Penetration of Ampicillin and Sulbactam in the Lower Airways during Respiratory Infections

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We studied the penetration of ampicillin-sulbactam in the alveolar lining fluid (ALF) of eight patients after intravenous administration of 2,000 mg of ampicillin and 1,000 mg of sulbactam three times daily over 30 min. Bronchoalveolar lavage was performed on day 3, 30 min after the end of the morning drug administration. The mean penetration ratios (i.e., the ratios of the concentrations in ALF versus those in serum) were 53% (standard error, 12%) and 61% (standard error 31%) for ampicillin and sulbactam, respectively. The concentration ratio of ampicillin versus sulbactam in serum was not significantly different from that in ALF. From a pharmacokinetic point of view, ampicillin-sulbactam is a good choice for treatment of infectious exacerbation of chronic obstructive pulmonary disease and community-acquired bacterial pneumonia, since the concentrations of both drugs in ALF exceed the MICs for the respiratory pathogens responsible.

The clinical outcome of bacterial lower respiratory tract infections is a function of two main factors: the efficacy of the host defense mechanisms and institution of appropriate antimicrobial therapy. The antimicrobial drug concentration at the site of infection is supposed to be a determinative element for effective antimicrobial therapy (2). This concentration should equal or exceed the MIC for the respiratory pathogen responsible (3, 17, 22). The final bioactive antibiotic concentrations in the bronchial wall and in secretions and in the alveolar lining fluid (ALF), which are the sites of infection in the lower respiratory tract, are the results of a complex and dynamic process (1, 2, 14).

We evaluated the pharmacokinetic behavior of parenterally administered ampicillin-sulbactam in serum and studied the disposition of both drugs in ALF by using the bronchoalveolar lavage (BAL) technique. BAL has been used to study the chemical compositions of ALF in both normal and pathologic situations, such as interstitial lung disease and asthma. With this technique, the concentrations of immunoglobulins, complement components, surfactant, α1-antitrypsin, and several mediators have been determined (8, 28). Only a few studies have used BAL for evaluation of the concentrations of drugs such as corticosteroids and antibiotics in ALF (5–7).

Sulbactam is a semisynthetic β-lactamase inhibitor (10, 20). In combination with ampicillin, it restores and extends the antibacterial activity of ampicillin to include some β-lactamase-producing strains of bacteria, such as Haemophilus influenzae and Branhamella catarrhalis, that would otherwise be resistant (10, 15, 24). The penetration and ampicillin/sulbactam ratio in ALF have not been studied previously.

MATERIALS AND METHODS

Patients. Seven males and one female (average age, 71 years) with bacterial respiratory tract infections were invited to participate in the study after giving informed consent. All patients had chronic obstructive pulmonary disease and were hospitalized because of infectious exacerbation. Five patients had radiographic signs of pneumonia, and three had bronchial infections.

Drug administration. A combination of ampicillin (2,000 mg) and sulbactam (1,000 mg) was administered intravenously over 30 min in 100 ml of a 5% glucose solution every 8 hr for 3 days.

Study procedure. The study was performed on day 3 of parenteral therapy, when intravenous infusion of ampicillin-sulbactam was given. Blood samples for determination of ampicillin and sulbactam concentrations in serum were taken just before and at 60, 90, 120, 180, and 240 min after the start of intravenous administration. Blood samples were taken in heparinized tubes. The tubes were centrifuged immediately (10 min, 3,000 × g, 4°C). The plasma was kept in glass tubes at −70°C until assay, which was done within 5 days. At 60 min, a blood sample for determination of blood urea was taken.

Fiber bronchoscopy with BAL was performed 60 min after the start of the intravenous drug infusion. BAL was done by wedging the tip of a bronchoscope into a subdivision of a segmental bronchus of the right middle lobe. Fifty milliliters of sterile isotonic saline was instilled through the aspiration channel of the bronchoscope and gently reaspirated, and this procedure was repeated twice. The three lavage samples were combined, and the BAL fluid was centrifuged immediately in a refrigerated centrifuge. The supernatant was separated into two aliquots. Both tubes were kept at −70°C until assay for ampicillin-sulbactam and urea concentrations.

Determination of ampicillin and sulbactam concentrations. Determination of the concentration of ampicillin in plasma and BAL fluid was performed by a high-performance liquid chromatographic assay as described earlier (21). Calibration curves for ampicillin were linear over a concentration range of 1 to 100 μg/ml in 0.1-ml plasma samples and over a range of 0.02 to 2 μg/ml in 0.5-ml samples of BAL fluid. The limits of detection were 0.05 and 0.01 μg/ml with 0.1 ml of plasma and 0.5 ml of BAL fluid, respectively. The within-day precision (coefficient of variation [CV]) was between 4.7 and 7.7% for 0.1-ml plasma samples (1 to 100 μg/ml; n = 5) and between 3.6 and 11.5% for 0.5-ml BAL fluid samples (0.02 to 2 μg/ml; n = 5). Analytical recovery was between 97 and 110% for plasma and between 97 and

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111% for BAL fluid. The between-day precision (CV) and accuracy of the assay method were 5.5% \((n = 6)\) and 107%, respectively, with plasma spiked with ampicillin (25 \(\mu\)g/ml) and 8.4% \((n = 6)\) and 99%, respectively, with BAL fluid spiked with ampicillin (0.75 \(\mu\)g/ml). For determination of sulbactam in plasma and BAL fluid, the highly sensitive high-performance liquid chromatography assay described by Haginata et al. was used (12). Sulbactam was extracted from acidified plasma (0.5 ml) and BAL fluid (2 ml) at pH 1.3 with ethyl acetate. After evaporation of the solvent, the product was reacted with 2 M 1,2,4-triazole solution at 50°C for 15 min in acetonitrile. Twenty microliters of the supernatant was then injected on a Spherisorb 5ODS reverse-phase column (25 cm by 4.6 mm; Chrompack). The mobile phase was a mixture of water-MeOH-acetonitrile (80:17:3) containing Pic\(^{4}\)A (0.005 M tetrabutylammonium phosphate) at 50°C at a flow rate of 1 ml/min. The detection wavelength was 326 nm. The calibration curves for sulbactam were linear over a concentration range of 0.25 to 25 \(\mu\)g/ml in 0.5-ml plasma samples and over a concentration range of 0.05 to 10 \(\mu\)g/ml in 2-ml samples of BAL fluid. The limits of detection were 0.2 and 0.04 \(\mu\)g/ml with 0.5-ml plasma and 2-ml BAL fluid samples, respectively. The within-day precision (CV) was between 3.3 and 7.7% \((n = 5)\) for a concentration range of 0.25 to 25 \(\mu\)g/ml in plasma and between 2.3 and 9.9% \((n = 5)\) for a concentration range of 0.05 to 10 \(\mu\)g/ml in BAL fluid. Analytical recovery was between 95 and 105% for both fluids. The between-day precision (CV) and accuracy of the assay were 6.4% \((n = 6)\) and 97.5%, respectively, with plasma spiked with sulbactam (10 \(\mu\)g/ml) and 6.8% \((n = 6)\) and 99%, respectively, with BAL fluid spiked with sulbactam (0.5 \(\mu\)g/ml).

Urea assay. Urea in serum was determined by an enzymatic kinetic UV method using urease and L-glutamic dehydrogenase as previously described (11, 13). The urea concentration in BAL fluid was determined by a modification of this method, increasing the sensitivity by increasing the sample volume. With this modification, linearity to at least 60 mg/dl was obtained. The sensitivity of this modified method for urea determination was tested on unconcentrated BAL fluid. The lowest detectable urea concentration which could be distinguished from zero with 95% confidence was 0.20 mg/dl. Between-run CVs of 9.86, 1.61, and 0.48% were obtained at urea concentrations of 0.50, 5, and 10 mg/dl, respectively \((n = 10)\).

Calculations. To determine the exact concentrations of ampicillin and sulbactam in ALF, the dilutional effect of the instilled BAL fluid was accounted for. Urea diffuses readily throughout the body compartments so that the concentration of urea in serum and the local concentration of urea in ALF are identical. Urea was shown to be a good marker of dilution to quantify the apparent volume of ALF obtained by the BAL procedure (19).

Thus, the concentration of both drugs in ALF can be calculated by using simple dilution principles: Drug\(_{\text{BAL}}^{*}\)/Urea\(_{\text{BAL}}\) = Drug\(_{\text{ALF}}^{*}\)/Urea\(_{\text{ALF}}\), in which Drug\(_{\text{BAL}}^{*}\) and Urea\(_{\text{BAL}}\) are the concentrations in BAL fluid and Drug\(_{\text{ALF}}^{*}\) and Urea\(_{\text{ALF}}\) are the concentrations in ALF. Since the concentration of urea in serum equals the concentration of urea in ALF (19), Drug\(_{\text{ALF}}^{*}\) = (Drug\(_{\text{BAL}}^{*}\) × Urea\(_{\text{serum}}\))/Urea\(_{\text{BAL}}\).

Pharmacokinetic analysis. Plasma half-lives were calculated by linear regression analysis, as the semilogarithmic plot of the concentration-versus-time curve showed a linear course.

Statistics. Values were compared by using the paired \(t\) test.

**RESULTS**

Concentration-time profiles of sulbactam and ampicillin in plasma. The concentrations of both ampicillin and sulbactam in serum before and after intravenous administration of the morning dose on day 3 are shown in Fig. 1. Low concentrations of both drugs were still present 8 h after the previous intravenous administration. The concentrations of both products in plasma rose sharply after intravenous administration of the combination of 2,000 mg of ampicillin and 1,000 mg of sulbactam, and maximal levels were found at the first sampling point, i.e., 30 min after the end of administration (Fig. 1).

The mean ampicillin/sulbactam ratios in serum just before and at 60, 90, 120, 180, and 240 min after the start of intravenous administration were 2.84 (standard error [SE], 0.76), 1.88 (SE, 0.08), 1.99 (SE, 0.13), 2.23 (SE, 0.19), 2.14 (SE, 0.12), and 2.26 (SE, 0.19), respectively. The calculated plasma half-lives of ampicillin and sulbactam were 87.7 and 80.0 min, respectively.

Concentrations of ampicillin and sulbactam in ALF. At 30 min after the end of the 30-min infusion of both drugs, at their expected peak concentrations in serum, BAL was performed. We calculated the concentrations of both ampicillin and sulbactam in ALF as described in Materials and Methods. The results are shown in Table 1.

The degrees of penetration of ampicillin and sulbactam from the vascular compartment through the alveolar-capillary membrane into the ALF are shown in Table 2. The mean penetration ratios (ratios of concentrations in ALF versus concentrations in serum) were 0.53 (SE, 0.12) and 0.61 (SE, 0.13) for ampicillin and sulbactam, respectively. The ratio of the ampicillin concentration to the sulbactam concentration in the ALF is also shown in Table 2, with a mean ratio of 1.70 (SE, 0.20).

There was a significant difference between the concentrations of ampicillin in serum and in ALF \((P < 0.05)\) and between the concentration of sulbactam in serum and in ALF \((P < 0.05)\). The ratio of the concentration of ampicillin to the concentration of sulbactam in ALF was 2.14 (SE, 0.12), respectively. The calculated plasma half-lives of ampicillin and sulbactam were 87.7 and 80.0 min, respectively.

FIG. 1. Concentrations (mean ± the standard error of the mean) of ampicillin and sulbactam in plasma at different times following a 30-min intravenous infusion (starting at time zero) of 2,000 mg of ampicillin and 1,000 mg of sulbactam. The concentrations of both drugs in ALF were determined at 60 min postinfusion.
TABLE 1. Individual and mean concentrations of urea, sulbactam, and ampicillin in serum and ALF 60 min after intravenous administration of 2,000 mg of ampicillin and 1,000 mg of sulbactam

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Urea concn (mg/dl)</th>
<th>Ampicillin concn (μg/ml)</th>
<th>Sulbactam concn (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>BAL</td>
<td>Serum</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>1.91</td>
<td>51.8</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>0.05</td>
<td>93.4</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>0.43</td>
<td>97.2</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>0.11</td>
<td>90.8</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>1.48</td>
<td>68.3</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>0.79</td>
<td>52.4</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>0.37</td>
<td>62.4</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>1.88</td>
<td>63.1</td>
</tr>
</tbody>
</table>

Mean 42 (4.0)a 0.88 (0.27) 72.4 (6.6) 0.48 (0.11) 43.4 (12.8) 39.4 (4.3) 0.29 (0.08) 26.6 (7.6)

a The numbers in parentheses are standard errors of the means.

the concentration of sulbactam was not significantly different between serum and ALF. There was also no significant difference between the degrees of penetration of ampicillin and sulbactam into ALF.

Clinical and bacteriological outcomes with respect to penetration ratios. All of our patients, except one, had uneventful clinical outcomes with clearing of infectious signs and symptoms and without relapses. In patients from whom respiratory pathogens were isolated, all of the pathogens but one (Pseudomonas aeruginosa) were eradicated. The penetration ratios of ampicillin and sulbactam in patients with pneumonia were 0.65 (SE, 0.37) and 0.79 (SE, 0.38), respectively. These ratios were substantially greater than in patients with bronchial infections, for whom the ratios were 0.33 (SE, 0.20) and 0.39 (SE, 0.35) for ampicillin and sulbactam, respectively. However, because of the limited number of patients studied, statistical analysis was not possible. The greater penetration of both drugs in the setting of pneumonia can be due to more extended interstitial inflammation, which increases vascular blood flow and permeability.

DISCUSSION

Community-acquired pneumonia and infectious exacerbation of chronic obstructive lung disease remain important causes of morbidity, mortality, and economic loss. Streptococcus pneumoniae, H. influenzae, and B. catarrhalis are the three most important bacterial pathogens that cause these diseases. The combination of ampicillin and sulbactam has been reported to be clinically effective and safe for treatment of various infections of the lower respiratory tract (10, 26). The kinetics of sulbactam are similar to those of ampicillin. After an initial rapid distribution phase, with a distribution half-life of approximately 15 min for both drugs, a terminal half-life of approximately 60 min is observed. A moderate degree of protein binding of sulbactam in human serum (38%), similar to that of ampicillin (28%), predicts extensive diffusion from blood to extravascular tissues. The apparent volumes of distribution were approximately 12 liters for the central compartment (blood and rapidly equilibrating tissues) and approximately 23 liters for the whole body (9).

Proper antimicrobial therapy demands appropriate concentrations of antimicrobial drugs at the site of infection (2, 10). Penetration of antibiotics into respiratory secretions is dependent on complex pharmacokinetic laws (1, 2, 14). Several studies on ampicillin concentration in the respiratory tract using sputum or bronchial secretions as the study material have been performed (3, 4, 16, 23). An oral dose of 1 g of ampicillin achieved a level of 0.15 μg/ml in sputum and a peak level of 3.2 μg/ml in serum, resulting in a ratio of 0.047. In another study, however, oral doses of 0.25 to 0.5 g of ampicillin resulted in peak concentrations in sputum ranging between 0.24 and 0.30 μg/ml (22). Intravenous administration of 1 to 2 g of ampicillin produced concentrations in plasma 8 to 10 times those observed after oral

TABLE 2. Relative penetration of ampicillin and sulbactam in ALF following intravenous administration of 2,000 mg of ampicillin and 1,000 mg of sulbactam 60 min before

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Type of lower respiratory tract infection</th>
<th>Ratio of concentrations of a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amp in ALF/Amp in serum</td>
<td>Sub in ALF/Sub in serum</td>
</tr>
<tr>
<td>1</td>
<td>Bronchial</td>
<td>0.14</td>
</tr>
<tr>
<td>2</td>
<td>Pneumonia</td>
<td>1.02</td>
</tr>
<tr>
<td>3</td>
<td>Pneumonia</td>
<td>0.47</td>
</tr>
<tr>
<td>4</td>
<td>Pneumonia</td>
<td>1.06</td>
</tr>
<tr>
<td>5</td>
<td>Bronchial</td>
<td>0.31</td>
</tr>
<tr>
<td>6</td>
<td>Pneumonia</td>
<td>0.47</td>
</tr>
<tr>
<td>7</td>
<td>Bronchial</td>
<td>0.53</td>
</tr>
<tr>
<td>8</td>
<td>Pneumonia</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Mean 0.53 (0.12)b 0.61 (0.13) 1.70 (0.20) 1.88 (0.08)

a Amp, Ampicillin; Sub, sulbactam.

b The numbers in parentheses are standard errors of the means.
administration, but there was no corresponding increase in penetration into sputum (27). The ratio between bronchial concentrations and simultaneous concentrations in serum usually ranged between 3 and 5%.

Local vasodilation and increased vascular permeability, which are features of inflammation, increase the penetration of ampicillin into bronchial secretions (3, 16). Single-dose oral administration of 750 mg of sulbactam in four patients with chronic respiratory tract infections resulted in ranges of peak levels in sputum of 0.2 to 1.3 and 0.13 to 0.52 μg/ml for sulbactam and ampicillin, respectively. The ratio of concentrations in plasma to the concentrations in sputum ranged from 3.8 to 17.3 for sulbactam and from 2.1 to 3.8 for ampicillin (25).

The main disadvantage of using sputum and bronchial secretions for determination of antibiotic concentrations is that the samples are pooled collections of secretions from the alveoli, the airway submucosal and mucosal glands, and the oral pharynx (17, 18). In view of the enormous surface of the alveolar-capillary membrane in contrast to the blood-bronchus barrier and the fact that the most important mechanism of transport of antibiotics across this membrane is passive diffusion across a concentration gradient, one may assume that antibiotic concentrations in the ALF more closely approximate concentrations in serum than do the concentrations in bronchial secretions (1, 27).

The best method to evaluate the concentrations of substances in the ALF is BAL. Using this technique necessitates the use of highly sensitive measuring techniques and a reference molecule (urea in our study) to calculate the dilutional effect of the instilled fluid (19).

In our study, we showed that BAL is a valuable and promising technique for determination of the concentrations of antibiotics in the ALF, which is the site of infection in pneumonia. At 1 h after intravenous administration of ampicillin and sulbactam, at their peak concentration in serum, penetration ratios of 53 and 61%, respectively, were achieved. These percentages are much higher than the known penetration ratios in sputum and bronchial secretions. In two patients, the ratios of both drugs exceeded 100%, suggesting drug accumulation. The great interindividual variability of the penetration rate could be explained by individual variations in the degree of inflammation in the lower respiratory tract. In patients with bronchopneumonia, the penetration ratio tended to be higher than in those with bronchitis. The higher degree of interstitial inflammation in pneumonia is probably responsible for these findings. However, statistical analysis of the registered values was impossible in view of the limited number of patients.

Furthermore, an important finding in our study was that ratio of the ampicillin concentration to the sulbactam concentration in serum was virtually identical to that in the ALF. This means that there will be a high enough concentration of β-lactamase inhibitor at the site of the bacterial infection.

The correlation between antibiotic concentrations in the ALF and the clinical and microbiological outcomes awaits further study.

LITERATURE CITED