Microdialysis Study of Cefotaxime Cerebral Distribution in Patients with Acute Brain Injury

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Central nervous system (CNS) antibiotic distribution was described mainly from cerebrospinal fluid data, and only few data exist on brain extracellular fluid concentrations. The aim of this study was to describe brain distribution of cefotaxime (2 g/8 h) by microdialysis in patients with acute brain injury who were treated for a lung infection. Microdialysis probes were inserted into healthy brain tissue of five critical care patients. Plasma and unbound brain concentrations were determined at steady state by high-performance liquid chromatography. *In vivo* recoveries were determined individually using retrodialysis by drug. Noncompartmental and compartmental pharmacokinetic analyses were performed. Unbound cefotaxime brain concentrations were much lower than corresponding plasma concentrations, with a mean cefotaxime unbound brain-to-plasma area under the curve ratio equal to 26.1 ± 12.1%. This result was in accordance with the brain input-to-brain output clearances ratio ($CL_{in,brain}/CL_{out,brain}$). Unbound brain concentrations were then simulated at two dosing regimens (4 g every 6 h or 8 h), and the time over the MICs (T>MIC) was estimated for breakpoints of susceptible and resistant *Streptococcus pneumoniae* strains. T>MIC was higher than 90% of the dosing interval for both dosing regimens for susceptible strains and only for 4 g every 6 h for resistant ones. In conclusion, brain distribution of cefotaxime was well described by microdialysis in patients and was limited.

Cefotaxime is a third-generation extended-spectrum cephalosporin and is recommended as an empirical therapy for bacterial meningitis, ventriculitis, and cerebral abscesses associated with metronidazole (1, 2). One of the limits of this urgent treatment is its central nervous system (CNS) penetration. The mechanism of penetration of microbial pathogens in the CNS remains unclear in bacterial meningitis (3), and it has been shown that some bacteria usually implicated in such infection could have the ability to bind and cross the vascular endothelium of the blood-brain barrier (BBB) (4). The exact location of bacteria in meningitis is still unclear. Few studies, limited to investigations in cerebrospinal fluid (CSF), were performed (5–8), and they found a low central distribution of cefotaxime (5, 6). In humans, the easiest way to study antibiotic penetration into CNS is to measure the concentration in CSF from lumbar puncture or external ventricular drainage. CSF concentrations are usually considered a good surrogate for brain target site concentrations (9). However, qualitative differences, such as anatomy, enzymatic activity, or bulk flow, exist between the BBB and blood-CSF barrier (BCSFB) (9), which could result in differences of drug distribution between CSF and brain extracellular fluid (ECF).

Intracerebral microdialysis is one of the *in vivo* techniques allowing brain ECF sampling to study ECF distribution of exogenous compounds, such as antibiotics. In humans, feasibility issues of brain microdialysis restrict its use, and this technique concerns only patients who require surgery for brain tumor resection (10) or cerebral metabolism monitoring in neurointensive care units (11). The main advantage in intracerebral microdialysis is to measure continuously brain unbound concentrations as a function of time; the comparison of these brain concentrations with unbound plasma concentrations may provide information on drug transport across the BBB (12).

The main goal of this study was to explore cerebral ECF distribution of cefotaxime in patients with acute brain injury by comparing unbound concentrations in brain and plasma.

MATERIALS AND METHODS

Patients. This study was performed in the neurointensive care unit at University Hospital of Poitiers (France) in accordance with the Declaration of Helsinki (Edinburgh, Scotland; October 2000) and was approved by the local ethics committee (CPP OUEST III, protocol no. 2008-003311-12). Written informed consent was obtained from a legal representative of the five patients enrolled. Patients (1 woman, 4 men), 39 to 67 years old, were brain injured, sedated with midazolam and fentanyl, and mechanically ventilated. The demographic characteristics are detailed in Table 1. All received cefotaxime (Panpharma, Fougères, France) for the clinical management of a lung infection at a dosing regimen of 2 g three times per day. The exclusion criteria were a renal failure (calculated creatinine clearance of <5 ml · min^{-1}) and cefotaxime contraindication. Routine monitoring for acute brain injury included brain-specific monitoring of intracranial pressure (ICP) (Microsensor ICP monitoring system; Codman & Shurtleff, Inc., Raynham, MA), measurement of the partial pressure of oxygen in brain tissue (PbO_2) (Licox; Integra Neurosciences, Lyon, France), and cerebral microdialysis (CMA-70 [20 kDa]; CMA, Stockholm, Sweden) for determination of metabolism parameter concentrations.

Microdialysis probe implantation. Upon admission in the unit (day 0), patients were equipped with microdialysis probe, ICP, and PbO_2 cath-

TABLE 1 Demographic patient characteristics

Characteristic	Value by patient no.:				
	1	2	3	4	5
Age (yrs)	64	43	56	39	67
Ht (cm)	172	164	180	175	175
Wt (kg)	85	58	85	95	100
Creatinine clearance (ml · min^{-1})	166	193	146	187	143
Serum albumin (g · liter^{-1})	28.9	28.0	30.0	28.4	24.4
Serum total proteins (g · liter^{-1})	52.0	51.5	60.0	52.8	52.0
Diagnosis[a]	TBI	TBI	SAH	TBI	SAH
No. of cefotaxime doses before study	14	9	9	5	9
Corticotherapy (3 × 1 mg · kg^{-1} · d^{-1})	No	Yes	Yes	No	No

[a] TBI, trauma brain injury; SAH, subarachnoid hemorrhage.

eters for clinical indications. All were inserted through a single triple lumen bolt (Licox; Integra Neuroscience, Lyon, France) in healthy brain tissue, confirmed by a routine computed tomography scan (CT scan) performed on day 2. After insertion, the microdialysis probe was perfused with CNS perfusion fluid (CMA, Stockholm, Sweden) using a microdialysis pump (CMA-106; CMA, Stockholm, Sweden) at a flow rate of 0.3 μl · min^{-1}.

Drug administration and samplings. Cefotaxime brain pharmacokinetic study was conducted at the steady state between days 3 and 5, after 5 to 14 cefotaxime administrations. After collection of baseline dialysates and blood samples, 2 g of cefotaxime was infused over 0.5 h. Brain dialysates were collected over a maximum period of 9 h at 30-min intervals during the first 3 h and at 1-h intervals for the rest of experiment. Seven to nine blood samples were collected over 8 to 10 h, maximum. Blood samples were centrifuged at 2,000 × g for 15 min at 4°C, and plasma was collected to determine total cefotaxime concentrations. Two blood aliquots for each patient were used to determine unbound plasma concentrations of cefotaxime. The first blood sample was chosen at early time (between 0.25 h and 0.75 h after the beginning of infusion), and the second at the end of pharmacokinetic experiment (between 6 and 10 h). Blood was centrifuged, and plasma was then ultrafiltrated (Centrifree; Millipore Corporation, Billerica, MA) at 2,500 × g for 15 min at 4°C. Directly after collection, dialysates, ultrafiltrate (UF), and plasma samples were kept at −80°C until analysis.

Recovery calculations. For each patient, *in vivo* probe recovery was determined using retrodialysis by drug over 2.5 h at the end of the experiment, as previously described (12, 13). The next cefotaxime injection was delayed to perform the recovery estimation. Briefly, the microdialysis probe was perfused with 20 μg · ml^{-1} solution of cefotaxime in CNS perfusion fluid, and after a 1-h equilibration period, three 30-min interval dialysates were collected. Cefotaxime concentrations were determined in the perfusate (Cin) and in dialysates (Cout). The *in vivo* relative recovery by loss was calculated for each dialysate collected, and the mean value was used to correct dialysate concentrations for pharmacokinetic calculations (13). In the present study, because the cefotaxime residual unbound concentration in the brain was always estimated as less than 1% of cefotaxime concentration administered in probe (Cin), this concentration was not taken into consideration to estimate recovery like it could be previously performed (14).

Cefotaxime assay. Cefotaxime concentrations were determined by high-performance liquid chromatography with UV detection. The chromatographic system consisted of a Xterra C$_{18}$ column (150- by 3.8-mm internal diameter [i.d.]; France), a Hitachi pump L-2130 (VWR, Fontenay sous bois, France), and a Hitachi L-2200 autosampler (VWR, Fontenay sous bois, France) connected to a UV detector (Schimadzu SPD 10A; Marne la Vallée, France) at 235 nm. Data were recorded and analyzed on an EZChrom integrator (VWR, Fontenay sous bois, France). The mobile-phase consisted of a solution of KH$_2$PO$_4$ 0.01 M mixed with acetonitrile (86:14 [vol/vol]) at a flow rate of 0.4 ml · min^{-1}. Brain dialysates and UF

samples were injected directly after dilution with an internal standard solution of dimetridazole (0.5 ng · ml^{-1}). A seven-point calibration standard curve with concentrations between 0.2 and 40 μg · ml^{-1} was performed. The dialysates and UF cefotaxime intra- and interday variability were, respectively, characterized at four (0.2, 0.5, 5, and 40 μg · ml^{-1}) and three (0.5, 5, and 30 μg · ml^{-1}) levels of concentrations, respectively, and were always lower than 15%. Plasma samples (100 μl) were treated by the addition of 200 μl of acetonitrile containing internal standard (2.5 μg · ml^{-1}) for deproteinization. A seven-point calibration standard curve was prepared with concentrations between 1 and 80 μg · ml^{-1}. The plasma intra- and interday variability were, respectively, characterized at four (1, 2, 10, and 80 μg · ml^{-1}) and three (2, 10, and 60 μg · ml^{-1}) levels of concentrations and were always lower than 20%.

Pharmacokinetic analysis. Ratios of cefotaxime concentrations in ultrafiltrate on corresponding total plasma concentrations allow for the obtainment of two individual unbound fraction values (fu) of cefotaxime for each patient. A mean was then determined and was used to convert total concentrations into unbound concentrations. Pharmacokinetic parameter values were estimated from unbound plasma and ECF brain unbound concentrations by individual noncompartmental analysis (NCA) and compartmental analysis (Phoenix WinNonlin 6.2; Pharsight).

Noncompartmental analysis. The areas under the plasma and brain ECF unbound concentration-time curves from dosing time to dosing interval (τ) (AUC$_{0 \to \tau}$) were calculated using the linear trapezoidal rule. The elimination rate constant k_{el} and corresponding half-lives ($t_{1/2}$) were determined by least-squares fit of data points (log concentration time) in the terminal phase of the decline. Steady-state unbound body clearance of cefotaxime (CL$_{ss,u}$) and volume of distribution ($V_{ss,u}$) were calculated according to standard procedures.

Compartmental analysis parameters. The compartmental analysis was performed in two steps as previously described (12). Briefly, unbound plasma drug concentration-time data were first analyzed using a two-compartment model. Plasma parameters estimated were then fixed to fit brain data. The brain was represented as a compartment characterized by a volume (V_b) which was fixed as a constant corresponding to 1% of the volume of distribution (15). This brain compartment was also characterized by brain input clearance (CL$_{in,brain}$) and brain output clearance (CL$_{out,brain}$) (Fig. 1). However, due to the small size of the brain, unbound plasma drug concentrations govern brain unbound drug concentrations but not the reverse. Therefore, drug transport into and out of the brain can be modeled with elimination from the brain, which does not influence plasma drug concentrations (15).

Cefotaxime dosing regimen simulations. Mean cefotaxime unbound concentrations in brain were simulated after multiple administrations at two dosing regimens, 4 g every 6 h (16 g per day) and 4 g every 8 h (12 g per day), considering that the drug was infused at a constant rate over 30 min using Berkeley Madonna software, version 8.3.18. (University of
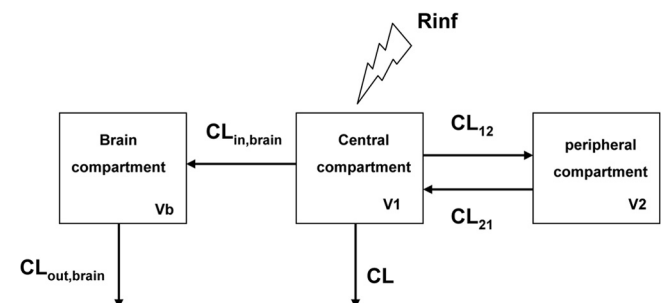

FIG 1 Pharmacokinetic model. V1, V2, and Vb correspond to respective volumes of central, peripheral, and brain compartments. CL$_{12}$ and CL$_{21}$ represent distribution clearances between central and peripheral compartments, CL$_{in,brain}$ and CL$_{out,brain}$, the brain input and output clearances. Rinf is the rate of infusion (2 g over 30 min).
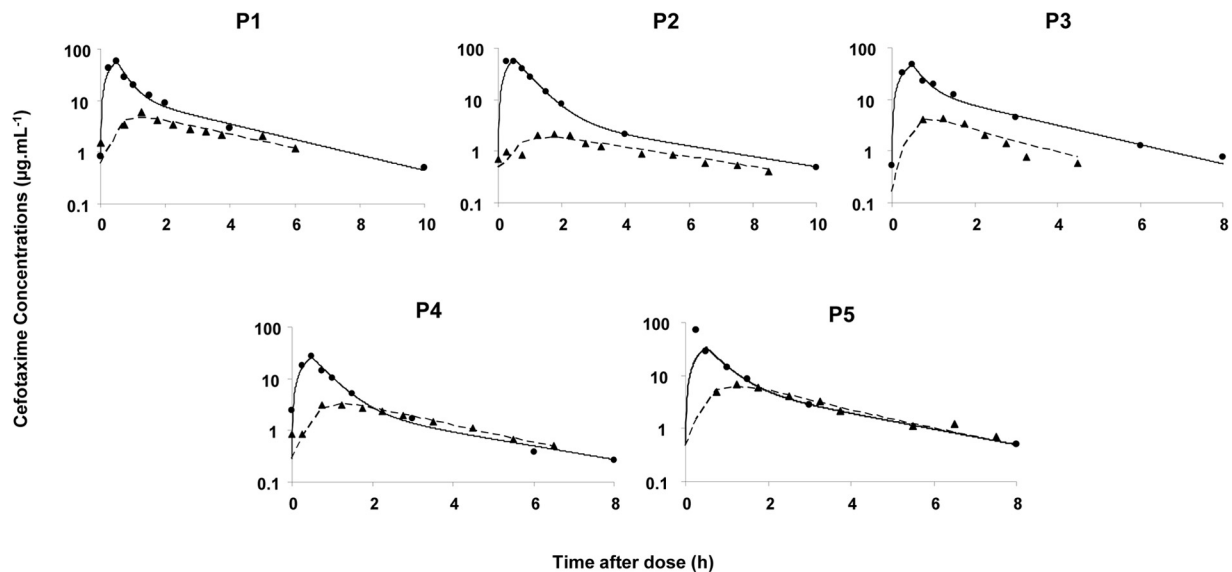
FIG 2 Individual plasma and brain cefotaxime concentrations versus time in five patients after a 30-min intravenous infusion of 2 g of cefotaxime administered every 8 h. ●, experimental unbound concentrations in brain obtained by microdialysis; ▲, experimental unbound concentrations in plasma. Full and dashed lines represent, respectively, estimated concentrations in plasma and in brain by the pharmacokinetic model.

California, Berkeley, CA). Simulations were performed using unbound plasma pharmacokinetic parameters and mean brain clearances ($CL_{in,brain}$ and $CL_{out,brain}$) obtained from the five patients. Simulated unbound brain ECF concentrations were then compared with MIC values equal to 0.5 and 2 $\mu g \cdot ml^{-1}$, corresponding to susceptible and resistant MIC breakpoints for *Streptococcus pneumoniae* (16). The time during which cefotaxime unbound concentrations in brain exceeded the MIC (T>MIC) was determined for each dosing regimen and MIC. T>MIC was expressed as a percentage of the dosing interval.

Statistical analysis. Results are expressed as means ± standard deviations (SD). Half-life and mean maximum unbound concentration values in plasma and brain were compared by a no-parametric Wilcoxon test at a significance level of P values of <0.05.

RESULTS

The *in vivo* probe recoveries estimated were ranging between 37.6 ± 0.1% and 54.8 ± 0.2%. The estimated mean unbound fraction of cefotaxime in plasma (fu) was ranging between 47.4 ± 3.3% and 68.0 ± 10.3% and did not seem affected by concentrations. Unbound cefotaxime concentration-time curves in the brain were below the corresponding curves in plasma and shifted to the right ($t_{max,brain}$ = 1.35 ± 0.25 h). Mean maximum unbound concentration in brain ($C_{max,ub}$ = 4.5 ± 1.7 $\mu g \cdot ml^{-1}$) was about 10-fold and significantly lower than the corresponding value in plasma ($C_{max,up}$ = 52.1 ± 13.7 $\mu g \cdot ml^{-1}$) (Fig. 2). The mean cefotaxime unbound brain-to-plasma AUC ratio was then equal to 26.1 ± 12.1%. Pharmacokinetic parameters obtained by NCA are presented in Table 2.

The pharmacokinetic model provided adequate data fitting both in plasma and in brain (Fig. 2). Estimations of $CL_{in,brain}$ and $CL_{out,brain}$ were good, with precision on parameters (CV%) always lower than 20%. For all patients, estimated $CL_{in,brain}$ was always lower than $CL_{out,brain}$, and a mean corresponding ratio of $CL_{in,brain}$ to $CL_{out,brain}$ equal to 33.1 ± 15.8 (from 13.4 to 52.0) was consistent with the mean brain-to-plasma AUC ratio of 26.1 ± 12.1% (11.9 to 38.3%) estimated by noncompartmental analysis.

DISCUSSION

In the present study, cefotaxime unbound clearance corrected by fu was almost the same as previous values in healthy volunteers (240 ml · min^{-1} or 14.4 liters · h^{-1}) (17), but patients in this study had a well-preserved renal function (Table 1 and 2). Unbound volume of distribution at steady state was relatively high (55.5 ± 20.9 liters), probably due to an increased extracellular water volume traditionally observed in critical care patients. The mean cefotaxime fu of 59.4% in the present study was in accordance with previous *in vitro* results in which a mean cefotaxime fu of 63% was found (17).

In vivo probe recovery determination is necessary for pharmacokinetic studies. In the present study, it was evaluated in each patient by the *in vivo* retrodialysis-by-drug method to adequately estimate cefotaxime brain ECF concentrations (12). Probe recoveries were ranging between 37.6 ± 0.1% and 54.8 ± 0.2%; in practice, a 20% *in vivo* recovery is usually as satisfactory for precise correction (18). Probe recoveries in this study were higher than those estimated in our previous investigation on meropenem

TABLE 2 Pharmacokinetic parameters obtained by noncompartmental analysis in patients after a 30-min intravenous infusion of 2 g of cefotaxime administered every 8 h[a]

Patient	$Vd_{ss,u}$ (liters)	$CL_{ss,u}$ (liters/h)	$t_{1/2\ plasma}$ (h)	$t_{1/2\ brain}$ (h)	Brain ECF-free plasma AUC ratio
P1	51.4	29.2	2.0	2.9	0.278
P2	40.0	27.4	2.1	3.3	0.119
P3	55.0	33.7	1.7	0.7	0.154
P4	88.6	68.2	1.5	1.9	0.383
P5	42.5	36.1	1.6	2.0	0.369
Mean	55.5	38.9	1.8	2.2	0.261
SD	20.9	19.2	0.3	1.2	0.121

[a] Individual and mean (±SD) results are presented.

brain distribution in two critical care patients (19% ± 7% and 29% ± 7%), even though larger cutoff probes (CMA 71, 100 kDa), were used with the same flow rate (12).

Brain distribution of antibiotics using microdialysis has been reported only in rare occasions with various molecules, including vancomycin, rifampin, fosfomycin, doripenem, and meropenem (12, 19–22). *In vivo* recovery was estimated in only two occasions, either by no net flux (12) or by retrodialysis by drug (20). In each of these studies, free brain-to-plasma AUC ratios were estimated lower than unity, attesting to the limited brain distribution of these antibiotics. However, without precise probe recovery estimation, these should be interpreted very carefully.

The present study is the first one to investigate cefotaxime extracellular brain concentrations by microdialysis. Previous studies in human have explored only cefotaxime distribution in cerebrospinal fluid (CSF) (5–8). Among them, two in adults (5, 6) and one in children (7) reported CSF-to-plasma AUC ratios lower than 20%, indicating poor penetration of cefotaxime in CSF as reported now in brain (26.1 ± 12.1% in the present study). It is well established that physicochemical properties lead partly to compound distribution in the CNS and that a low molecular size, an appropriate lipophilicity, and a large amount of nonionized form facilitate compound penetration in brain (23). Cefotaxime exhibits a relatively low molecular mass (425.5 kDa), an intermediate protein binding, and a moderate lipophilicity, and it is a weak acid with a pKa at 3.4, meaning that at pH 7.4 most of cefotaxime is ionized, which should restrict its penetration in CNS. But limited cefotaxime CNS penetration should be explained mostly by active transport systems located on barriers between blood and CNS, like the blood-brain barrier (BBB) and the blood-CSF barrier (BCSFB). Some β-lactam antibiotics were shown to be transported *in vitro* or *in vivo* by different transporters, like organic anion transporter 3 (OAT-3), peptide transporter 2 (PEPT-2), and multidrug-resistant associated protein 4 (MRP4) (24–26). Among these transporters, MRP4 is present at the luminal side of both brain capillary endothelial cells and epithelial cells of choroid plexus (blood sides) (9). Cephalosporins were shown to have a high *in vitro* affinity for MRP4 transporters, with ceftriaxone exhibiting one of the highest affinities, but cefotaxime was not tested (24). OAT-3 is both expressed in the brush border membrane of choroid plexus (9, 27) and at the brain level of the BBB (9, 27, 28) and is involved, with coactivation of transporters localized at the blood level, in drug efflux from CNS to blood (25). PEPT-2 is present at the apical (luminal) side of the choroidal epithelium (29), but its presence was not described in endothelial cells of the BBB (30). In the present study, the cefotaxime active transport out of brain ECF was responsible for higher brain efflux clearances ($CL_{out,brain}$) than influx clearances ($CL_{in,brain}$) estimated in all patients. In fact, if only passive diffusion processes were involved in cefotaxime brain distribution, $CL_{in,brain}$ and $CL_{out,brain}$ should be equal (13). Higher $CL_{out,brain}$ than $CL_{in,brain}$ values explain the lower cefotaxime unbound brain ECF than unbound plasma concentrations observed in the present study, with a mean brain-to-plasma AUC ratio equal to 26.1 ± 12.1% (13).

Cefotaxime, like other β-lactams, is a time-dependent agent, meaning that efficacy is correlated with the time during which concentrations are over the MIC (T>MIC). Cefotaxime in the present study was administered at a usual dose to treat lung infection due to mechanical ventilation in critical care patients (2 g every 8 h). However, traditional cefotaxime dose regimens for
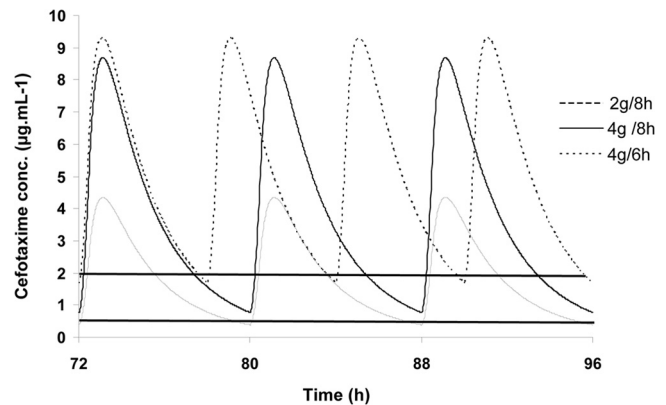

FIG 3 Simulations of mean cefotaxime unbound concentrations in brain, at steady state, after drug infusion at a constant rate over 30 min at different dosing regimens, 2 g every 8 h and 4 g every 6 h or 8 h. The horizontal lines at 0.5 and 2 µg · ml⁻¹ represent, respectively, the MIC of susceptible and resistant strains of *Streptococcus pneumoniae*.

treatment of bacterial meningitis are 4 g every 6 h or 4 g every 8 h for adults (31), and the major issue in the treatment of bacterial meningitis is the risk of antibiotherapy failure. Consequently, simulations of mean brain ECF concentrations were performed from mean patient pharmacokinetic parameters using cefotaxime doses used in meningitis treatment. The simulated cefotaxime brain concentrations in the present study were consistent with those previously observed in a critical care patient treated by cefotaxime at the meningitis dose (4 g three times per day) (32). The experimental C_{max} observed in this patient (11.4 µg · ml⁻¹) was close to the present simulated value for the same dosing regimen (8.7 µg · ml⁻¹). Considering MIC breakpoints of *Streptococcus pneumoniae*, T>MIC values were estimated for susceptible strains to be 99.9% and 99.8% of the 4 g/8 h and 4 g/6 h dosing intervals, respectively, and estimated to be 63.7% and 89.8% of the same dosing intervals for resistant strains (Fig. 3). For β-lactams used in meningitis, a time over minimal bactericidal concentration (MBC) at least equal to 50% of the dosing interval (33) and even greater than 90% (34) has been required for a bacterial eradication. Considering that for bactericidal agents like β-lactams the MBC value is usually the same as the MIC value or two times higher (35), the target was reached for susceptible strains whatever the dosing regimens (4 g/6 h and 4 g/8 h), while 4 g every 6 h was required for resistant strains. However, three limitations should be taken into consideration with these simulations. First, it should be assumed that brain distribution is linear in the range of concentrations used. Second, the desacetylcefotaxime metabolite of cefotaxime was not estimated in this study because of difficulties of quantification by microdialysis. Since this metabolite is active, it should be necessary to characterize its brain distribution to evaluate complete treatment efficacy. Third, our patients were all brain injured, but none of them were treated for meningitis. Antibiotic penetration through inflamed meninges is increased compared to noninflamed ones, and it should be expected to get higher cefotaxime concentrations in case of meningitis (36).

In conclusion, this study is the first one to quantify brain distribution of cefotaxime in patients by microdialysis. In CSF, cephalosporins are known to have a limited distribution, due mainly to active transporters. This study describes also a limited distribution of cefotaxime in the extracellular fluid.

ACKNOWLEDGMENTS

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