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Pharmacokinetics and brain uptake of lactoferrin in rats

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Abstract

Lactoferrin (Lf) is an iron-binding glycoprotein belonging to the transferrin (Tf) family. Lf was reported to cross the blood brain barrier (BBB) via receptor-mediated transcytosis in an in vitro model of the BBB. In the present study, we compared the in vivo brain uptake of Lf with that of OX26, an anti-Tf receptor antibody, and Tf. These three proteins were radiolabeled with ^{125}I and administered to rats by i.v. injection. We found that Lf was more rapidly eliminated from the blood compared with OX26 and Tf (The half-life of Lf was approximately 8 and 6 times shorter than that of OX26 and Tf, respectively; the area under the blood concentration–time curve of Lf was approximately 15 and 17 times smaller than that of OX26 and Tf, respectively), and mainly accumulated in the liver, spleen, and kidney. Markedly high brain uptake was observed for Lf relative to Tf and OX26. Lf might be useful as a ligand for facilitating drug delivery into the brain.

Keywords: Lactoferrin; Pharmacokinetics; Blood brain barrier; Transport

Introduction

Brain targeting is an important requirement for drug delivery to the central nervous system. Attaching pilot molecules to the surface of the drug carrier, such as liposome, or the therapeutic molecule itself so as to allow the drug to pass through the BBB is one of the strategies for brain targeting (Qian et al., 2002).

Lactoferrin (Lf) is a mammalian cationic iron-binding glycoprotein belonging to the transferrin (Tf) family. Many physiological roles of Lf have been indicated, particularly in the host defense against infection and severe inflammation. This broad spectrum of biological functions relies on the interaction of Lf with numerous cells. The Lf receptor (LfR), which mediates the uptake of Lf into cells, is expressed on the surface of cells, such as platelets (Leveugle et al., 1993), megakaryocytes (Nillesse et al., 1994), dopaminergic neurons and endothelial cells of mesencephalic microvessels (Faucheux et al., 1995) for the uptake of Lf. Lf is produced by exocrine glands (Levay and Viljoen, 1995; Sanchez et al., 1992), and is mainly stored in specific granules of neutrophilic leukocytes (Masson et al., 1969). However, as for the physiological roles of Lf in the brain, it is not clear whether it contributes to the transport of iron into the brain. Using co-culture of bovine brain capillary endothelial cells and astrocytes as an in vitro model of the BBB, Lf has been demonstrated to cross the BBB via receptor-mediated transcytosis (Fillebeen et al., 1999). Therefore, it was of interest to attempt to determine whether Lf can be transported into the brain across the BBB in vivo. In the present study, the pharmacokinetics and brain uptake of Lf were investigated and compared with those of Tf and OX26, an anti-Tf receptor antibody, in in vivo conditions.

Materials and methods

Male Sprague–Dawley rats (250–300 g) were purchased from Japan SLC Inc. (Shizuoka, Japan). Lf from bovine colostrums and human holo-Tf were purchased from Sigma (St. Louis, MO). OX26 was purchased from BD Biosciences (Franklin Lakes, NJ). Na^{125}I (2.2 mCi/nmol) was purchased from Amersham Co. (Arlington Heights, IL). All other chemicals were of analytical grade.

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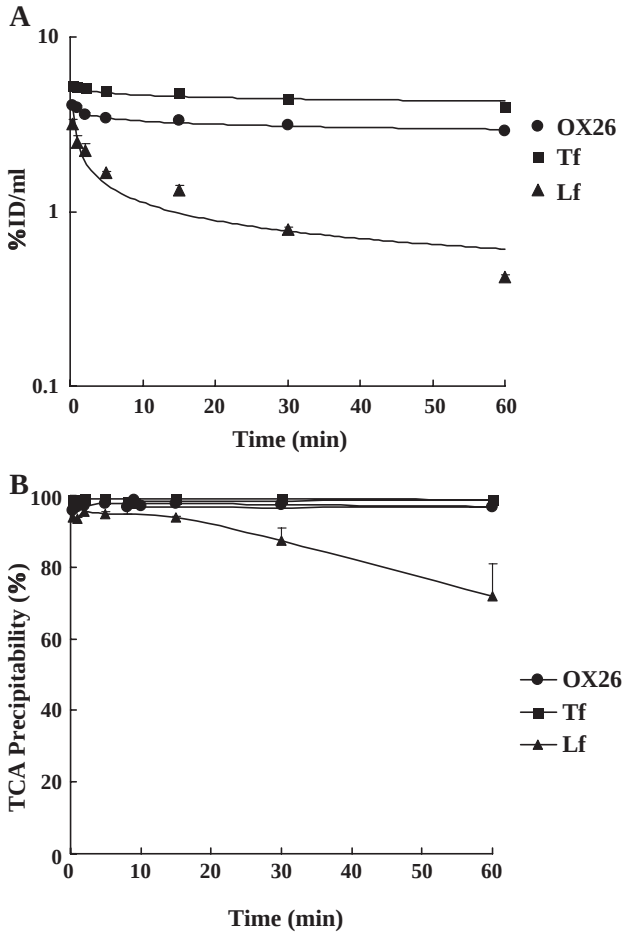

Fig. 1. (A) Time profiles of blood concentrations of ^{125}I–Lf, ^{125}I–OX26 and ^{125}I–Tf after i.v. administration in rats. Blood concentrations were corrected by TCA-precipitability. (B) Metabolic stability of ^{125}I–Lf, ^{125}I–OX26 and ^{125}I–Tf. Metabolic stability was presented by TCA-precipitable radioactivities. Data are mean ± SE (n = 3).

For iodination, OX26 (1 nmol), Tf (1 nmol), and Lf (1 nmol) were dissolved in 50 μl of 0.2 M sodium phosphate buffer (pH 7.4) and mixed with 200 μCi of Na^{125}I. The reaction was initiated by the addition of 8.4 nmol of chloramine-T, carried out at room temperature for 2 min, and stopped by the addition of 12.5 nmol of sodium metabisulfite. The iodinated proteins were eluted from a 2.6 × 10 cm Sephadex G-25 column with 5 ml of 0.2 M sodium phosphate buffer containing 0.1% bovine serum albumin (pH 7.4). The trichloroacetic acid (TCA)-precipitability of OX26, Tf, and Lf was >95%, 95%, and 90%, respectively.

0.3 ml of PBS containing 2 μCi of iodinated OX26, Tf or Lf was injected via the tail vein of rats. Blood samples (0.2 ml) were collected via a heparinized PE45 cannula implanted in the femoral artery, at 0.25, 1, 2, 5, 10, 30, and 60 min after the injection. The blood volume was replaced with an equal volume of saline. The organs including heart, liver, lung, kidney, spleen, and brain were extirpated at 60 min. The radioactivity of ^{125}I in the blood and organ samples was counted using a Beckman γ-counter. Aliquots of blood samples were precipitated with TCA for examination of the metabolic stability of the labeled proteins. Brain TCA precipitability was measured by homogenized brain tissue in 6-fold weight of cold 15% TCA, followed by centrifugation.

The pharmacokinetic parameters were calculated by fitting the blood TCA-precipitable radioactivity data to a biexponential equation, $C(t)=Ae^{-k_A t}+Be^{-k_B t}$ (Fillebeen et al., 1999), where $C(t)$=% injected dose (ID)/ml of blood. The organ volume of distribution (V_d) at 60 min was determined from the ratio of the dpm (disintegrations per minute) per gram tissue to the dpm per microliter of terminal blood. The area under the blood concentration–time curve (AUC) was calculated by the linear trapezoidal rule, and blood clearance (CL_{tot}) and organ uptake clearance (CL_{organ}) were calculated by the following equations: $CL_{tot}=Dose/AUC_{0-60\ min}$, and $CL_{organ}=[V_d-V_0]C_{(60\ min)}/AUC_{0-60\ min}$. V_0 is the intrinsic blood volume of each organ (brain, heart, liver, lung, kidney, and spleen, approximately 21,148, 196, 190, 114, and 253 μl/g tissue, respectively), which was determined with ^{125}I-labeled mouse native IgG2a in rat (Triguero et al., 1991). The organ uptake of all three proteins was expressed as percentage of ID/g tissue, and was determined as follows: $\%ID/g=[V_d-V_0]C_{(60\ min)}$.

Results

In comparison with ^{125}I–Tf and ^{125}I–OX26, ^{125}I–Lf was rapidly eliminated from the blood circulation after i.v. administration (Fig. 1A). ^{125}I–Lf was metabolically less stable (approximately 70%) compared to ^{125}I–OX26 (approximately 95%) or ^{125}I–Tf (approximately 95%) after 60 min of observation (Fig. 1B). The results of the pharmacokinetic analysis are shown in Table 1. The elimination half-life ($T_{1/2,\ B}$) of ^{125}I–Lf was approximately 8 and 6 times shorter than that of ^{125}I–OX26 and ^{125}I–Tf, respectively. $AUC_{0-\infty}$ of ^{125}I–Lf was approximately 15 and 17 times smaller than that of ^{125}I–OX26 and ^{125}I–Tf, respectively (Table 1). CL_{organ}, as well as the percentage of ID per gram tissues, calculated from AUC data and tissue V_d, showed that ^{125}I–Lf was mainly taken up by the liver, spleen, and kidney (Fig. 2). The uptake of ^{125}I–Lf to these organs was approximately 10-fold greater than those of ^{125}I–OX26 and ^{125}I–Tf. The brain V_d of ^{125}I–Lf was approximately 40 μl/g brain, and was higher than those of ^{125}I–OX26 (approximately 20 μl/g brain) and ^{125}I–Tf (approximately 18 μl/g brain) (Fig. 3A). As the brain V_0 estimated

Table 1
Pharmacokinetics parameters for ^{125}I–Lf, ^{125}I–Tf, and ^{125}I–OX26 in rats

Parameter	^{125}I–Lf	^{125}I–OX26	^{125}I–Tf
A (%ID/ml)	1.43 ± 0.097	0.81 ± 0.07	0.26 ± 0.10
B (%ID/ml)	1.82 ± 0.10	3.43 ± 0.04	5.06 ± 0.20
K_A (min^{-1})	0.60 ± 0.14	0.70 ± 0.11	1.01 ± 0.13
K_B (min^{-1})	0.025 ± 0.002	0.004 ± 0.002	0.004 ± 0.001
$T_{1/2,\ A}$ (min)	1.26 ± 0.26	1.06 ± 0.19	0.71 ± 0.09
$T_{1/2,\ B}$ (min)	28.11 ± 1.97	233.41 ± 17.05	179.3 ± 12.33
AUC_{0-60} (%ID·min/ml)	59.97 ± 1.19	189.73 ± 2.35	270.5 ± 8.37
$AUC_{0-\infty}$ (%ID·min/ml)	77.07 ± 1.44	1159.8 ± 72.51	1306.0 ± 34.36
CL_{tot} (ml/min/rat)	1.30 ± 0.03	0.087 ± 0.009	0.077 ± 0.002

The pharmacokinetic parameters were estimated from the data shown in Fig. 1. The analyses were carried out as described in Materials and methods. Data are presented as mean ± SE (n = 3).

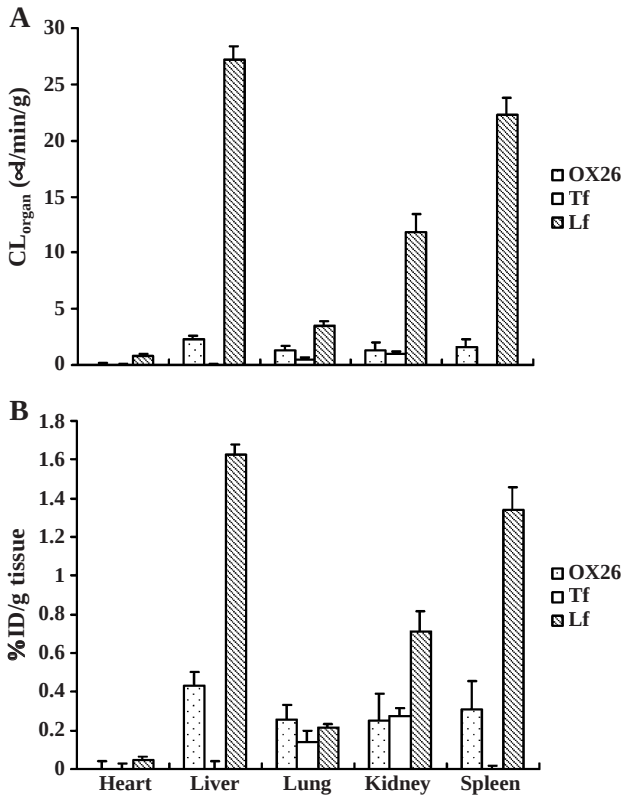

Fig. 2. Comparison of the CL_{organ} (A) and organ uptake (B), at 60 min after i.v. injection for ^{125}I–Lf, ^{125}I–Tf, and ^{125}I–OX26 in the rat. The analyses were carried out as described in Materials and methods. Data are mean ± SE (n = 3).

in our preliminary experiment using ^{3}H–inuline (approximately 20 μl/g brain) was in good agreement with published values using ^{125}I-labeled mouse native IgG2a (Triguero et al., 1991), we conceived that the brain V_d values of ^{125}I–OX26 and ^{125}I–Tf were at a level similar to the intrinsic blood volume in the brain. The brain uptake of ^{125}I–Lf, represented as %ID/g brain at 60 min, was approximately 0.016%, while those of ^{125}I–OX26 and ^{125}I–Tf were negligible (Fig. 3B). However, the brain uptake of Lf did not increase in a time-dependent manner (data not shown), likely due to its relatively low blood concentration at >60 min after injection.

Discussion

Receptor-mediated endocytosis/transcytosis was considered as the main mechanism of uptake of Lf by organs/cells. LfR has been identified in many tissues, including monocytes, lymphocytes, liver, and mammary epithelial cells (Suzuki and Lonnerdal, 2002). The expression of LfR in monocytes, lymphocytes, and liver is in good agreement with the high accumulation of Lf in spleen and liver observed in the present study.

Tf is also taken up via receptor-mediated endocytosis/transcytosis (Broadwell et al., 1996). TfR has been identified on the brain microvessels, and is considered to contribute to iron transport into the brain across the BBB (Moos and Morgan, 2000). However, the trans-endothelial transfer of Tf into the brain through the BBB in vivo is still controversial. Some investigators have reported that the transcytosis of Tf through cerebral endothelia in vivo (Skarlatos et al., 1995) and in vitro (Descamps et al., 1996) is prominent, while others claim that although some Tf molecules do enter the endothelia, most of them are recycled to the blood (Strahan et al., 1992; Taylor et al., 1991). Other studies have found no transport of Tf from the blood to the brain (Morris et al., 1992; Roberts et al., 1992). Our study showed that there was no detectable amount of Tf entering the brain after i.v. administration. The concentration of circulating Tf is about 25 μM, while the dissociation constant of Tf is approximately 10^{-6} M (Sawyer and Krantz, 1986). Therefore, cellular TfR might steadily be saturated with endogenous Tf. By contrast, the low plasma concentration of endogenous Lf (approximately 16 nM: 1000-fold lower than Tf) seems beneficial in regard to its receptor.

OX26 is a mouse monoclonal antibody against rat TfR. It is reported to cross the BBB in a TfR-mediated manner and is used to deliver plasmid to the brain in vivo (Shi and Pardridge, 2000). However, our present experiment showed that there was

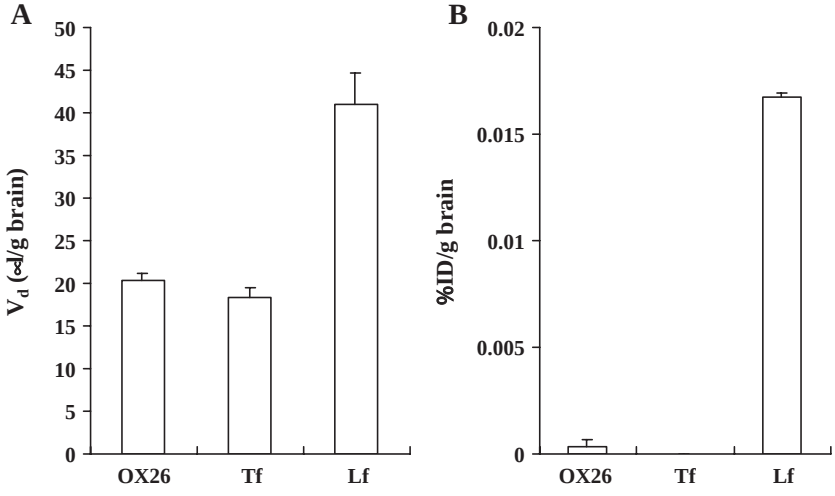

Fig. 3. V_d values (A) and brain uptake (B) of ^{125}I–Lf, ^{125}I–Tf, and ^{125}I–OX26 at 60 min after i.v. injection. The analyses were carried out as described in Materials and methods. Data are mean ± SE (n = 3).

only a slight amount of OX26 in the brain at 1 h after i.v. injection (Fig. 3). The reason for this discrepancy is yet to be elucidated. One possible explanation is that efficient transcytosis of immunoliosomes by the brain endothelia is attributable to cooperative effects of OX26 and liposome. In fact, it has been reported that unconjugated OX26 can be endocytosed but not readily transcytosed by brain capillary endothelial cells (Moos and Morgan, 2001). Hence, OX26 that is taken up by the endothelial cells may either stay inside the cells or be recycled to the blood. Another possibility is that experimental conditions, including injected dose, may influence the tissue uptake of OX26. Radiolabeled OX26 in previous quantitative examinations was administered to rats at a dose ranging 0.7 to 18 μg (Friden et al., 1991; Moos and Morgan, 2001), and the dosage was indicated to have no significant effects on the brain uptake. However, the injected dose of ^{125}I–OX26 in our present work (approximately 0.3 μg per rat) was considerably lower than the above-mentioned dose, and thus may produce pharmacokinetic properties that differ from those in previous investigations. It also might be possible that a subset of commercially available OX26 antibodies are less prone to uptake into the brain tissue, unlike the one purified by the researchers themselves (Friden et al., 1991; Moos and Morgan, 2001). Indeed, recent in situ perfusion study has demonstrated that commercial OX26 enters the brain capillary endothelial cells but not the postcapillary brain tissue (Gosk et al., 2004).

The attachment of ligand to the specific target of the delivery system, such as liposome (Shi et al., 2001) or bioconjugate (Wu et al., 1997), allows the selective delivery of gene and antisense to the target organs/cells. Considering the difficulty in brain delivery using existing systems, the present result indicates possible application of Lf to the construction of a delivery system to the brain, although transfer of Lf to non-brain organs should also be taken into account. The brain-specific effect could further be increased by constructing Lf-conjugated liposome containing neuronal promoter-combined therapeutic genes, as demonstrated elsewhere (Shi et al., 2001). Agents composed of Lf and neuroreceptor ligand would also permit exploitation of brain-targeted therapies without causing adverse effects on peripheral organs. The rapid brain uptake of Lf may be useful for a brain-targeting delivery system, particularly for those requiring prompt delivery within limited measurement time, such as imaging with a positron nuclide. In addition, the delivery system with Lf attachment might be more efficient under certain pathological conditions, such as Parkinson's disease (Faucheux et al., 1995) and Alzheimer's disease (Kawamata et al., 1993), as increased expression of LfR on microvessels and neurons has been reported. In conjunction with these facts, our present results provide possible neuroprotective approaches to diverse neurodegenerative disorders.

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