*Drug Development and Industrial Pharmacy*, 2011; 37(11): 1306–1310

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.571696

informa
healthcare

**RESEARCH ARTICLE**

# Delivery of cefotaxime to the brain via intranasal administration

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**Abstract**

The purpose of this study was to investigate the plausibility of delivery of cefotaxime to the brain via intranasal administration. *In vitro* permeation studies were carried out using Franz diffusion cells, and the effect of different concentrations of chitosan (0.1% w/v and 0.25% w/v) on drug permeation across the bovine olfactory mucosa was determined. Samples were collected from the receiver compartment at different time points and analyzed using HPLC. The amount of cefotaxime that permeated across the olfactory mucosa when 0.25% w/v of chitosan was used as a permeation enhancer was ~1.5- and ~2-fold higher at the end of the first hour and second hour, respectively, over control (29.56 ± 6.18 μg/cm$^2$). There was no significant enhancement in drug permeation when 0.1% w/v chitosan was used as the permeation enhancer. Pharmacokinetic studies were carried out using Sprague-Dawley rats. Cefotaxime solution with 0.25% w/v chitosan (40 mg/kg) was administered intravenously (i.v.) to rats in groups 1 and 3 and intranasally to those in group 2 and 4. The time course of drug in the brain was investigated by performing microdialysis in rats of groups 1 and 2. Blood samples were withdrawn from rats in groups 3 and 4, and cefotaxime in plasma was analyzed using HPLC after extraction with a hydrochloric acid–chloroform:1-pentanol (3:1) and phosphate buffer solvent system. Pharmacokinetic parameters were calculated using the trapezoidal rule. The results imply that the drug levels attained in the brain following i.v. and intranasal administrations were comparable. These results suggest that intranasal administration of cefotaxime could be a potential method of delivering antibacterial agents because of it being noninvasive and patient compliant.

**Keywords:** Cefotaxime, chitosan, nose to brain, bioavailability, microdialysis, pharmacokinetics

## Introduction

Meningitis is an infection of the cerebrospinal fluid and meninges that surround the brain and spinal cord. Bacterial meningitis is primarily caused by three main bacterial species (*Neisseria meningitidis*, *Streptococcus pneumonia*, and *Haemophilus influenzae* type B) and it accounts for three-fourth of meningitis infection around the world. Bacterial meningitis is more common in African, American, and Asian countries. Generally, it is treated using large doses of antibiotics such as chloramphenicol and cephalosporins[1–4]. Effective levels of antibiotics should be maintained in the brain all through the treatment period for successful treatment of meningitis. In most developing countries, the use of cephalosporins is limited, despite their greater potency, due to high cost.

Cefotaxime is one of the cephalosporins recommended for the treatment of bacterial meningitis and is generally administered via the parenteral or oral route[5–7]. Systemic delivery of cephalosporins including cefotaxime is associated with the potential risk of causing severe systemic side effects[2,8,9]. Moreover, the drug has short elimination half-life in the brain, hence, frequent administration is inevitable during the treatment. Therefore, there is need for a patient compliant method to deliver cefotaxime to the brain.

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*(Received 30 November 2010; revised 07 March 2011; accepted 07 March 2011)*

It is well established that there exists a direct pathway from nose to brain via the olfactory region[10–13]. This route has been investigated as a potential route for delivery of several small and large molecular weight therapeutic agents[11,14–17]. Nose to brain pathway could deliver drugs directly to the brain, bypassing the blood–brain barrier (BBB)[18]. Moreover, intranasal delivery would be noninvasive and allows frequent administration. Apart from this, treatment with nasal drops would be less expensive than parenteral or oral therapy. Nasal drops can be self-administered and do not require physician supervision during administration unlike in the case of parenteral formulations. Oral therapy of cefotaxime requires administration of large doses. Hence, nasal formulations are likely to reduce overall cost of the therapy. Therefore, in this study, the plausibility of delivering cefotaxime to the brain via intranasal route was investigated.

Olfactory mucosa acts as a significant barrier to the delivery of drugs via nose to brain. Therefore, several strategies have been investigated to enhance the permeability of the olfactory mucosa. It was reported by Vaka and coworkers[16] that chitosan leads to reversible permeabilization of olfactory mucosa. It was also reported by Smith and coworkers as well as Dodane and coworkers[19,20] that chitosan in other membranes is capable of enhancing the permeability of mucous membranes by opening of the tight junctions. The bioavailability of nerve growth factor (NGF) in the brain following intranasal administration of a chitosan-containing formulation was found to be ~14-fold higher than the formulation that did not contain chitosan[16]. Therefore, in this study, chitosan was used as a permeability enhancer of olfactory mucosa.

## Materials and methods

### Chemicals

Cefotaxime sodium salt, chitosan (MW ~250 kDa, 75–80% deacetylation), Krebs-Ringer bicarbonate (KRB) buffer (premixed powder), 1-butanol, and potassium phosphate (monobasic) were procured from Sigma Chemicals (St. Louis, MO). Chloroform, phosphoric acid, and acetonitrile were obtained from Fischer Scientific (Atlanta, GA).

### Olfactory mucosa

Bovine olfactory mucosa was used for *in vitro* studies and was purchased from Pel-Freez Biologicals (Rogers, AR). Bovine olfactory mucosa is similar to human olfactory mucosa with respect to drug transport and metabolic pathways[21]. Freshly excised frozen tissue was obtained from the supplier and used within 24 h of excision. It was thawed for a period of 30 min by immersing in sterile KRB buffer before carrying out the permeation studies.

### *In vitro* permeation studies

*In vitro* permeation studies across the bovine olfactory mucosa were carried out using vertical Franz diffusion apparatus (Logan Instruments, Somerset, NJ). The olfactory mucosa with a diffusion area of 0.64 $cm^2$ was sandwiched between the donor and receiver compartments such that the dorsal side of the tissue is facing the donor compartment and the ventral side is facing the receiver compartment. The donor and receiver compartments were fitted with Ag/AgCl electrodes (procured from Alfa Aesar, Ward Hill, MA) in the form of a circular ring with a diameter of 0.5 mm. They were placed 2 mm away from the tissue in both the donor and receiver compartments. The electrical resistance of the mucosa was measured by placing a load resistor $R_L$ (100 kΩ) in series with mucosa after filling the donor and receiver compartments with 500 µL and 5 mL of KRB buffer, respectively. Waveform generator and multimeter (Agilent Technologies, Santa Clara, CA) were used to measure voltage drop across the whole circuit ($V_o$) and across the mucosa ($V_E$). The resistance of the tissue was measured by applying a small voltage of about 100 mV at 10 Hz and the resistance in kΩ $cm^2$ was approximated[16].

The effect of different concentrations of chitosan (0.1% and 0.25% w/v) on the permeation of cefotaxime was investigated (Figure 1). Drug–chitosan solution was prepared by dissolving the required quantity of chitosan in 1% glacial acetic acid solution in KRB followed by the addition of cefotaxime (1 mg/mL). This solution (500 µL) was placed in the donor compartment and the receiver compartment was filled with KRB buffer. Control set of experiments were run without incorporation of chitosan in donor solution. The amount of cefotaxime that permeated across the olfactory mucosa was determined by analyzing the samples collected from the receiver compartment using HPLC.

### Analytical method

An HPLC system (Waters, 1525) consisting of a Phenomenex C-18 analytical column (4.6 mm × 150 mm, Luna 5.0 µm) and a variable wavelength detector

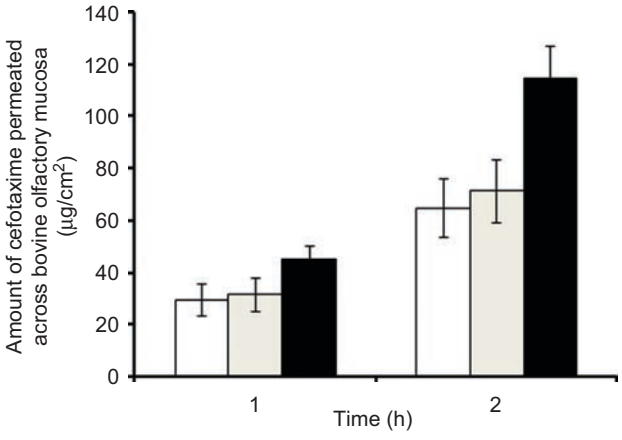


Figure 1. Effect of different concentrations of chitosan on *in vitro* permeability of cefotaxime across the bovine olfactory mucosa (unfilled, gray filled, and black filled bars represent control, 0.1%, and 0.25% w/v chitosan, respectively). Data points provided are an average of four trails and error bars represent the standard error of mean (SEM).

(Waters, 2487) was used. The mobile phase was made up of 0.007 M phosphoric acid in water–acetonitrile (85:15 v/v), flow rate was 1.3 mL/min, and the column effluent was monitored at 254 nm. Range of calibration curve was 0.025 to 1 μg/mL ($R^2 = 0.99$)[22].

### Extraction of cefotaxime

Blank plasma obtained from untreated rats was spiked with known concentrations of cefotaxime and the calibration curve was plotted using the analytical method as mentioned in the previous section.

The rat plasma samples containing cefotaxime were extracted initially by vortexing 1 mL of plasma with 0.5 mL of 0.4 M HCl. Then, 7 mL of chloroform–1-pentanol (3:1) was added to the mixture and vortexed for 30 min followed by centrifugation at 4°C for a period of 10 min at 1000*g*. From this the bottom organic phase (5 mL) was collected and 350 μL of phosphate buffer (pH 7) was added so as to back extract (30 min) the cefotaxime, which was then centrifuged at 4°C for a period of 10 min. The upper aqueous phase was quantified by HPLC[23].

### Calibration of microdialysis probe

Microdialysis probes (CMA 12) were calibrated *in vitro* by immersing the probes in dialysis media containing cefotaxime (concentrations 25, 50, and 75 μg/mL). A syringe pump controller (BASi, West Lafayette, IN) was used to continuously perfuse the KRB buffer at a flow rate of 2.0 μL/min. The probes were equilibrated for a period of 30 min and then samples were collected at regular intervals for a period of 6 h. The percentage recovery was calculated as the ratio of dialysate concentration to its concentration in the dialysis medium surrounding the probe

$$\text{Percentage recovery} = \frac{\text{Concentration of the dialysate}}{\text{Concentration of the dialysis membrane surrounding the probe}}$$

### Pharmacokinetic studies

Pharmacokinetic studies were carried out in Sprague-Dawley rats (male, 250–300 g; Harlan Company, Indianapolis, IN). The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Mississippi (protocol no. 10-017). Rats were categorized into four groups ($n = 3$ for groups 1 and 2 and $n = 6$ for groups 3 and 4) and anesthetized using ketamine (80 mg/kg) and xylazine (10 mg/kg) (intraperitoneal injection).

Cefotaxime solution with 0.25% w/v chitosan (40 mg/ kg) was administered intravenously (i.v.) to rats in groups 1 and 3 and intranasally (i.e. administration of drug directly into the posterior segment of the nose using a microsyringe connected with a soft polymer capillary) to rats in groups 2 and 4.

In case of rats belonging to groups 1 and 2, the time course of drug in the brain was investigated by microdialysis. A microdialysis probe (CMA 12) was inserted into the brain (anterior-posterior = 5.6 mm, mediolateral = 5 mm, dorsoventral = 7 mm, from bregma) after securing the rat on a stereotaxic frame (Harvard Instruments, Holliston, MA). The microdialysis probes were equilibrated by perfusing KRB buffer at 2 μL/min for a period of 30 min with the aid of a microinjection pump. Following i.v. or intranasal administration of cefotaxime solution (10 mg in 100 μL), the microdialysis fractions were collected at 15, 30, 60, 90, 120, 180, 240, 300, and 360 min and quantified using HPLC.

In case of rats belonging to groups 3 and 4, blood samples were collected at 15, 30, 60, 90, 120, 180, 240, 300, and 360 min from retroorbital plexus using heparinized capillary tubes. Plasma was collected from blood samples by centrifuging at 1500 rpm for 15 min and cefotaxime was analyzed using HPLC after extraction with HCl, chloroform–1-pentanol (3:1) and phosphate buffer solvent system.

### Data analysis

Statistical analysis was carried out using GraphPad Prism 5 software. *T*-test was selected as the test of significance, and $P < 0.05$ was considered statistically significant.

### Pharmacokinetic data analysis

AUC was calculated using the trapezoidal rule, and $C_{max}$ and $t_{max}$ were directly obtained from the concentration–time graphs.

## Results and discussion

### *In vitro* permeation studies

Chitosan (0.25% w/v) enhanced the permeation of cefotaxime across the bovine olfactory mucosa by ~1.5-fold and ~2-fold at the end of the first hour and second hour, respectively, over control (29.56 ± 6.18 μg/cm$^2$). However, a significant enhancement of cefotaxime permeation at 0.1% w/v concentration of chitosan was not noted. Overall, the enhancement in the permeability of drug by chitosan was not as dramatic as that observed in case of NGF[16]. Therefore, a solution of drug in 0.25% w/v chitosan was used for intranasal and i.v. administration in *in vivo* studies. Furthermore, higher concentrations of chitosan were not considered as the increased viscosity of the formulation is likely to cause discomfort upon intranasal administration in rats.

### Pharmacokinetics of cefotaxime in the brain

*In vitro* recovery of microdialysis probes was found to be 12.84 ± 1.42%, and the recovery of cefotaxime from plasma was 39.60 ± 3.25%.

The $C_{max}$ of cefotaxime in the brain following intranasal administration (1.477 ± 0.19 μg/mL) was found to be less than that observed upon i.v. administration (3.286 ± 1.17 μg/mL). This difference in $C_{max}$ is likely a

result of drug loss caused by absorption of a greater fraction of drug from nasal mucosa into the systemic circulation followed by elimination via normal clearance. Similar kind of observations using tetramethylpyrazine were reported earlier by Hussian and coworkers as well as by Illum[12,24].

Moreover, the bioavailability ($AUC_{0-\infty}$) of cefotaxime in the brain following intranasal administration ($0.38 \pm 0.06 \times 10^3$ min·μg/mL) was not significantly different from the bioavailability ($AUC_{0-\infty}$) following i.v. administration ($0.45 \pm 0.06 \times 10^3$ min·μg/mL) (Figure 2).

However, the time required to achieve maximum concentration ($t_{max}$) was less upon intranasal administration (30 min) compared with i.v. administration (180 min). Similar observations have been reported in case of tetramethylpyrazine. Frey and coworkers[14] suggested that rapid absorption of drug via the olfactory region could be due to convective transport of drugs along the nose–brain pathway. The ability of intranasally administered drugs to reach the brain relatively faster than those given via the i.v. route was previously reported by Illum[12] in case of hydrophilic drugs.

The minimum concentration at which a drug has the ability to inhibit bacterial growth is defined as minimum inhibitory concentration (MIC). MIC levels of cefotaxime against most bacteria causing meningitis range from 0.01 to 0.6 μg/mL[9]. From Figure 2 it is evident that the drug delivered via intranasal route attained MIC levels rapidly and remained over MIC through a 6-h duration similar to that observed upon i.v. administration.

Provided these data are translatable to humans, effective levels of drug could be achieved in the brain rapidly via intranasal administration and frequent administration would keep the drug levels fairly consistent throughout the treatment duration. Although the brain levels of cefotaxime are higher following i.v. administration (Figure 2), intranasal route may be preferred over i.v. route because of noninvasiveness and potentially minimal side effects. Moreover, overshooting the MIC levels in the brain, as seen in the case of i.v. route, is not likely to improve the treatment but could cause toxicity in case of certain drugs with small therapeutic window.

Bioavailability of the drug in the brain following i.v. administration was expected to be higher than that following intranasal administration. However, the overall brain uptake following either route of administration was found to be comparable. This could be attributed to the rapid hepatic metabolism of drug in the systemic circulation. Liver is the major metabolic organ for cefotaxime[25,26] where it was enzymatically metabolized to desacetylcefotaxime[25,26]. It was also reported that desacetylcefotaxime is only 12% as active as cefotaxime[27]. Earlier, Tsai et al. reported a $C_{max}$ of ~1.9 μg/mL in the brain following i.v. administration, which is ~2-fold less compared with the present case. However, the bioavailability ($AUC_{0-t}$) did not differ significantly between these two studies.

## Pharmacokinetics of cefotaxime in plasma

Drug levels in the plasma following intranasal administration were almost an order of magnitude less when compared with those after i.v. administration (Figure 3). The $AUC_{0-\infty}$ levels following intranasal administration and i.v. administration were $15.32 \pm 3.42 \times 10^3$ min·μg/mL and $371.30 \pm 191.02 \times 10^3$ min·μg/mL, respectively. These data suggest that the drug is likely to undergo metabolism in the mucosal membrane. However, low systemic bioavailability following intranasal administration is likely to result in less systemic side effects compared with parenteral administration.

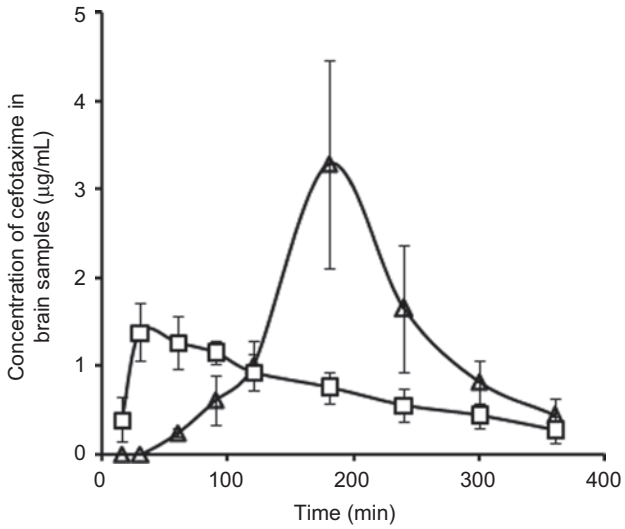


Figure 2. Concentration–time profile of cefotaxime in rat brain following intravenous (Δ) and intranasal (□) administration. Data points represent baseline-adjusted values and are average of values obtained from three animals with SEM as error bars.

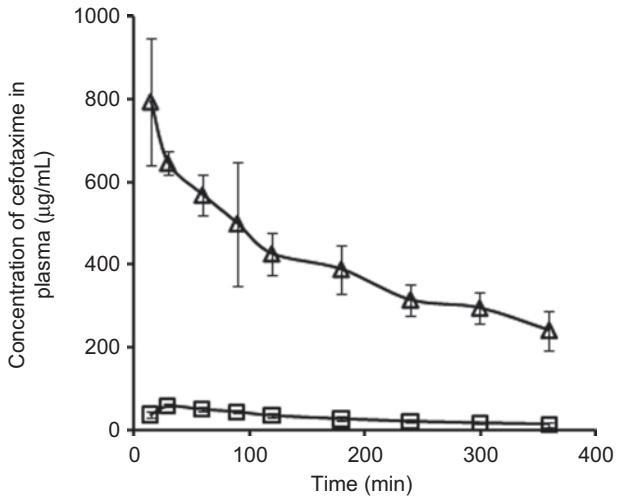


Figure 3. Concentration–time profile of cefotaxime in rat plasma following intravenous (Δ) and intranasal (□) administration. Data points represent baseline-adjusted values and are average of values obtained from six animals with SEM as error bars.

These results indicate that intranasal route could be utilized as one of the potential routes for delivery of cefotaxime to the brain. Effective drug levels could be achieved by increasing the dose or frequency of administration. Intranasal administration of cefotaxime was also found to result in relatively low systemic bioavailability. These results suggest that intranasal delivery of cefotaxime could be developed as a potential treatment approach for bacterial meningitis.

## Declaration of interest

The project was funded by grant no. 5P20RR021929 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or National Institutes of Health. The authors report no conflicts of interest.

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