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Direct Drug Transport from the Rat Nasal Cavity to the Cerebrospinal Fluid: the Relation to the Molecular Weight of Drugs

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Abstract

To clarify the relationship between the direct transport from the rat nasal cavity to the cerebrospinal fluid (CSF) and the molecular weight of the drug, the transport of fluorescein isothiocyanate-labelled dextran (FD) with various molecular weights was investigated. FDs (average molecular weights 4400 (FD4); 9400 (FD10); 18 900 (FD20); 40 500 Da (FD40)) were administered nasally or intravenously to rats, and the concentrations in the plasma and the CSF were measured and compared.

None of the FDs were detected in the CSF after intravenous administration. However, FD4, FD10 and FD20 were observed to appear in the CSF after nasal administration, whereas the concentration in the plasma was much lower than that after intravenous administration. FD40 was not detected even after nasal administration. In addition, the concentration of these FDs in the CSF decreased with the increase in the molecular weight of FDs.

These findings show that drugs with a molecular weight up to at least 20 000 Da can be directly transported from the nasal cavity to the CSF and that the transport of FDs to the CSF is dependent on their molecular weights.

There exists much evidence that supports the connection of the nasal mucosa with the cerebrospinal fluid and the central nervous system (Jackson et al 1979; Bradbury et al 1981). Arnold et al (1973) reported that the tracer injected into the cisterna magna of experimental animals appeared within seconds to minutes in the cervical lymph node by way of the nasal submucosa. Tolley & Schwartz (1991) reported spontaneous rhinorrhea in man. This is, though rare, a phenomenon in which CSF leaks through the nasal mucosa to the nasal cavity without any underlying causes, when the intracranial pressure is elevated (for example, nose-blowing). Furthermore, it has been reported that an infectious organism can penetrate the nasal mucosa and reach olfactory nerves (Yoffey & Courtice 1970). Ectromelia virus infection can extend from the olfactory fibres to the olfactory bulb (Johnson & Mims 1968).

Kumar et al (1974, 1976, 1982) have described the possibility of direct drug transport from the nasal cavity to the CSF. They found that progesterone (Kumar et al 1982), oestradiol (Kumar et al 1974) and dopamine (Kumar et al 1976) reached higher levels in the CSF after nasal, as compared with intravenous administration. Stimulated by these reports, we started the investigation on the direct transport pathway from the nasal cavity to the CSF with the aim of drug delivery to the brain through the nasal route. In the previous studies, we showed that a drug (cephalexin) can be transported directly from the nasal cavity to the CSF (Sakane et al 1991a) and that the degree of transport is dependent on the lipophilicity (Sakane et al 1991b) and the ionization of the drug (Sakane et al 1994). In this study, we investigated the transport of macromolecules to clarify the relationship to the molecular weight of the drug, using fluorescein isothiocyanate-labelled dextran (FD) as a model macromolecule.

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Materials and Methods

Materials

FDs were purchased from Sigma Chemical Co. (St Louis, MO). Their average molecular weights were 4400 (FD4, Lot 70H0599), 9400 (FD10, Lot 90F5018), 18 900 (FD20, Lot 21H0975) and 40 500 Da (FD40, Lot 125F0124). Other chemicals were of analytical grade and were commercially available.

Animal experiments

Under sodium pentobarbitone anaesthesia (Nembutal, 50 mg kg^{-1}), the right femoral artery of male Wistar rats, 230–280 g, was cannulated with polyethylene tubing (SP-31, Natsume Co., Ltd, Tokyo, Japan) for blood sampling.

Intravenous administration. FDs were dissolved in physiological saline. FD solution (50 mg mL^{-1}, 0·2 mL) was administered through the left femoral vein and blood was collected 15, 30, 45 and 60 min thereafter. CSF was obtained immediately after the last sampling of blood.

Nasal administration. FDs were dissolved in pH 7·4 isotonic phosphate buffer (FD4, 100 mg mL^{-1}; FD10, 300 mg mL^{-1}; FD20 and FD40, 500 mg mL^{-1}). According to the method of Hirai et al (1981), the surgery was performed on the

oesophagus and the trachea of the rat. Fifty microlitres of the dosing solution was administered into the nasal cavity through the nostril by means of a micropipette or a microsyringe. Thereafter, blood and CSF were obtained as described above.

Collection of CSF. CSF was obtained by cisternal puncture as previously described (Sakane et al 1991b). Briefly, an incision was made in the skin over the occipital bone and the first layer of the muscle was cut. The sharp end of a 27-gauge needle connected with polyethylene tubing (PE-50, approx. 1 m length) was carefully inserted into the cisterna magna and CSF was withdrawn into the tubing by a disposable syringe. Collection was terminated as soon as blood appeared in the tubing. CSF was divided into two parts in terms of the length of the tubing containing CSF, and the latter half which was withdrawn later 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.

Analytical procedure

Blood was centrifuged to separate plasma. An equal volume of 10% trichloroacetic acid was added to the plasma and the mixture was centrifuged once more. The concentration of FDs in the supernatant and the CSF was determined as previously reported (Sakane et al 1991b). FD was detected by a fluorescence spectromonitor (RF-530, Shimadzu, Kyoto, Japan) at 495 nm (excitation) and 514 nm (emission).

Determination of the kinetic parameters

It was assumed that the kinetics of FDs follows the one-compartment model. The total body clearance (CL_{tot}, mL min^{-1}) and the apparent distribution volume (Vd, mL) were obtained by fitting the plasma concentration-time profiles after intravenous administration to the following equation with the computer program MULTI for nonlinear least squares regression analysis (Yamaoka et al 1981).

$$C_p = (100/Vd) \cdot e^{-(CL_{tot}/Vd)t} \quad (1)$$

where C_p and t are the plasma concentration (% dose mL^{-1}) and time (min) after administration, respectively.

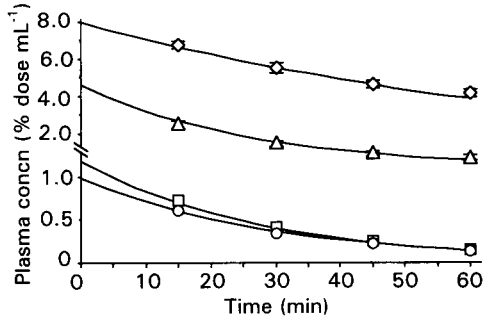

FIG. 1. Plasma concentration–time profiles after intravenous administration of FD4 (○), FD10 (□), FD20 (△) and FD40 (◇) to rats. The curves are predicted from the kinetic parameters of Table 1. The concentrations are standardized with the dose. Data are expressed as means with s.e.m. (n = 4–7).

Table 1. Kinetic parameters of fluorescein isothiocyanate-labelled dextrans.

Dextran	CL_{tot} (mL min^{-1})	Vd (mL)
FD4	3·32 ± 0·33	101·2 ± 10·3
FD10	3·02 ± 0·29	84·7 ± 4·0
FD20	0·780 ± 0·088	21·8 ± 2·1
FD40	0·153 ± 0·008	12·5 ± 0·3

Results and Discussion

Fig. 1 shows the plasma concentration-time profiles of FDs after intravenous administration. The plasma level increased with the increase in the molecular weight of the FDs.

Table 1 lists CL_{tot} and Vd values. Both parameters decreased with the increase in molecular weight of FDs. Vd seems to approach the value of the total plasma volume as the molecular weight of FD increases.

None of the FDs were detected in the CSF after intravenous administration (data not shown). Generally, drugs are transported from the blood to the CSF through the choroid plexus (i.e. blood-CSF barrier), or from the blood to the brain parenchyma and subsequently to the CSF. Therefore, the permeability of the blood-brain and the blood-CSF barriers to FDs is shown to be very small. This is reasonable, since inulin, which has an average molecular weight of 5000 Da, has been used as a vascular marker in an evaluation of the cerebrovascular permeability of drugs (Ohno et al 1978).

Plasma concentration–time profiles of FDs after nasal administration are shown in Fig. 2. The concentrations of FD in the CSF at the end of the experimental period are also presented in Fig. 2. Although the levels of FDs in the plasma (0·5–10 μg mL^{-1}) was much smaller than those after intravenous administration (100–500 μg mL^{-1}), FD4, FD10 and FD20 were detected in the CSF after nasal administration, showing that these FDs can be directly transported from the nasal cavity to the CSF through the nasal mucosa. The concentration of FD40 in the CSF was below the detection limit. Therefore, it was also shown that the upper limit of the molecular weight in the transport to the CSF is at least 20 000 Da. The nasal perfusion experiment, in which the drug concentration in the nasal cavity is constant, revealed that the concentration of low molecular weight drugs (< 400 Da) in the CSF reached a level which was 0·01–0·1% (10^{-4}–10^{-3}) of that in the nasal perfusion fluid (Sakane et al 1991b). Assuming that the decrease in the concentration of the FD in the nasal cavity can be ignored, the concentration ratio of the CSF to the nasal cavity is calculated to be 10^{-6}. Consequently, the degree of the direct transport of FD to the CSF is two or three orders of magnitude smaller than that of the low molecular weight drug.

The concentration in the CSF decreased with the increase in the molecular weight of the FDs. According to McMartin et al (1987), drugs with a molecular weight up to 1000 Da have good bioavailability. On the other hand, the bioavailability of drugs with a molecular weight above 1000 Da declines with the increase in molecular weight. They concluded that the nasal route is suitable for the efficient and

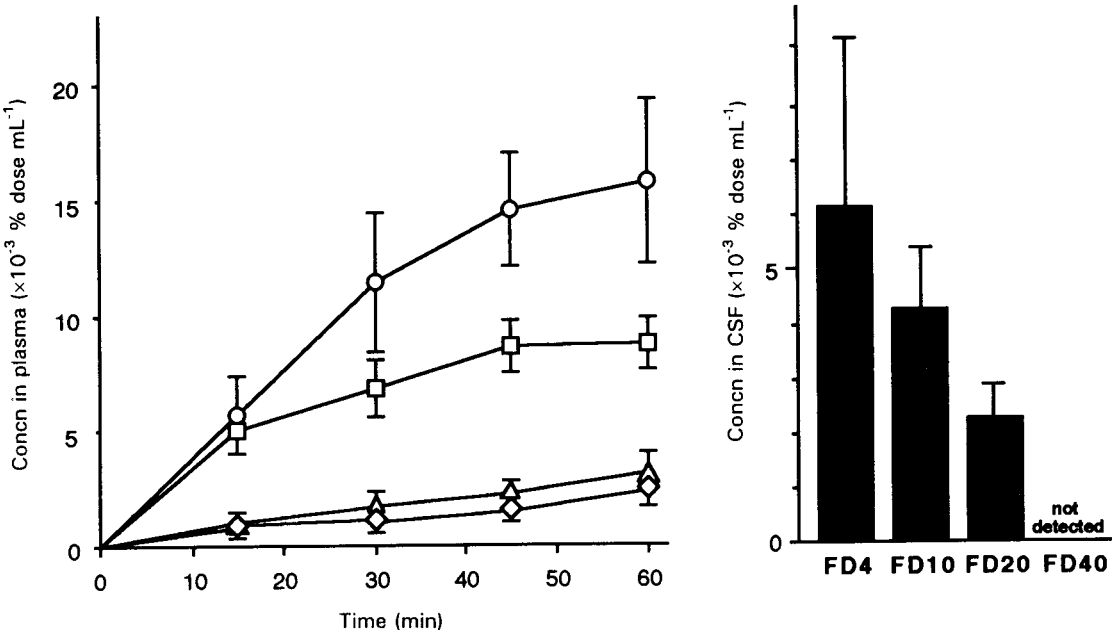

FIG. 2. Plasma concentration–time profiles (left) and concentrations in the CSF at the end of the study (right) after nasal administration of FD4 (○), FD10 (□), FD20 (△) and FD40 (◇) to rats. The concentrations are standardized with the dose. Data are expressed as means with s.e.m. (n = 6–7).

rapid administration of drugs with a molecular weight up to 1000 Da. In our study using gel filtration chromatography, the molecular weight distribution of FDs detected in the CSF was similar to those of FDs before administration (data not shown). Therefore, our data on the direct transport to the CSF are essentially consistent with the results of McMartin et al (1987) and suggest that the rate-limiting process in the direct transport to the CSF is the same as that in the nasal absorption into the systemic circulation.

In conclusion, drugs with a molecular weight up to at least 20 000 Da can be directly transported from the nasal cavity to the CSF, although the degree of the FD transport is very small compared with those of low molecular weight drugs.

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