of therapy were clearly adequate for treatment of *B. catarrhalis*, with all isolates being eradicated by this therapy. For *H. influenzae*, 7 days appeared to be adequate, although two patients became recolonized with the same organism when therapy was discontinued.

ACKNOWLEDGMENT

Financial support for this study was provided by Beecham Laboratories, Bristol, Tenn.

LITERATURE CITED

1. **Back, A. E., and T. R. Oberhofer.** 1978. Use of the Minitek system for biotyping *Haemophilus* species. J. Clin. Microbiol. **7**:312–313.
2. **Beeuwkes, H., and V. H. Rutgers.** 1981. A combination of amoxicillin and clavulanic acid in the treatment of respiratory tract infections caused by amoxicillin-resistant *Haemophilus influenzae*. Infection **9**:244–248.
3. **Brown, A. G., D. Butterworth, M. Cole, G. Hanscomb, J. D. Hood, C. Reading, and G. N. Rolinson.** 1976. Naturally-occurring β-lactamase inhibitors with antibacterial activity. J. Antibiot. **29**:668–669.

3a. **Chapman, A. J., D. M. Musher, S. Jonsson, J. E. Clarridge, and R. J. Wallace, Jr.** 1985. Development of bactericidal antibody during *Branhamella catarrhalis* infection. J. Infect. Dis. **152**:878–882.

4. **Diamond, L. A., and B. Lorber.** 1984. *Branhamella catarrhalis* pneumonia and immunoglobulin abnormalities: a new association. Am. Rev. Respir. Dis. **129**:876–878.
5. **Elliot, P. J., D. S. Reeves, H. A. Holt, S. T. Chapman, and M. J. Bywater.** 1980. Evaluation of Augmentin in respiratory tract infections, p. 269–274. *In* Proceedings of the 1st symposium on Augmentin clavulanate-potentiated amoxycillin.
6. **Farmer, T., and C. Reading.** 1982. β-Lactamases of *Branhamella catarrhalis* and their inhibition by clavulanic acid. Antimicrob. Agents Chemother. **21**:506–508.
7. **Geckler, R. W., D. H. Gremillion, C. K. McAllister, and C. Ellenbogen.** 1977. Microscopic and bacteriological comparison of paired sputa and transtracheal aspirates. J. Clin. Microbiol. **6**:396–399.
8. **Havard, C. W. H., A. Fernando, W. Brumfitt, and J. M. T. Hamilton-Miller.** 1982. A pilot study of "Augmentin" in lower respiratory tract infections: pharmacokinetic and clinical results. Br. J. Dis. Chest **76**:255–260.
9. **May, J. R., and D. M. Delves.** 1965. Treatment of chronic bronchitis with ampicillin. Lancet **i**:929–933.
10. **McNeely, D. J., C. S. Kitchens, and R. M. Kluge.** 1976. Fatal *Neisseria* (*Branhamella*) *catarrhalis* pneumonia in an immunodeficient host. Am. Rev. Respir. Dis. **114**:399–402.
11. **Mehtar, S.** 1980. Respiratory tract infections caused by beta-lactamase producing organisms treated with Augmentin, p. 259–264. *In* Proceedings of the 1st symposium on Augmentin clavulanate-potentiated amoxycillin.
12. **Penn, R. L., K. L. Manocha, J. G. Burford, R. B. George.** 1984. Prospective comparison of Augmentin and Cefaclor as therapy for lower respiratory infections. Postgrad. Med. **12**(Suppl.): 251–256.
13. **Shurin, P. A., C. D. Marchant, C. H. Kim, G. F. Van Hare, C. E. Johnson, M. A. Tutihasi, and L. J. Knapp.** 1983. Emergence of beta-lactamase-producing strains of *Branhamella catarrhalis* as important agents of acute otitis media. Pediatr. Infect. Dis. **2**:34–38.
14. **Slevin, N. J., J. Aitken, and P. E. Thornley.** 1984. Clinical and microbiological features of *Branhamella catarrhalis* bronchopulmonary infections. Lancet **i**:782–783.
15. **Srinivasan, G., M. J. Raff, W. C. Templeton, S. J. Givens, R. C. Graves, and J. C. Melo.** 1981. *Branhamella catarrhalis* pneumonia: report of two cases and review of the literature. Am. Rev. Respir. Dis. **123**:553–555.
16. **Tan, J. S., and T. M. File, Jr.** 1984. Double-blind efficacy and safety study of Augmentin versus Cefaclor in the treatment of lower respiratory tract infection. Postgrad. Med. **12**(Suppl.): 129–132.
17. **Thornley, P. E., J. Aitken, C. J. Drennan, J. MacVicar, and N. J. Slevin.** 1982. *Branhamella catarrhalis* infection of the lower respiratory tract: reliable diagnosis by sputum examination. Br. Med. J. **285**:1537–1538.
18. **Wald, E. R., G. J. Milmoe, A'D. Bowen, J. Ledesma-Medina, N. Salamon, and C. D. Bluestone.** 1981. Acute maxillary sinusitis in children. N. Engl. J. Med. **304**:749–754.
19. **Wong, L. K., A. L. Barry, and S. M. Horgan.** 1982. Comparison of six different criteria for judging the acceptability of sputum specimens. J. Clin. Microbiol. **16**:627–631.
20. **Yogev, R., C. Melick, and W. J. Kabat.** 1981. In vitro and in vivo synergism between amoxicillin and clavulanic acid against ampicillin-resistant *Haemophilus influenzae* type b. Antimicrob. Agents Chemother. **19**:993–996.