Continuous Sampling for Determination of Pharmacokinetics in Rat Cerebrospinal Fluid

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A method for determining drug concentration relationships between plasma and cerebrospinal fluid (CSF) in rats is described. Continuous CSF samples were collected directly from the third anterior ventricle with an indwelling cannula inserted through the bregma point, and drug concentrations were determined by high-pressure liquid chromatography and radioimmunoassay micromethods. Three antibiotics with different abilities to cross the blood-CSF barrier (chloramphenicol, piperacillin, and gentamicin) were tested. This method was found to be reproducible for each drug even if the antibiotic levels were low and the sample volumes very small. Peak CSF concentrations occurred between 0.75 and 1.25 h after injection for all three antibiotics. Percent penetration values at 1 h were 50, 1.2, and 5.4% for chloramphenicol, piperacillin, and gentamicin, respectively.

Several techniques have been described for collecting cerebrospinal fluid (CSF) from various animals (13). In larger animals such as rabbits and dogs, continuous sampling of CSF in pharmacokinetic studies is done with a cannula implanted generally in the cisterna magna. The present report presents a simple, rapid cannulation technique for repeated sampling of CSF in rats. The CSF samples were collected directly from the third anterior ventricle with an indwelling cannula.

This technique was tested and applied to study the pharmacokinetics in the CSF of three antibiotics from different families (chloramphenicol, piperacillin, and gentamicin) chosen for their varying ability to cross the blood-CSF barrier in mammalian species (2-4, 6).

MATERIALS AND METHODS

Animals. Male Wistar rats (350 g) were anesthetized by intraperitoneal injection of urethane (1.25 g kg⁻¹), and a catheter was inserted in the aortic arch by the carotid artery to allow blood sampling and antibiotic injection. The animals were then placed on a stereotactic table or in a surgical head holder. During deep anesthesia a subcutaneous injection of 2% Xylocaine was given locally. Five minutes later a hole was made in the skull at the bregma point (Fig. 1), and a cannula (1-mm external diameter) with a mandrel was placed into the third ventricle 6.5 mm under the brain surface. The mandrel was removed, and a needle (0.5-mm external diameter) was inserted into the cannula. This needle connected with polyethylene tubing to a peristaltic pump (Gilson) (flow rate, 2 ml min⁻¹). Under these conditions, the CSF flow rate was 1 μl min⁻¹ and was stable throughout the course of the experiment, which lasted 3 h. CSF was sampled every 15 min in small conical polypropylene tube (Ependorff). Arterial blood pressure was controlled and remained stable during the experiment. Mono- or bisuccinate sodium chloramphenicol (165 mg kg⁻¹), piperacillin sodium (20 mg kg⁻¹), and gentamicin sulfate (20 mg kg⁻¹) were injected in a bolus in the arterial catheter.

High-pressure liquid chromatography assay. Concentrations of chloramphenicol and piperacillin in plasma and CSF samples were measured by high-pressure liquid chromatography with micromethods (8, 10). Standard dilutions of both drugs were prepared with drug-free rat plasma and artificial CSF and stored at −80°C with unknown samples. For the assay of chloramphenicol in plasma, 0.05 ml of drug-free rat plasma was added to 0.05 ml of unknown rat plasma and 0.4 ml of ethyl acetate. The preparation was then mixed for 10 min and centrifuged at 4°C for 10 min at 1,000 x g. 0.25 ml of the supernatant was accurately pipetted and evaporated to dryness. The residue was rediluted with 0.1 ml of chloramphenicol (mobile phase), and 0.025 ml of this solution was injected into the chromatograph. For the assay of chloramphenicol in CSF, 0.01 ml of CSF was injected directly into the chromatograph. For the determination of piperacillin, a different extraction procedure was performed. Plasma (0.05 ml) was added to 0.150 ml of drug-free rat plasma and 0.25 ml of dimethylformamide. This preparation was mixed for 20 min at a temperature of 60°C and then centrifuged at room temperature for 10 min at 1,000 x g. A 0.02-ml sample of the supernatant was injected into the chromatograph. For the assay of piperacillin in CSF, 0.012 ml of CSF was injected directly into the chromatograph. For chloramphenicol and piperacillin assays, a μ-Bondapak C-18 column was used.

The mobile phase for chloramphenicol was ethanol-water-acetic acid (37:63:1, vol/vol); the flow rate was 1.5 ml min⁻¹, and detection was at 280 nm. For piperacillin, the mobile phase was acetonitrile-0.05 M phosphate buffer (16.5:83.5, vol/vol) adjusted to pH 6.5. The flow rate was 1.5 ml min⁻¹, and detection was at 254 nm.

Radioimmunoassay. Radioimmunoassay of gentamicin was performed in duplicate with rabbit antiserum obtained by carbodiimide conjugation of gentamicin on bovine serum albumin (4, 9). The antisera titer was 1:200.

Iodinated gentamicin was purchased from New England Nuclear Corp. (Boston, Mass.), and a precipitating antiserum reagent was purchased from C.E.A. (Saclay, France). Gentamicin standard dilutions were prepared with drug-free rat plasma and artificial CSF and stored at −30°C with unknown samples. A 0.1-ml sample of diluted plasma (1/100)
or 0.1 ml of diluted CSF (1/10) was incubated with 0.1 ml of labeled rabbit gentamicin (0.04 μCi ml⁻¹) at 4°C for 12 h. Precipitating antiserum reagent (0.2 ml) was added to the mixture, and incubation lasted for 2 h at room temperature. The solution was centrifuged (10 min, 100 × g, 4°C), and the supernatant was removed. After two washings the precipitate containing the radioactive bound gentamicin was measured in an automatic LKB gamma counter with a 10⁶ cpm precount.

Calculations. Plasma drug decays were studied in the elimination phase by a monoeponential model. Data were fitted with a classical least-squares method. Permeability surface products were calculated by the method of Bradbury (3). A plot of the concentration of plasma-free drug minus the concentration of CSF divided by the plasma-free drug concentration versus time on semilogarithmic paper gave a straight line (times, 0, 15, and 30 min). A linear least-squares method gave the slope (K_out - K_in). The formula K_out = P*S was used, where P is the permeability of the drug, and S is the exchange surface.

RESULTS

The mean arterial pressure was 100 ± 10 mm Hg (n = 15) in all animals and remained stable. The CSF flow rate was stable in each animal at 1 μl min⁻¹ (1.03 ± 0.14 μl min⁻¹; n = 65 points for five rats). The CSF was clear, sterile, and without blood contamination.

Under the high-pressure liquid chromatographic conditions described above, the retention time of chloramphenicol was 5.6 min. The glucuroconjugated chloramphenicol was not detectable in this high-pressure liquid chromatography assay and did not interfere. The sensitivity of the method was 1 mg liter⁻¹ for plasma and 0.1 mg liter⁻¹ for CSF. The reproducibility and recovery errors were less than 7% over the range studied (500 to 1 μg ml⁻¹ for plasma; 50 to 0.1 μg ml⁻¹ for CSF). For piperacillin the retention time was 5.4 min. This drug has no metabolites in rats. The sensitivity of the method was 1 mg liter⁻¹ for plasma and 0.1 mg liter⁻¹ for CSF. The reproducibility and recovery errors were below 6.5% over the range studied (200 to 1 μg ml⁻¹ for plasma; 1 to 0.1 μg ml⁻¹ for CSF). The limit of sensitivity for the gentamicin radioimmunoassay was 10 pg per tube with reproducibility and recovery errors below ±7% over the concentration range studied (20 to 0.1 μg ml⁻¹ for plasma; 1 to 0.01 μg ml⁻¹ for CSF). Under the described conditions, the limit of detection was 1 ng ml⁻¹ for CSF and 0.1 ng ml⁻¹ for plasma. Concentrations of the three antibiotics in plasma are shown in Fig. 2. According to the plasma drug decay time of between 30 and 150 min, a monoeponential model gave a t½ of 90 min for chloramphenicol, 80 min for piperacillin, and 90 min for gentamicin. The drug concentrations in CSF are shown in Fig. 3. The peak chloramphenicol concentration (23 mg liter⁻¹) was observed at 0.75 h and remained unchanged until 1.75 h after injection. At this
point, the level decreased rapidly, reaching a concentration of 3 mg liter⁻¹ during the third hour. The peak piperacillin level was observed at 1.25 h (0.32 mg liter⁻¹) and thereafter decreased slowly, reaching a concentration of 0.13 mg liter⁻¹ during the third hour. A maximum concentration of gentamicin (0.35 mg liter⁻¹) was observed at 0.75 h, thereafter decreasing to a level of 0.13 mg in the third hour. One hour after injection, the percent penetration values were, respectively, 50, 1.2, and 5.38% for chloramphenicol, piperacillin, and gentamicin (CSF concentration/plasma concentration). Drug-protein binding percentages were, respectively, 0, 24, and 25% for gentamicin, piperacillin, and chloramphenicol for rat plasma by the equilibrium dialysis method at 4°C. Permeability surface products were 0.0088 min⁻¹ for chloramphenicol, 0.00048 min⁻¹ for piperacillin, and 0.0005 min⁻¹ for gentamicin.

**DISCUSSION**

Cannula implantations are usually based on stereotaxic atlases based on rats of specified sex, weight, and strain. In this report, we used a simple technique based on easy localization of the bregma point to localize the third ventricle (12). This point is easily found in rats irrespective of the sex, weight, and strain. This double-cannula technique is simple and rapid and enables the quantity and sampling rate of the CSF to be controlled. The physiological parameters of rats are easily monitored during the experiments. In rats, CSF production has been determined to be 1.9 μl min⁻¹ by infusion manometric studies or tracer experiments (1, 7). This production permitted a turnover of 45% of the total fluid value per hour at CSF pressure (100 mm H₂O). Our CSF sampling was done at atmospheric pressure in the third ventricle near the choroid plexus with a 1-μl min⁻¹ rate which did not disturb physiological production and reabsorption of CSF, as previously noted in catecholamine studies (5, 11). These authors found a flow rate of 1 μl min⁻¹ with another cannula and demonstrated that the mean rate of accumulation of 5-hydroxyindole acetic acid after probenecid administration is exactly similar to the rate obtained with serial puncture in the cisterna magna of rats; this fact clearly demonstrates that a continuous sampling at 1 μl min⁻¹ did not greatly modify the physiological movement of CSF. The mean drug concentration was reproducible and stable for each sampling time in various rats (Fig. 3), and standard deviations were low during pharmacokinetic experiments.

Permeability and percent penetration values for these three antibiotics are in agreement with previous studies in rabbits and humans (2, 6) in which chloramphenicol is mentioned as a good penetrating drug and piperacillin and gentamicin as poorly penetrating drugs with an intact CSF barrier.

In conclusion, the continuous sampling technique, coupled with modern analytical techniques which are very sensitive, enabled us to obtain reliable results for each antibiotic. Very small sampling volumes of CSF permitted us to follow the kinetics of drug entry in the CSF in each animal.

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**LITERATURE CITED**


