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Penetration of Amoxicillin and Potassium Clavulanate into the Cerebrospinal Fluid of Patients with Inflamed Meninges

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A single intravenous dose of 2.0 g of amoxicillin and 0.2 g of potassium clavulanate was given to patients with bacterial meningitis, and the pharmacokinetics of both drugs in the cerebrospinal fluid (CSF) and plasma were evaluated. Twenty-one patients aged 14 to 76 years were studied. Both amoxicillin and potassium clavulanate were detectable in the CSF as early as 1 h and reached peak concentrations by approximately 2 h. The highest mean CSF concentrations were 2.25 μg/ml for amoxicillin and 0.25 μg/ml for potassium clavulanate and were found in patients with moderately or severely inflamed meninges. The CSF penetration relative to plasma for amoxicillin and potassium clavulanate was 5.8 and 8.4%, respectively. These levels suggest that the amoxicillin-potassium clavulanate combination may be effective for the treatment of bacterial meningitis caused by β-lactamase-producing pathogens.

Potassium clavulanate is a naturally occurring β-lactamase inhibitor. In vitro studies showed that potassium clavulanate has inhibitory activity against a variety of plasmid and chromosomally produced β-lactamases (1). Clinical studies with amoxicillin and potassium clavulanate in combination given orally have demonstrated efficacy in the treatment of infections caused by β-lactamase-producing *Haemophilus influenzae* and *Staphylococcus aureus* (9, 10, 15, 23). The cerebrospinal fluid (CSF) pharmacokinetics of orally administered potassium clavulanate alone, as well as of the 2:1 (wt/wt) combination of amoxicillin and potassium clavulanate, were previously evaluated in patients with noninflamed meninges (7, 11). A 10:1 combination of amoxicillin and potassium clavulanate for intravenous (i.v.) use has recently become available for clinical trials. This investigation evaluates the pharmacokinetics of these two compounds in CSF and plasma after a single i.v. dose given to patients suffering from bacterial meningitis who were at the same time receiving standard therapy with benzylpenicillin or chloramphenicol or both.

MATERIALS AND METHODS

A total of 21 patients (11 men and 10 women) were included in the study. Their ages ranged from 14 to 76 years (mean, 34.6 years) and their weights ranged from 36 to 85 kg (mean, 65.9 kg). Permission was obtained from all patients or their guardians before the study. All patients were suffering from bacterial meningitis diagnosed by clinical findings and one or more of the following: CSF leukocyte count, CSF/plasma glucose concentration ratio, or the demonstration of bacteria in CSF or blood. The following pathogens were identified (the number of isolates is given in parentheses): *Neisseria meningitidis* group B (8), *N. meningitidis* group C (2), *Streptococcus pneumoniae* (3), *H. influenzae* type b (2), and β-hemolytic streptococci group A (1). "*Streptococcus milleri*" and *Bacteroides fragilis* were cultured from the CSF of a patient with a ruptured brain abscess. There was no growth of bacteria in CSF or blood cultures from four patients (two had received antibiotics before admission). Based on the results of counterimmunoelectrophoresis of CSF, *S. pneumoniae* was identified as the pathogen in one of the four patients. The bacterial isolates were tested for susceptibility to amoxicillin and potassium clavulanate by the standard International Collaborative Study technique (4) on 5% blood-supplemented PDM agar plates (AB Biodisc; Solna, Sweden) with amoxicillin-potassium clavulanate-containing tablets (Neosensitabs, 30/15 μg; A/S Rosco, Taastrup, Denmark). All strains, with the exception of *B. fragilis*, were highly susceptible to the amoxicillin-potassium clavulanate combination, with amoxicillin MICs in the presence of potassium clavulanate of 0.08 μg/ml for all the streptococci, 0.2 μg/ml for *N. meningitidis*, and 0.9 μg/ml for β-lactamase-producing *H. influenzae* type b. The *B. fragilis* strain died before detailed species identification and susceptibility testing in broth could be performed.

The degree of meningeal inflammation was determined by criteria (slightly modified) previously published by Netland, Müller, and Andrews (12). These criteria were as follows. (i) Mildy inflamed meninges: CSF leukocyte count, $<100 \times 10^6$/liter; CSF/plasma glucose ratio, >0.4; CSF total protein concentration, <750 mg/liter. (ii) Moderately inflamed meninges: CSF leukocyte count, $<1{,}000 \times 10^6$/liter; CSF/plasma glucose ratio, <0.4; CSF total protein concentration, <1,500 mg/liter. (iii) Severely inflamed meninges: CSF leukocyte count, $>1{,}000 \times 10^6$/liter; CSF/plasma glucose ratio, <0.4; CSF total protein concentration, >1,500 mg/liter. At least two criteria in one category were required to assign a patient to that category of meningeal inflammation.

Each patient received amoxicillin (50 mg/kg of body weight; maximum dose, 2.0 g) and potassium clavulanate (5 mg/kg of body weight; maximum dose, 0.2 g) i.v. combined in an infusion solution of 100 ml of 0.9 N saline at a constant rate over 30 min. Each patient was randomly allocated to one of four study groups by drawing a sealed envelope, and a CSF sample was obtained after a second clinically indicated lumbar puncture (LP) at either 1, 2, 3, or 4 h after the infusion began, 48 to 96 h after standard antibiotic therapy was initiated. The degree of meningeal inflammation was

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TABLE 1. Parameters used for classifying the degree of meningeal inflammation of 21 patients with bacterial meningitis

Meningeal inflammation	No. of patients	Leukocyte count (10^6)/liter of CSF[a]	Glucose concn ratio (CSF/plasma)[a]	CSF protein (mg/liter)[a]
Mild	5	206 ± 125	0.52 ± 0.08	638 ± 196
Moderate	7	512 ± 287	0.46 ± 0.16	1,218 ± 442
Severe	9	11,575 ± 19,518	0.19 ± 0.10	3,211 ± 2,345

[a] Mean values ± standard deviations.

also determined from data obtained by the second LP. Blood was collected by venipuncture in heparinized plastic tubes at 0, 30, 60, 120, and 240 min. A simultaneous blood sample was taken from patients who had the LP at 180 min. After centrifugation in a Sorvall GLC-2B at 1,800 rpm for 20 min, the plasma was carefully separated and frozen with the CSF sample at −70°C until the assay. All samples were analyzed within 30 days of collection. There were no untoward effects on any of the patients of the amoxicillin-potassium clavulanate infusions. The LPs were difficult to perform in two patients, resulting in slight blood contamination of both CSF samples, as well as a delay in the CSF sample collection time of one patient (290 min).

The drug concentration assays of amoxicillin and potassium clavulanate in ultrafiltrated plasma and undiluted CSF samples were performed by high-performance liquid chromatography. Amoxicillin chromatography was performed on a μBondapak C18 column (25 cm by 4.6 mm inside diameter; Waters Associates, Inc., Milford, Mass.), and the mobile phase was a mixture of water, methanol, and acetic acid. Potassium clavulanate samples were chromatographed on a Spherisorb-5-ODS column (Waters Associates), and the mobile phase contained methanol in tetrabutyl ammonium hydrogen sulfate in phosphate buffer. Detection was achieved after postcolumn derivatization by using fluorescence and UV light for amoxicillin and potassium clavulanate, respectively. The lowest standards of detection for amoxicillin and potassium clavulanate in plasma were 0.5 and 0.28 μg/ml, respectively, and the respective values in CSF were 0.06 and 0.05 μg/ml. The reproducibility of the methods was evaluated by analyzing five aliquots at each of three concentrations. Variations between the peaks for amoxicillin and potassium clavulanate, expressed as coefficients of variation, were less than 5%. The areas under the concentration-time curves (AUCs) from 0 to 4 h (AUC_{0-4}) for amoxicillin and potassium clavulanate in plasma were calculated by the trapezoidal method. The AUCs in CSF were calculated by the mean drug concentration values measured at 1 to 4 h. The Student t test was used for all statistical calculations.

TABLE 2. Amoxicillin and potassium clavulanate concentrations in plasma and concentration ratios[a]

Time (min)	No. of patients	Mean concn (μg/ml) ± SD in plasma of:		Concn ratio
		Amoxicillin	Potassium clavulanate	
30	21	101.9 ± 46.8	8.19 ± 3.09	12.44
60	21	43.3 ± 15.7	3.89 ± 1.48	11.13[b]
120	21	17.0 ± 6.71	1.73 ± 0.65	9.82[b]
180	5	7.07 ± 4.15	0.69 ± 0.53	10.24[b]
240	21	4.86 ± 1.51	0.67 ± 0.36	6.87[b]
290	1	5.07	ND[c]	

[a] Concentrations and ratios were determined after a 30-min i.v. infusion of amoxicillin-potassium clavulanate (50 and 5 mg/kg of body weight and a maximum dose of 2.0 and 0.2 g, respectively).
[b] $P > 0.10$.
[c] ND, Not detected.

TABLE 3. Mean concentrations of amoxicillin and potassium clavulanate in CSF and concentration ratios

Hour of LP	No. of patients	Mean concn (μg/ml) ± SD in CSF of:		Concn ratio
		Amoxicillin	Potassium clavulanate	
1	5	1.12 ± 0.87	0.15 ± 0.10	7.47
2	5	2.32 ± 0.79	0.28 ± 0.21	8.29[a]
3	4	1.57 ± 1.08	0.24 ± 0.15	6.54[a]
4	5	2.53 ± 0.59	0.27 ± 0.04	9.37[a]

[a] $P > 0.20$.

RESULTS

The mean values of the parameters used in classifying the degree of meningeal inflammation and the number of patients included in each category are shown in Table 1. The mean concentrations in plasma of amoxicillin and potassium clavulanate and the drug concentration ratios are displayed against time in Table 2. Although the ratio decreased toward the end of the study period, the change was not statistically significant ($P > 0.10$). The mean concentrations in CSF of amoxicillin and potassium clavulanate against time (hour of LP) are shown in Table 3. Neither amoxicillin nor potassium

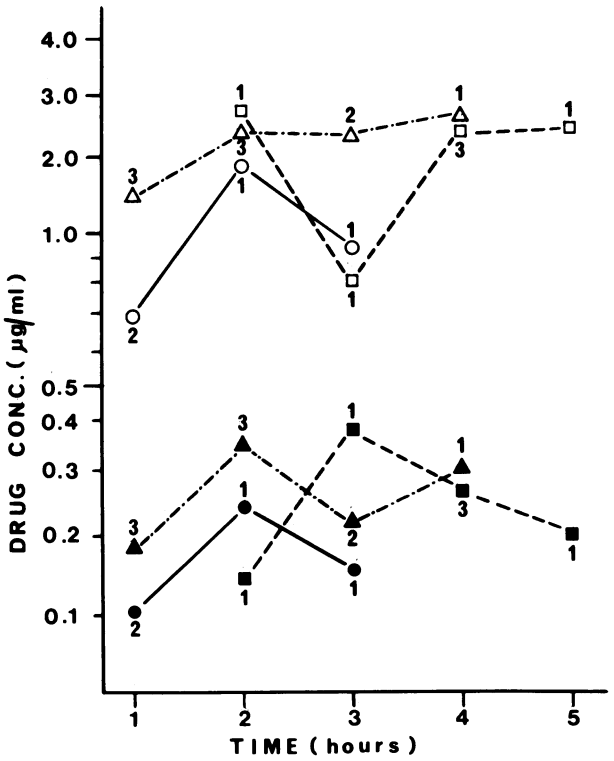

FIG. 1. Influence of time and degree of meningeal inflammation on the mean concentrations of amoxicillin (top) and potassium clavulanate (bottom) in the CSF of patients with bacterial meningitis. The numbers adjacent to each symbol indicate the number of patients entered in each category of meningeal inflammation. Degree of meningeal inflammation: ○ and ●, mild; □ and ■, moderate; △ and ▲, severe.

TABLE 4. Amoxicillin and potassium clavulanate concentrations in CSF and concentration ratios from 19 patients with various degrees of meningeal inflammation

Meningeal inflammation	Mean concn (μg/ml) ± SD in CSF of:		Concn ratio
	Amoxicillin	Potassium clavulanate	
Mild	1.04 ± 0.59	0.15 ± 0.06	6.93
Moderate	2.25 ± 0.84[a]	0.25 ± 0.08	9.00
Severe	2.05 ± 0.99[a]	0.26 ± 0.18	7.88

[a] $P < 0.05$.

clavulanate could be detected in the CSF samples of two patients. In one case, this was the result of CSF sample mishandling before analysis. We have no ready explanation for the failure to detect either drug in the CSF of the other patient. An analytical technical error is the most likely explanation. Both patients were omitted from the CSF calculations. For the remaining 19 patients, both amoxicillin and potassium clavulanate were detectable in the CSF as early as 1 h postinfusion; drug concentrations reached peaks at about 2 h and reached plateaus at 3 and 4 h (Table 3). Fig. 1 portrays the 19 concentration values of amoxicillin and potassium clavulanate in CSF against the time of LP and the degree of meningeal inflammation. The concentrations of amoxicillin and potassium clavulanate were higher in patients with moderately or severely inflamed meninges compared with those in patients with mild inflammation (Table 4), but the differences reached statistical significance only for amoxicillin (amoxicillin, $P < 0.05$; potassium clavulanate, $P < 0.10$). The ratio of amoxicillin to potassium clavulanate concentrations in CSF did not vary significantly with time ($P > 0.20$; Table 3) or with the degree of meningeal inflammation ($P > 0.10$; Table 4). Based on the AUCs, the ratios of amoxicillin and potassium clavulanate concentrations in CSF to the drugs in plasma (CSF penetration) were 0.058 and 0.084, respectively (Table 5). When CSF penetration was expressed as the ratio of the simultaneously measured concentrations of the drugs in CSF and plasma, the entry of either drug into the CSF appeared considerably more extensive (Table 5).

DISCUSSION

Most authors agree that β-lactam penetration into CSF varies proportionally with protein content and degree of pleocytosis (2, 3, 8, 16, 18–20). Netland et al. (12) classified meningeal inflammation into three very general categories. We attempted to be more precise in classification, demonstrating (Table 1) that changes in CSF leukocyte count, protein content, and glucose concentration vary predictably with increasing degrees of meningeal inflammation. This is important because many β-lactam drugs are removed from the CSF by both active and passive mechanisms (5, 13). The relative proportion of the drug removed by the active, probenecid-susceptible secretory process in the choroid plexus is inversely related to the degree of inflammation. β-Lactam compounds that attain high concentrations in CSF, like moxalactam, ceftazidime, and ceftriaxone, share the common property of not having their renal (and probably choroid plexus) clearance mechanisms influenced by probenecid (16, 17). The renal excretion of potassium clavulanate occurs primarily by glomerular filtration (21). The low molecular weight, low protein binding, and slight acidic ionic charge, combined with no active CNS secretory mechanism, should allow theoretically for high concentrations in CSF.

The lowest standard of detection of potassium clavulanate in the study performed by Münch et al. (11) was 0.16 μg/ml, and only 1 of 15 patients had a measured concentration in CSF above the lower detection limit. In spite of only modest concentrations in CSF in their investigation, Kosmidis et al. (7) emphasized that potassium clavulanate levels in CSF of 0.11 to 0.18 μg/ml should be of sufficient magnitude to give synergistic action with amoxicillin against β-lactamase-producing *H. influenzae*. Potassium clavulanate concentrations in CSF in this investigation ranged from 0.07 to 0.67 μg/ml, and five (26%) of the concentrations were lower than 0.15 μg/ml. The amoxicillin concentrations in CSF in our study ranged from 0.69 to 3.46 μg/ml. Sixty-eight percent of the concentrations were higher than 1.0 μg/ml, levels sufficiently high to be effective in combination with potassium clavulanate against β-lactamase-producing pathogens. The in vitro susceptibility data from Hunter et al. (6) supports this assumption.

Like Münch et al. (11) we observed a wide variation in drug concentrations in plasma and CSF, as well as in the ratio of amoxicillin to potassium clavulanate over time, indicating considerable individual differences in the distribution and elimination of the two drugs. The slightly higher amoxicillin to potassium clavulanate concentration ratios in plasma observed against time (Table 2) compared with those in CSF (Table 3) would suggest that potassium clavulanate either penetrates somewhat better into CSF or has a longer CSF half-life than amoxicillin. This is corroborated by the calculated percent CSF penetration values [(CSF AUC/plasma AUC) × 100] for amoxicillin (5.8%) and potassium clavulanate (8.4%). We believe, as do other investigators (2, 14), that CSF penetration is more correctly expressed by the ratio between the total amounts of the drug registered, rather than by a static ratio calculated from concentrations in CSF and plasma measured at one particular time. Although the use of AUCs in calculating the CSF penetration ratios of the drugs would tend to underestimate the amount of each drug, it is clearly in the best interest of the patient that estimates remain conservative until more information about the two drugs becomes available. Penetration expressed by the static CSF/plasma ratios may give misleading results (Table 5).

Our results do not permit firm conclusions about the usefulness of potassium clavulanate and amoxicillin in the

TABLE 5. Pharmacokinetics of amoxicillin and potassium clavulanate in CSF (19 patients) and plasma (12 patients) of patients with bacterial meningitis

Drug	AUC_{1-4} in CSF (mg · h/liter)	AUC_{0-4} in plasma (mg · h/liter)	CSF penetration[a]	Mean CSF/plasma concn ratio ± SD[b]
Amoxicillin	6.21	107.1	0.058	0.30 ± 0.29
Potassium clavulanate	0.81	9.64	0.084	0.26 ± 0.28

[a] AUC_{1-4} in CSF/AUC_{0-4} in plasma.
[b] Determined at the time of LP.

therapy of bacterial meningitis. The CSF penetration of potassium clavulanate varies less with the degree of meningeal inflammation than does that of amoxicillin. Reducing the dose ratio, combined with high-dosage administration, should raise the steady-state level of potassium clavulanate in CSF sufficiently to make it, combined with amoxicillin, useful in the therapy of meningitis caused by β-lactamase-producing organisms. Further clinical studies are warranted to investigate this possibility.

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