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Transport of cephalexin to the cerebrospinal fluid directly from the nasal cavity

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Abstract—The aim of the present study has been to confirm the existence of a transport pathway for a drug (cephalexin) to the cerebrospinal fluid (CSF) directly from the nasal cavity, by comparing the drug's concentrations in CSF after intranasal (i.n.), intravenous (i.v.) and intraduodenal (i.d.) administration. Higher levels of the drug were found in CSF following i.n. administration compared with the i.v. and i.d. routes, even though its plasma concentrations were similar. These findings suggest the existence of a direct transport pathway for cephalexin from the nasal cavity to the CSF. The concentration of drug in CSF at 15 min after i.n. administration was higher than that at 30 min. In contrast, its concentrations in CSF at 15 min after i.v. and i.d. administration were not significantly different from those at 30 min. The results confirm the presence of a direct transport pathway to CSF from the nasal cavity. This pathway may represent a new delivery route to CSF and possibly to brain parenchyma.

Increasing attention has been directed to the nasal cavity as a route for administering systemically active drugs. Some drugs such as propranolol (Hussain et al 1979), progesterone (Hussain et al 1981) and enkephalins (Su et al 1985) can be absorbed effectively. Furthermore, peptides and proteins such as insulin (Hirai et al 1981b), in combination with absorption enhancers, appear to be absorbed. The use of the intranasal (i.n.) route avoids hepatic first pass elimination, gut wall metabolism, and destruction in gastrointestinal tracts; also the rate and extent of absorption and the plasma concentration vs time profiles are comparable with those obtained intravenously (i.v.) (Chien et al 1989).

Pardridge 1985, 1986) reviewed the strategy for the delivery of peptides to the brain and suggested that i.n. administration was a possible route. Kumar et al (1974, 1982) have shown that progesterone and oestradiol, which are lipid soluble, achieve higher levels in cerebrospinal fluid (CSF) after i.n. than after i.v. administration. Some physiological data show that the cerebral perivascular space and subarachnoid space of olfactory lobes are connected with the submucous bases of the nose (Jackson et al 1979; Bradbury et al 1981). However, the transport pathway of a drug to CSF has not been investigated systematically.

Cephalexin is a water-soluble antibiotic with low permeability to the blood-brain barrier. Therefore, it is suitable for the study of the drug transport pathway to CSF since its CSF level after non-i.n. administration could be expected to be low. In the present study, we set out to confirm a direct transport pathway for the drug to CSF from the nasal cavity using the antibiotic as a model drug. The finding also provides information on the drug's side effects on the CNS when the i.n. route is used.

Materials and methods

Reagents. Cephalexin was from Wako pure chemical industries (Osaka, Japan). Other reagents were of analytical grade and commercially available.

Animal preparation. Male Wistar rats, 210–260 g, were anaesthetized with intraperitoneal (i.p.) pentobarbitone (40 mg kg^{-1}). The femoral artery was cannulated with polyethylene tubing (SP-31, Dural Plastics & Engineering, Australia) for the collection of blood samples.

Collection of CSF. CSF was obtained as reported by Chou & Levy (1981). An incision was made in the skin over the occipital bone and the first layer of the muscle was cut. CSF was obtained by cisternal puncture with the sharp end of a 25-gauge needle connected with about 1 m length of PE-50 tubing. CSF was withdrawn by a disposable syringe. Collection was terminated as soon as blood appeared in the tubing. When cephalexin was administered (i.v.) or intraduodenally (i.d.), 100 μl of CSF was taken for assay of the antibiotic. When cephalexin was administered i.n., the length of the tubing containing CSF was measured and CSF was divided into two parts. Since it was shown in a preliminary experiment that CSF withdrawn initially did not contain drug, 70 μL of CSF withdrawn later (latter half) was taken for the analysis. If blood appeared immediately or if the total volume of CSF was less than 150 μL, the plasma and CSF concentration data were excluded from the results.

I.n. administration. The oesophagus and trachea were prepared according to Hirai et al (1981a). An incision was made in the neck and trachea was cannulated with a polyethylene tube (O.D. 2 mm, No. 6, Hibiki, Tokyo, Japan). Another tube was inserted from the oesophagus to the posterior part of the nasal cavity. The nasopalatine was closed with an adhesive agent. The drug solution (4 mg mL^{-1}, 0·1 mL) was administered into each nostril by a micropipette. Blood was taken from the femoral artery at 5, 10, 15 min or 10, 20, 30 min. At the end of the experiment, the sample of CSF was collected.

I.v. administration. Cephalexin (0·8 mg mL^{-1}, 0·25 mL) was administered into the femoral vein and the blood was taken at intervals from the femoral artery. The CSF sample was taken at the end of experiments.

I.d. administration. The intestine was exposed through the midline incision and cannulate were inserted into the portions just after the ligament of Treitz and before the caecum. The mucosal side of the intestine was washed out twice with 20 mL of pH 6·5 isotonic phosphate buffer. The drug solution (0·2 mg mL^{-1}, 7 mL) was introduced into the loop and the intestine was put back into the peritoneal cavity. After that, blood and CSF samples were collected as described above.

Analytical procedure. Cephalexin in CSF and plasma was fluorized according to the method of Barbhaiya et al (1978). The fluorized product of cephalexin was determined by HPLC (LC-6A, Shimadzu, Kyoto, Japan) equipped with a fluorescence spectromonitor (RF560, Shimadzu, Kyoto, Japan).

Results

Cephalexin concentrations in CSF and in plasma. Tables 1 and 2 show the concentrations of cephalexin in plasma and in CSF after i.n., i.v. and i.d. administration. The concentration in plasma was increased rapidly after i.n. administration. The

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Table 1. Concentrations of cephalexin in plasma at 5, 10 and 15 min and in cerebrospinal fluid (CSF) at 15 min after intranasal (i.n.), intravenous (i.v.) and intraduodenal (i.d.) administration. Cephalexin was administered i.n. (0·8 mg), i.v. (0·2 mg) and i.d. (1·4 mg). Figures are the mean ± s.d. of three rats.

	Plasma (μg mL^{-1})			
	5 min	10 min	15 min	CSF (μg mL^{-1})
i.n.	0·510 ± 0·233	0·763 ± 0·184	1·03 ± 0·11	4·492 ± 2·480*
i.v.	2·520 ± 0·171	2·440 ± 0·584	2·08 ± 0·40	0·027 ± 0·008
i.d.	0·291 ± 0·142	0·756 ± 0·391	1·15 ± 0·64	0·028 ± 0·019

* $P < 0·05$ vs i.v. and i.d. by Student's t-test.

Table 2. Concentrations of cephalexin in plasma at 10, 20 and 30 min and in cerebrospinal fluid (CSF) at 30 min after intranasal (i.n.), intravenous (i.v.) and intraduodenal (i.d.) administration. Cephalexin was administered i.n. (0·8 mg), i.v. (0·2 mg) and i.d. (1·4 mg). Figures are the means ± s.d. of three rats.

	Plasma (μg mL^{-1})			
	10 min	20 min	30 min	CSF (μg mL^{-1})
i.n.	0·619 ± 0·332	1·00 ± 0·28	1·56 ± 0·23	2·401 ± 1·261*
i.v.	2·186 ± 0·331	1·89 ± 0·49	1·63 ± 0·37	0·021 ± 0·016
i.d.	0·636 ± 0·178	1·43 ± 0·32	1·98 ± 0·68	0·025 ± 0·011

* $P < 0·05$ vs i.v. and i.d. by Student's t-test.

concentration in CSF after i.n. administration was significantly higher both at 15 and at 30 min than those after i.v. and i.d. administration, although the plasma concentrations were similar, suggesting the existence of a direct transport pathway to CSF from the nasal cavity. No significant difference was observed between the concentrations in CSF after i.v. and i.d. administrations. When cephalexin was administered i.n., its concentration in CSF at 30 min was low compared with that at 15 min. On the other hand, its concentrations in CSF at 30 min after i.v. and i.d. administration were not significantly different from those at 15 min.

Discussion

There exists much evidence of a connection of the nasal mucosa with the subarachnoid space of the nose. Chiro et al (1972) showed that ^{125}I-human serum albumin injected cisternally appeared in the nasal submucosal space and the nasal cavity and that a considerable amount of CSF leaked as rhinorrhea in the normal dog. It was demonstrated by Arnold et al (1973) that the tracers injected into the cisterna magna of experimental animals appeared within seconds to minutes in the cervical lymph node by way of the perineural spaces of the olfactory nerves. There is also evidence that an infectious organism can penetrate the nasal mucosa and olfactory nerve (Yoffey & Courtice 1970). Johnson & Mims (1968) showed that ectromelia virus infection could extend from the olfactory fibres to the olfactory bulbs.

The present study has shown the existence of a direct transport pathway to CSF from the nasal cavity. Cephalexin achieved hundredfold higher levels in CSF within 15 min after i.n. administration as shown in Table 1. As the concentration in CSF at 15 min was higher than that at 30 min, cephalexin in the nasal cavity was thought to be decreased with time, and may diffuse passively into CSF.

Kumar et al (1976) showed that the concentration of dopamine in CSF after spraying it into the nostril of a rhesus monkey was high compared with that after i.v. administration. However, factors such as the circulation and secretion of CSF must be taken into account in comparing out results with those of Kumar et al (1976). In our preliminary experiment, it was shown that the concentration of cephalexin in the initial samples of CSF was negligible compared with that in CSF withdrawn later. The CSF level of the drug is perhaps high only in CSF near the nasal mucosa because the cisterna magna is located far from the subarachnoid space above the nasal mucosa. In addition, differences in the CSF circulation and secretion among animal species have been reported. Schurr et al (1953) demonstrated the differences in the CSF circulation among dog, cat and rhesus monkey and emphasized that care should be taken in comparing the CSF circulation of lower animals with that of man. Net CSF production rates of man, dog, rabbit and rat are 0·35, 0·0286, 0·01 and 0·0021 mL min^{-1}, equivalent to turnover rates of 0·38, 0·40, 0·43 and 0·75% min^{-1}, respectively (Davson et al 1987). The CSF flow of the rat is high compared with that of other larger animals. Therefore, the drug in CSF near the nasal mucosa of the rat is thought to be cleared to blood rapidly compared with the rhesus monkey.

In conclusion, a direct transport pathway of cephalexin to CSF from the nasal cavity has been confirmed. I.n. administration may represent a valuable delivery route to CSF and possibly to brain parenchyma with an appropriate dosage form design.

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References

Arnold, W., Ritter, R., Wagner, W. H. (1973) Quantitative studies on the drainage of the cerebrospinal fluid into the lymphatic system. Acta Otolaryngol. 75: 156–161

Barbhaiya, R. H., Brown, R. C., Payling, D. W., Turner, P. (1978) Isolation and identification of the fluorescent degradation product of some β-lactam antibiotics. J. Pharm. Pharmacol. 30: 224–227

Bradbury, M. W. B., Cserr, H. F., Westrop, R. J. (1981) Drainage of cerebral interstitial fluid into deep cervical lymph of the rabbit. Am. J. Physiol. 240: F329–F336

Chien, Y. W., Su, K. S. E., Chang, S.-F. (1989) Nasal Systemic Drug Delivery. Marcel Dekker, New York.

Chiro, G. D., Stein, S. C., Harrington, T. (1972) Spontaneous cerebrospinal fluid rhinorrhea in normal dogs. J. Neuropathol. Exp. Neurol. 31: 447–453

Chou, R. C., Levy, G. (1981) Effect of heparin and salicylate infusion on serum protein binding and on concentration of phenytoin in serum, brain and cerebrospinal fluid of rats. J. Pharmacol. Exp. Ther. 219: 42–48

Davson, H., Welch, K., Segal, M. B. (1987) The Physiology and Pathophysiology of the Cerebrospinal Fluid. Churchill Livingstone, London.

Hirai, S., Yashiki, T., Matsuzawa, T., Mima, H. (1981a) Absorption of drugs from the nasal mucosa of rats. Int. J. Pharm. 7: 317–325

Hirai, S., Yashiki, T., Mima, H. (1981b) Effects of surfactants on the nasal absorption of insulin in rats. Ibid. 9: 165–172

Hussain, A., Hirai, S., Bawarshi, R. (1979) Nasal absorption of propranolol in rats. J. Pharm. Sci. 68: 1196

Hussain, A., Hirai, S., Bawarshi, R. (1981) Nasal absorption of natural contraceptive steroids in rats—progesterone absorption. Ibid. 70: 466–467

Jackson, R. T., Tigges, J., Arnold, W. (1979) Subarachnoid space of the CNS, nasal mucosa, and lymphatic system. Arch. Otolaryngol. 105: 180–184

Johnson, R. T., Mims, C. A. (1968) Pathogenesis of viral infections of the nervous system. N. Engl. J. Med. 278: 23–30

Kumar, T. C. A., David, G. F. X., Umberkoman, B., Saini, K. D. (1974) Uptake of radioactivity by body fluids and tissues in Rhesus monkeys after intravenous injection or intranasal spray of tritium-labelled oestradiol and progesterone. Contraception 43: 435–439

Kumar, T. C. A., David, G. F. X., Kumar, K., Umberkoman, R., Krishnamoorthy, M. S. (1976) A new approach with neuroendocrine pathways. In: Kumar, T. c. A. (ed.) Neuroendocrine Regulation of Fertility. Krager, Basel, pp. 314–322

Kumar, T. C. A., David, G. F. X., Sankaranarayanan, A., Puri, V., Sundram, K. R. (1982) Pharmacokinetics of progesterone after its administration to ovariectomized rhesus monkeys by injection, infusion, or nasal spraying. Proc. Natl. Acad. Sci. 79: 4185–4189

Pardridge, W. M. (1985) Strategies for drug delivery through the blood-brain barrier. In: Borchardt, R. T, Repta, A. J., Stella, V. J. (eds) Directed Drug Delivery, Humana Press, Clifton, pp 83–96

Pardridge, W. M. (1986) Receptor-mediated peptide transport through the blood-brain barrier. Endocr. Rev. 7: 314–330

Schurr, P. H., Maclaurin, R. L., Ingraham, F. D. (1953) Experimental studies on the circulation of the cerebrospinal fluid. J. Neurosurg. 10: 515–525

Su, K. S. E., Campanale, K. M., Mendelsohn, L. G., Kerschner, G. A., Gries, C. L. (1985) Nasal delivery of polypeptides I: Nasal absorption of enkephalins in rats. J. Pharm. Sci. 74: 394–398

Yoffey, J. M., Courtice, F. C. (1970) Lymphatics, Lymph and the Lymphomyeloid Complex. Academic Press, New York

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Microemulsions as topical drug delivery vehicles: in-vitro transdermal studies of a model hydrophilic drug

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Abstract—Microemulsions with a 58:42 weight ratio of dioctyl sodium sulphosuccinate:octanol and containing 15, 35, and 68% water have been tested for their ability to transport glucose across human cadaver skin. A flow-through multisample skin diffusion cell showed that both the 35 and 68% water microemulsions caused enhanced (approximately 30-fold) transport of glucose. No transport was discernible for the 15% water microemulsion. Differences in percutaneous glucose transport were shown to parallel differences in the diffusion of water within the microemulsion vehicles before application to the skin.

Microemulsions offer advantages over traditional creams and lotions as topical drug delivery formulations. Since microemulsions are thermodynamically stable, the properties of the formulation would not be dependent upon process, and the product will not phase-separate, provided temperature and pressure conditions remain reasonably constant. In addition to improved physical stability, microemulsions often function as "supersolvents" for certain compounds. Thus, these clear, fluid liquids may dramatically increase the solubility/solubilization of poorly soluble drugs. While microemulsions have significant potential as drug delivery vehicles, few well-characterized surfactant systems have been systematically studied (Florence 1981).

In a previous investigation (Osborne et al 1988a), the effect of microemulsion composition upon the in-vitro skin transport of water from a microemulsion system was investigated. The three microemulsions evaluated had a fixed weight ratio of surfactant to cosurfactant, and water concentrations of 15, 35, and 68 weight %. The study concluded that most of the water in the 15% microemulsion is bound to the surfactant headgroups and is not available for transport across the skin. Thus, the transport of water across the skin from that microemulsion is less than the transport for water itself. For the microemulsions with higher water content an approximately sixfold enhancement in water transport occurred. Pretreatment studies using the surfactant, cosurfactant, and surfactant/cosurfactant mixtures indicated that the enhancement in water penetration from the high water content microemulsions was a result of a synergistic effect between the surfactant, dioctyl sodium sulphosuccinate (DSS) and the cosurfactant octanol.

With this background established, it was considered important to complete and in-vitro transdermal transport study of a hydrophilic compound delivered from the same microemulsion vehicles. It was hypothesized that a hydrophilic drug would not be available for percutaneous transport from a microemulsion, unless water from the microemulsion is freely transported percutaneously. If this hypothesis is verified, then the topical formulator desiring to deliver hydrophilic drugs must assure 1) sufficient mobility of water within the microemulsion vehicle and 2) sufficient percutaneous transport of water across the skin barrier.

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Materials and methods

Dioctyl sodium sulphosuccinate USP (DSS) from American Cyanamid Company (Bridgewater, NJ) and 1-octanol (Aldrich 99%) were used as received. USP purified water was treated in a Millipore MILLI-Q filtration system before use, while tritiated water (5 mCi mL^{-1}) and D-[^{14}C(U)]glucose (3·7 mCi $mmol^{-1}$) were obtained from Amersham Corporation (Arlington Heights, IL). The in-vitro skin permeation studies were conducted on an apparatus as described by Holland et al (1984). This flow-through cell has a small-volume receiving chamber and a skin surface dosing area of 2 cm^2. Unlike the method described by Holland, 0·9% NaCl in distilled water was used as the receiving medium (flow rate 1·66 mL h^{-1}), and no attempts were made at maintaining skin viability. All dosing chambers were occluded with parafilm to prevent evaporation. Volumes of the donor phases were checked at the end of the experiment to assure that significant diffusion of the receiving fluid into the donor phase did not occur. Dermatomed human cadaver skin was obtained from a 57 year old caucasian female. The skin was frozen within 24 h of death and kept frozen until use. The skin was allowed to thaw gradually to room temperature (23°C) and thoroughly rinsed with purified water. After the skin had been mounted, water was used to maintain its hydration and to eliminate potential osmotic effects. This soaking was for a minimum of 4 h. The receiving medium was thermostated at 35°C.

Microemulsion samples for the skin permeation study were prepared by adding 25 μL [^{3}H]H_2O (50 mCi mL^{-1}) to a tared