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Analysis of Ceftriaxone and Ceftazidime Distribution in Cerebrospinal Fluid of and Cerebral Extracellular Space in Awake Rats by In Vivo Microdialysis

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In vivo microdialysis was used to estimate the extracellular concentrations of ceftazidime and ceftriaxone, two expanded-spectrum cephalosporins commonly used in the treatment of bacterial meningitis, in two brain regions (the right corpus striatum and the left lateral ventricle) of awake, freely moving rats. Antibiotics were administered by constant intravenous infusion at 18 mg/h until steady-state levels were reached. Ceftriaxone levels measured at the steady state in the extracellular space of the corpus striatum (0.80 ± 0.17 μg/ml) were statistically equivalent to those obtained in the cerebrospinal fluid of the lateral ventricle (0.71 ± 0.15 μg/ml). The ratios of these levels in the brain to the steady-state levels in plasma were 0.5 ± 0.1% for both regions. The postinfusion concentrations of ceftriaxone in the brain declined monoexponentially, with an elimination half-life similar to that obtained in plasma. However, the mean antibiotic concentration of ceftazidime in the striatum (2.2 ± 0.4 μg/ml) was lower ($P < 0.001$) than that in the lateral ventricle (3.8 ± 1.8 μg/ml), and the ratios of concentrations in the striatum and ventricle to the concentration in plasma (2.4 ± 0.5% and 4.0 ± 1.8%, respectively) were higher than those obtained with ceftriaxone. Moreover, the half-life of ceftazidime elimination from plasma was lower than that obtained in the two brain regions. It was concluded that the in vivo microdialysis technique yields useful data on antibiotic distribution in the extracellular space of the brain, that the distribution may not be homogeneous, and that the decay of postinfusion concentrations in the brain may be different from the decay of postinfusion concentrations in plasma.

Treatment of bacterial cerebral infections requires the use of bactericidal antibiotics that penetrate the cerebrospinal fluid (CSF) and cerebral tissues. Although intracellular pathogens can cause meningitis, several microorganisms causing central nervous system infections are located principally in the extracellular space of the brain (8, 13). In these cases, measurement of the antibiotic concentrations in the cerebral extracellular space (CES) may be more important than measurement of total concentrations in brain tissues.

In vivo microdialysis sampling has commonly been used to monitor the composition of the extracellular fluid in the central nervous system. The recent application of this technique to pharmacokinetic and metabolism studies has made it possible to measure the free extracellular concentrations of drugs, as well as their metabolites, in blood and tissues (14, 15). Before the advent of the microdialysis technique, data on the distribution of antibiotics and drugs in general into the CES of experimental animals were scarce. Since its introduction, several reports about the transport of drugs across the blood-brain barrier and their distribution in different brain regions have been published (16, 17, 19). A recent report described a study in which microdialysis was used to investigate the distribution of rifampin in the brains of anesthetized rats (9). However, microdialysis has not been used to measure the penetration of antibiotics into the brains of awake, freely moving animals.

In the present study, we used the in vivo microdialysis technique to investigate the penetration of ceftazidime (CFZ) and ceftriaxone (CFX), two cephalosporin antibiotics commonly used in the treatment of bacterial meningitis, into the brains of awake, freely moving rats. We also analyzed the possible existence of regional differences in antibiotic concentrations by measuring, simultaneously, the drug levels in two different areas (the corpus striatum and the lateral ventricle) of the rat brain.

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MATERIALS AND METHODS

Drugs and animals. Vials of CFX monohydrate were kindly supplied by Roche Laboratories (Madrid, Spain). A commercially available preparation of CFZ pentahydrate (Fortam; Glaxo Laboratories, Madrid, Spain) was used in the experiments. Vials of CFX and CFZ were reconstituted with sterile water for injection in accordance with the manufacturer's recommendations, and subsequent dilutions were made with sterile normal saline. All of the other chemicals used were of analytical or high-pressure liquid chromatography grade.

Male Wistar rats weighing 310 to 370 g were used throughout the study. Rats had free access to food and water, and each rat was used only once.

Surgical procedures. Animals were anesthetized with chloral hydrate (400 mg/kg given intraperitoneally) and subjected to surgical cannulation of both jugular veins with medical-grade silicone tubing (Silastic 602-135; Dow Corning Co., Midland, Mich.) for perfusion of antibiotic solutions and collection of blood samples at fixed times after drug administration. After that, rats were securely positioned in a stereotaxic instrument (David Kopf Instruments) and two I-shaped dialysis cannulae (12), localized in the right corpus striatum and the left lateral ventricle, were implanted. The dialysis probes (inside diameter, 0.22 mm; outside diameter, 0.31 mm) were prepared with polyacrylonitrile-sodium methalylsulfonate copolymer (AN 69; Hospal, Bologna, Italy) capped with epoxy. The exposed tip of the dialysis membrane was 4 mm long for the striatum cannula and 1 mm long for the cannula implanted in the lateral ventricle. The positions of the Bregma and dura were used as reference points. The coordinates were +6 mm A/P, −28 mm L/M, and −60 mm V/D for the striatum and −10 mm A/P, +14 mm L/M, and −35 mm V/D for the lateral ventricle, in accordance with the rat stereotaxic atlas of Paxinos and Watson (11).

After surgery, the animals were put in individual cages with free access to food and water for 18 to 24 h prior to the experiment.

When the experiment was terminated, each rat was given an overdose of

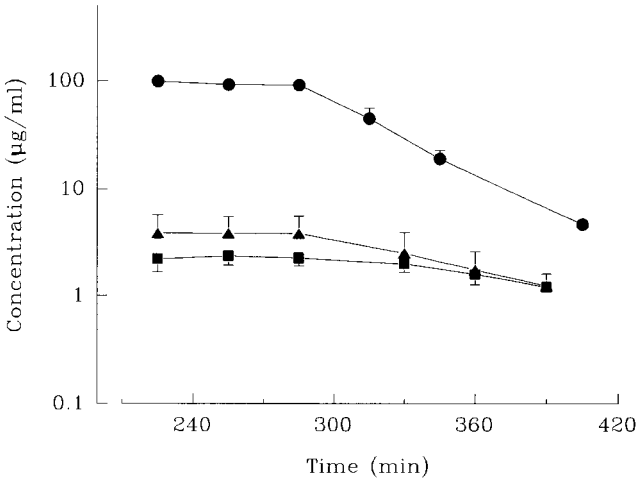

FIG. 1. Mean levels and standard deviations of CFZ in plasma (●), the extracellular space of the striatum (■), and the CSF of the lateral ventricle (▲) after administration of a constant-rate intravenous infusion of 18 mg/h to rats.

chloral hydrate and the brain was fixed with 4% paraformaldehyde via intracardiac perfusion. Coronal sections (40 μm thick) were made to check the correct placement of dialysis probes in accordance with the atlas of Paxinos and Watson (11).

Drug administration. A solution of each antibiotic (36 mg/ml) was prepared in normal saline, and each rat was infused with one of these solutions at a constant flow rate of 0.5 ml/h through the left jugular vein cannula. Prior to the infusion, an initial bolus of 0.5 ml of the antibiotic solution was administered.

Brain perfusions were started simultaneously with the intravenous infusion, with Ringer solution at a constant flow rate of 1.25 μl/min (Perfusor Secura; Braun, Melsungen, Germany). The Ringer solution contained 140 mM NaCl, 4.0 mM KCl, 1.2 mM $CaCl_2$, and 1.0 mM $MgCl_2$.

After steady-state levels of the antibiotic were reached (210 min of infusion), brain dialysis samples were collected during three 30-min intervals (210 to 240, 240 to 270, and 270 to 300 min) and blood samples (0.3 ml) were withdrawn through the right jugular vein cannula at the midpoint of each brain dialysis collection period. Intravenous antibiotic infusion was stopped at 300 min. Dialysates were collected during three additional 30-min intervals (315 to 345, 345 to 375, and 375 to 405 min), and three blood samples were collected at 315, 345, and 405 min.

Blood samples were centrifuged (1,000 × *g* for 5 min), and plasma was stored at −20°C until analysis.

Analytical techniques. CFX and CFZ concentrations in plasma and brain dialysates were determined by high-pressure liquid chromatography with UV detection. Brain dialysis samples (30 μl) were injected directly into the chromatographic system. Plasma samples (150 μl) were subjected to an extractive procedure (6) prior to injection into the chromatograph. The mobile phase consisted of 50 mM phosphate buffer (pH 6.5), 40 mM tetradecyltrimethylammonium bromide, acetonitrile, and methanol (45:15:30:10; vol/vol/vol/vol) for CFX analysis and 100 nM acetate buffer (pH 3.5), 40 mM tetradecyltrimethylammonium bromide, and acetonitrile (78:2:20; vol/vol/vol) for CFZ analysis. The mobile phase was always delivered at a flow rate of 1.0 ml/min with a Perkin Elmer Series 4 delivery pump. Detection of antibiotics was performed at 270 nm in a Hitachi 2000-S detector. The coefficient of variation of the analytical method was less than 3%, the detection limit was about 0.05 μg/ml, and the assay was linear up to 300 μg/ml ($r > 0.99$) for CFX. The respective values for CFZ were 3.7%, 0.1 μg/ml, and 250 μg/ml ($r > 0.99$).

Microdialysis probe characterization. To determine the extracellular concentrations in the striatum and the lateral ventricle, in vivo recovery was experimentally estimated in awake rats. We used the extrapolation-to-zero flow rate method described by Jacobson et al. (4) as follows. Six rats, cannulated and given CFZ and CFX as described above, were used to estimate the recovery of each antibiotic. After steady-state antibiotic levels in plasma and brain dialysates, the rate of brain perfusion (Q) was changed and five different values (0.60, 1.25, 2.20, 4.40, and 9.00 μl/min) were used. The concentration of the antibiotic in the brain dialysates (Cd) was determined at each brain perfusion rate. The dialysate concentration-perfusion rate data were then fitted by means of a nonlinear least-squares program (5) to the equation $Cd = C(1 - e^{-rA/Q})$, where C represents the actual concentration of the antibiotic in the brain extracellular medium, r is the mass transport coefficient, and A represents the surface area of the dialysis membrane. In vivo recoveries in the striatum and the lateral ventricle at a perfusion rate of 1.25 μl/min were calculated by dividing the observed antibiotic concentrations in brain dialysates by the estimated concentrations in the striatum and the lateral ventricle.

Pharmacokinetic methods and statistics. The elimination half-lives ($t_{1/2}$) of the antibiotics from plasma, the striatum, and the lateral ventricle were estimated by fitting a monoexponential equation to the postinfusion plasma and brain concentration-time data (5).

The extracellular concentrations of each antibiotic that were obtained at the steady state in the striatum and the lateral ventricle were compared by means of Student's t test for paired data. A repeated-measures analysis of variance test, followed by the Newman-Keuls multiple-comparison test, was used to compare the $t_{1/2}$ values for plasma and the two brain regions. A probability level of 0.05 was chosen for hypothesis testing.

RESULTS

In vivo recoveries varied for the different brain regions and the antibiotic perfused. In our experimental conditions, recovery in the lateral ventricle was 25% for CFZ and 51% for CFX, whereas in the striatum recovery was 39% for CFZ and 73% for CFX.

Figure 1 shows the plots of the CFZ concentrations versus time in plasma, ventricular CSF, and the striatum (corrected for the respective recoveries). Data obtained in the CFX experiments are represented in Fig. 2. Table 1 summarizes the mean steady-state levels and the $t_{1/2}$ values. As can be seen, the steady-state levels in brain regions and the ratio of these to the steady-state levels in plasma were higher for CFZ than for CFX.

The mean CFX concentration in the striatum was similar to that found in the lateral ventricle. However, in the experiments with CFZ a statistically significant difference between the two brain regions was found. The mean CFZ concentration in the striatum was only 58% of that in the lateral ventricle.

The postinfusion concentrations of CFX in the brain declined monoexponentially, with elimination half-life values similar to those in plasma (Fig. 2 and Table 1), suggesting rapid equilibration between the brain compartments and plasma. However, there were statistically significant differences between the $t_{1/2}$ values obtained for CFZ in plasma and in the brain regions studied. The $t_{1/2}$ in plasma was 3.6 times lower than in the striatum and 2 times lower than in the lateral ventricle, which means that there are differences in the equilibration kinetics of CFZ between plasma, the CES of the striatum, and the CSF of the lateral ventricle.

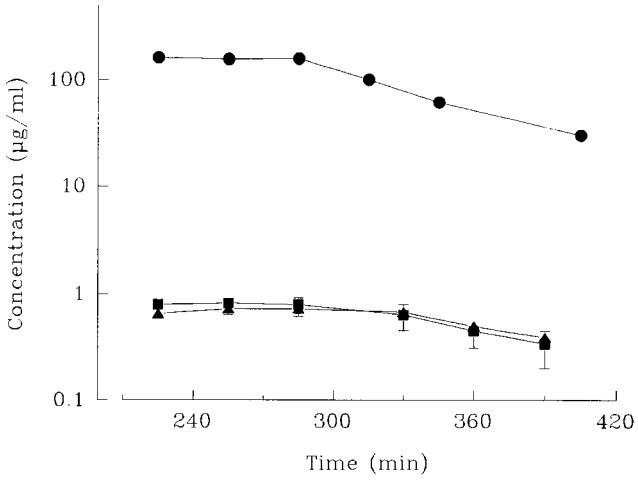

FIG. 2. Mean levels and standard deviations of CFX in plasma (●), the extracellular space of the striatum (■), and the CSF of the lateral ventricle (▲) after administration of a constant-rate intravenous infusion of 18 mg/h to rats.

TABLE 1. Steady-state concentrations, ratios of concentrations in the brain and plasma, and $t_{1/2}$ values of CFZ and CFX after administration of a constant-rate intravenous infusion of 18 mg/h

Parameter[a]	Mean ± SD for CFZ (n = 6) in:			Mean ± SD for CFX (n = 5) in:		
	Plasma	Striatum	Ventricle	Plasma	Striatum	Ventricle
C_{ss} (μg/ml)	94 ± 6	2.2 ± 0.4	3.8 ± 1.8[b]	159 ± 12	0.80 ± 0.17	0.71 ± 0.15
R (%)		2.4 ± 0.5	4.0 ± 1.8		0.5 ± 0.1	0.5 ± 0.1
$t_{1/2}$ (min)	24.4 ± 3.1	88.5 ± 19.2[c]	49.0 ± 12.6[c]	42.5 ± 10.0	51.9 ± 6.0	64.7 ± 15.7

[a] C_{ss}, steady-state concentration; R, ratio of concentrations in the brain and plasma.
[b] Statistically significantly different from the corresponding mean value for the striatum ($P < 0.001$).
[c] Statistically significantly different from the mean value for plasma ($P < 0.05$).

DISCUSSION

Penetration of CFZ and CFX into the CSF through noninflamed meninges has already been evaluated in humans by several investigators. Both antibiotics show poor penetration of the CSF, although they achieve bactericidal concentrations against the commonest pathogens (1). The reported levels of CFZ in CSF show a large interindividual variation. Fong and Tomkins (3) reported CFZ concentrations in the CSF that ranged from 0 to 1.8 μg/ml (mean, 0.66 μg/ml) 2 to 3 h after administration of a 3-g intravenous dose. These levels represented 0.8% of the CFZ levels in serum. In another study (18), CFZ concentrations in CSF were less than 1 μg/ml. Modai et al. (10) reported CFZ concentrations of 5.4 and 23.5% in the CSF 2 and 3 h, respectively, after an intravenous dose of 2 g had been given to patients with meninges supposedly healed after 11 to 20 days of treatment.

CFX penetration through noninflamed meninges seems to be similar to that reported for other expanded-spectrum cephalosporins, although this antibiotic exhibits a very high degree of plasma protein binding (84 to 96% for concentrations in plasma of 70 to 300 μg/ml) (1). After administration of a single intravenous dose of 2 g of CFX, the mean penetration of the CSF by the drug was approximately 1.5% (2).

The ratios of the concentration in CSF to the concentration in plasma obtained in this study for CFZ and CFX (4.0 and 0.5%, respectively) are similar to those reported in humans, which suggests that rats may be an adequate animal model for studying the penetration of the brain by cephalosporin antibiotics.

No data have been previously published on the distribution of CFZ or CFX in the extracellular space of the brain. CFX levels measured in the extracellular space of the corpus striatum were equivalent to those obtained in the CSF of the lateral ventricle, and the $t_{1/2}$ values for the two brain regions were similar. These results show that with CFX, the two brain regions are indistinguishable from a pharmacokinetic point of view. However, with CFZ there were striking differences between the levels of this antibiotic measured in the lateral ventricle and in the corpus striatum. As can be seen in Table 1, the antibiotic concentration in the lateral ventricle was statistically significantly higher than that obtained in the striatum. On the other hand, the $t_{1/2}$ values for CFZ varied with the compartment studied. The $t_{1/2}$ found in the striatum was somewhat higher than that found in the lateral ventricle, and both were higher than the value obtained in plasma (statistically significant differences). All of these features indicate the existence of kinetic differences between the compartments studied with CFZ. Modai et al. (10) showed that the percent penetration of CFZ into the CSF of patients determined 2 h after administration was statistically significantly lower than that obtained for samples collected 3 h after administration. No explanation was given for these results. In our opinion, the differences reported by these investigators could be explained by the fact that the $t_{1/2}$ values found in our study for the brain compartments are higher than those obtained for plasma.

Antibiotic concentrations in the brains of humans and animals have usually been measured by CSF sampling via lumbar puncture or by using a cannula implanted in the cisterna magna. The use of concentrations in CSF is attractive, for it has been demonstrated that, at least for certain drugs, CSF is pharmacokinetically indistinguishable from other brain regions. In fact, our findings on the penetration of the CES and CSF of rats by CFX confirm this assumption. However, for other drugs, the concentration in CSF is different from the concentration in other brain regions. After intracerebroventricular or even intravenous administration, processes like efflux of the drug out of the ventricular compartment and transport of the drug into the brain parenchyma may result in spatial differences between drug concentrations. Wong et al. (19) reported that the concentrations of zidovudine in thalamus extracellular fluid of rabbits were about half of those in the CSF. Mandema et al. (7) showed that baclofen concentrations in the CSF did not overlap the effect profile as measured by electroencaphalographic recordings, indicating large differences in equilibration kinetics between plasma and the effect site compared with the equilibration between plasma and CSF. Mindermann et al. (9) also found unusually low concentrations of rifampin in the extracellular space of the rat brain after intraperitoneal administration of the antibiotic with the compared total concentration in the CSF or brain, and they suggest that the low rifampin levels attained in the CES could be insufficient for treatment of some infections. Our findings on CFZ distribution in the brains of rats, along with the above reports, suggest that the use of CSF concentrations alone to evaluate the penetration of the blood-brain barrier by antibiotics and, therefore, to check their efficiency in the treatment of bacterial cerebral infections may not be enough. The use of animals models can give important information about differences in the distribution of drugs in brain regions. In this sense, the in vivo microdialysis technique is a useful tool for continuous and simultaneous monitoring of the levels of drugs in different regions of the brains of rats.

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