International Journal of Pharmaceutics 377 (2009) 85–91

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

The transnasal delivery of 5-fluorouracil to the rat brain is enhanced by acetazolamide (the inhibitor of the secretion of cerebrospinal fluid)

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ARTICLE INFO

Article history:
Received 16 January 2009
Received in revised form 1 May 2009
Accepted 6 May 2009
Available online 14 May 2009

Keywords:
Nasal absorption
Cerebrospinal fluid
Brain delivery
Brain uptake clearance
Acetazolamide
5-Fluorouracil

ABSTRACT

The purpose of the research is to evaluate the effect of acetazolamide (AZA), an inhibitor of the secretion of cerebrospinal fluid (CSF), on the direct drug transport from the nasal cavity to the CSF and the brain uptake of a model drug, 5-fluorouracil (5FU). 5FU was infused intravenously or perfused nasally in the presence and absence of intravenously administered AZA. Concentrations of 5FU in plasma, CSF and the cerebral cortex were measured. The AUC and the concentration of 5FU in the brain were used to calculate the apparent brain uptake clearance (CL_{up}) of 5FU, which is an index of drug delivery to the brain under the two experimental conditions. Intravenous AZA markedly increased the concentration of 5FU in the CSF and brain following the nasal perfusion of 5FU, although the plasma concentrations of 5FU were similar with intravenous infusion and nasal perfusions of 5FU. CL_{up} of 5FU after the nasal perfusion with AZA was significantly increased by 104% and 46% as compared to intravenous infusion and nasal perfusion without AZA, respectively. AZA enhanced the 5FU delivery to the brain through a nose-to-brain pathway by increasing the concentration of the nasally applied drug in the CSF.

1. Introduction

The transport of solutes between the blood and the brain is different from those between the blood and most other organs. In contrast to systemic capillaries, brain microvessel endothelial cells are joined by intercellular tight junctions, possess few endocytic vesicles and are devoid of fenestrae (Demeule et al., 2004; Pardridge, 1995). These endothelial cells restrict the transport of hydrophilic compounds into the brain and form the transport barrier generally called blood–brain barrier (BBB). Although the BBB creates and maintains the required brain environment by limiting the transport of certain substances, it is an obstacle to the development of therapeutic agents for brain disorders. In order to overcome this barrier, many strategies have been designed and investigated (Pardridge, 2007; Ochiai et al., 2006; Ohtsuki and Terasaki, 2007; Obradovic et al., 2007; Gynther et al., 2008; Vyas et al., 2006). One of these is intrathecal administration (direct injection of the drug into the cerebrospinal fluid) (Ochiai et al., 2006), since the cerebrospinal fluid (CSF) flows over the surface of the brain, and molecules in the CSF have rapid access to the extracellular space throughout the CNS (within 5–10 min). This movement along the intraparenchymal vascular network, with its accompanying spread into the cerebral interstitium, appears to be facilitated by the pulsation of penetrating arterioles within their perivascular space with each cardiac contraction (Rennels et al., 1990). Moreover, the drug can diffuse into the extracellular fluid of the brain more easily from the CSF than from the systemic blood.

On the other hand, many reports support the presence of a connection between the nasal cavity and the CSF. Consequently, the attention of many researchers has recently been focused on the potential of the nasal route for drug delivery to the brain (Vyas et al., 2006; Yang et al., 2005; Kandimalla and Donovan, 2005; van den Berg et al., 2004; Bagger and Bechgaard, 2004; Illum, 2000). For one decade from 1995, it was controversial if the nasal route was actually effective for drug delivery to the brain. However, many pieces of evidences and data in animals and human regarding the transport of proteins (Illum, 2000; Thorne et al., 2004, 2008), metals (Perl and Good, 1987; Czerniawska, 1970; Trombley, 1998; Hastings and Evans, 1991), and small molecules (Sakane et al., 1991a,b, 1994, 1995), including our previous report using 5-fluorouracil (5FU) as a model compound (Sakane et al., 1999), have been accumulated in support of the feasibility of transnasal drug delivery to the brain. Also, recent publications have presented evidence in humans that

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doi:10.1016/j.ijpharm.2009.05.009

drugs can be delivered to the brain and CSF and that intranasal administration with peptide produces a profound CNS effects including improved memory (Born et al., 2002; Benedict et al., 2004, 2007; Reger et al., 2008a,b). As a result, it is now generally accepted that the nasal route is applicable to the delivery of drugs to the brain.

According to Illum (2000), Thorne et al. (2004, 2008) and Dhanda et al. (2005), drugs are believed to undergo transport from the nasal cavity to the brain via multiple pathways, i.e. transaxonal, transepithelial and systemic pathways. In the transaxonal pathway, xenobiotics, drugs, metals (Perl and Good, 1987; Czerniawska, 1970; Trombley, 1998; Minoshima and Cross, 2008) and large proteins such as horseradish peroxidase (Persson and Kristensson, 1979), undergo adsorptive or receptor-mediated endocytosis on the surface of olfactory sensory neurons. A fraction of the endocytosed compound is subsequently transcytosed to axon terminals in the glomeruli of the olfactory sensory neurons as well as the trigeminal nerve that innervates the nasal mucosa, which has been shown to provide an additional pathway. Consequently, the transport of drugs and xenobiotics via the transaxonal pathway is slow when compared to the transepithelial pathway. On the other hand, in the transepithelial pathway, drugs having small molecular weights (Sakane et al., 1991a,b, 1994, 1995) and most therapeutic proteins (Dhanda et al., 2005) permeate the nasal epithelium through the transcellular or paracellular routes and reach the CSF and the brain parenchyma simultaneously via the perineural channel opening along the intercellular clefts that surround the olfactory and trigeminal nerves. The systemic pathway involves entry of the drug into the blood stream from various regions of the nose with subsequent translocation across the BBB. From the CSF, the drug can reach the brain parenchyma easily because, as opposed to the BBB, no tight barrier exists between the brain and the CSF. Small molecules are primarily delivered to the brain through this pathway (Sakane et al., 1991a). The important factor that determines the delivery through this pathway is the concentration of the drug in the CSF. The higher the CSF concentration of the nasally administered drug, the greater the amount of drug delivered to the brain. The concentration of the nasally applied drug that reaches the CSF is determined from the influx (i.e. from the nasal cavity to the CSF) and efflux (i.e. from the CSF to the venous blood). The efflux from the CSF is primarily governed by the bulk flow of the CSF. The reduction of the bulk flow of the CSF (i.e. secretion of CSF) is expected to improve the delivery to the brain.

Acetazolamide (AZA), a carbonic anhydrase inhibitor, has been shown to decrease CSF production in both *in vivo* and *in vitro* animal models and both human adult and children patients during the treatment of pseudotumor cerebri and hydrocephalus (Carrion et al., 2001). AZA therapy may result in hypokalaemia and hyperchloraemia, and, by altering blood flow to the choroid plexus, decreases CSF production by 40–90%, depending on the experimental model (Holloway and Cassin, 1972; Knuckey et al., 1991; Watanabe et al., 1976). The purpose of this study was to determine whether inhibition of CSF secretion in the choroid plexus by AZA leads to a higher enhances the concentration of 5FU in the CSF and, ultimately in the brain.

2. Materials and methods

2.1. Materials

^{3}H-5-Fluorouracil (5FU, Fluorouracil, 5-[6-^{3}H]-, specific activity, 555 GBq/mmol) was obtained from PerkinElmer (Waltham, MA, USA) and used without further purification. Acetazolamide (AZA) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of reagent grade and commercially available.

2.2. Animal experiments

All the animal experiments were performed in accordance with the Guideline of Setsunan University for the Care and Use of Laboratory Animals. Male Wistar rats weighing 200–300 g were anesthetized with intraperitoneal pentobarbital (40 mg/kg, Nembutal®, Abbott, Abbott Park, IL, USA).

2.3. Concentration of 5FU in the CSF after intravenous infusion and nasal perfusion

The right femoral artery of all rats was cannulated with polyethylene tubing (SP-31, Natsume, Tokyo, Japan) for collecting blood samples and this was previously filled with heparinized saline. After starting the intravenous infusion or nasal perfusion of 5FU, blood samples were taken at certain time points to obtain the plasma concentration time courses.

For the intravenous infusion (i.v. infusion), the left femoral veins were cannulated with polyethylene tubing (SP-31). ^{3}H-5FU (555 kBq/mL in physiological saline, i.e. 0.3 nmol/rat at 20 min) was infused at a flow rate of 15 μL/min by an infusion pump (No. 944, Harvard Apparatus, Holliston, MA, USA) through this cannula.

For the nasal perfusion group (perfusion(−AZA) and perfusion(+AZA)), the trachea and esophagus were surgically operated as previously reported by Hirai et al. (1981). Briefly, an incision was made in the neck and the trachea was cannulated with polyethylene tubing (No. 6, Hibiki, Tokyo, Japan) for simultaneous respiration. Another tube was inserted from the esophagus to the posterior part of the nasal cavity. The nasopalatine was closed with an adhesive agent.

In the group of nasal perfusion of 5FU (perfusion(−AZA)), 5FU dissolved in 8 mL of pH 7.4 isotonic phosphate buffer at the concentration of 1850 kBq/mL (26.7 nmol/rat) was recirculated through the nasal cavity at a flow rate of 1.0 mL/min by a peristaltic pump (SJ-1211H, ATTO, Tokyo, Japan). In the group in which the nasal perfusion of 5FU was accompanied with intravenous administration of AZA (i.e. nasal perfusion(+AZA)), AZA (25 mg/kg) was injected through the left femoral cannula 15 min before starting the nasal perfusion. Then, the nasal cavity of the rat was perfused as was done in the group that received no intravenous AZA (i.e. nasal perfusion(−AZA)). During the nasal perfusion, AZA was infused intravenously at a rate of 0.5 mg/min to maintain the desired plasma concentration.

At 20 min after i.v. infusion or nasal perfusion with 5FU, sampling of the CSF was performed by cisternal puncture as previously reported by Chou and Levy (1981). 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 (TERUMO, Tokyo, Japan) was directly connected to a piece of silicone tubing (SILASTIC TUBING®, O.D.; 0.9 mm, I.D.; 0.5 mm, DOW CORING, Midland, MI, USA), 5 cm long, which was also connected to a piece of polyethylene tubing (O.D.; 1 mm, No. 3, Hibiki) approximately 100 cm long via a 22 gauge needle (TERUMO, Tokyo, Japan), was carefully inserted into the cisterna magna. The CSF was withdrawn into the tubing by a 1 mL disposable syringe (TERUMO, Tokyo, Japan). Collection was terminated as soon as blood appeared in the tubing, and only those CSF samples that exceeded 150 μL were accepted for analysis. The CSF was divided into 2 parts, an initial and a latter half. The initial half of CSF was discarded and only the concentration of 5FU in the latter half was determined (Sakane et al., 1991a).

2.4. Change of concentration of 5FU in the plasma and the cerebral cortex during intravenous infusion and nasal perfusion

For the i.v. infusion group, the right femoral artery and left femoral vein were cannulated, and ^{3}H-5FU was infused in the same

manner as described above in the presence and absence of intravenous AZA (25 mg/kg) through the left femoral vein 15 min before starting the ^{3}H-5FU infusion. Brain tissue (the cerebral cortex) samples were taken at the end of the infusion period (Fig. 3). In the 30 min experiment, blood samples were taken at certain time points to obtain a representative plasma concentration–time course during the i.v. infusion (Fig. 2).

For the nasal perfusion(−AZA) and perfusion(+AZA) groups, the same surgical operation was utilized, and ^{3}H-5FU was perfused in the same manner as described above.

Brain tissue (the cerebral cortex) samples were taken at the end of the perfusion period (Fig. 3). In the nasal perfusion experiments for 60 min in the presence and absence of AZA, blood samples were taken at certain time points to draw profiles during nasal perfusion (Fig. 2). Perfusate samples (10 μL) were also taken periodically to determine the change in the 5FU concentration in the nasal perfusion fluid (Fig. 4).

2.5. The theory for calculation of apparent brain uptake clearance

A two-compartment system in which the plasma is compartment 1 and the brain is compartment 2 was used to describe the drug distribution in the brain (Ohno et al., 1978). Following drug application, the drug uptake (per gram of brain) from the plasma is given by the following equation.

$$\frac{dC_2}{dt} = CL_{up}\,(C_1 - (C_2/V_e)) \quad (1)$$

where C_1 and C_2 are drug concentrations in the plasma and in the brain. CL_{up} and V_e are the apparent brain uptake clearance (per gram of brain) and the brain/plasma concentration ratio at steady state, respectively. When $C_1 \gg C_2$, C_2/V_e is negligible and the above equation is simplified to

$$\frac{dC_2}{dt} = CL_{up}C_1 \quad (2)$$

Integration of the above equation results in the following equation.

$$C_2 = CL_{up}AUC_t \quad (3)$$

where AUC_t is the area under the plasma concentration (C_1)–time profiles up to time t. The above equation indicates a linear relationship between the brain concentration at time t and AUC_t when C_1 is much greater than C_2 ($C_1 \gg C_2$) with CL_{up} as the proportionality constant.

In this study, brain tissues were taken at various time intervals after starting the intravenous infusion or nasal perfusion with 5FU. The values obtained from the individual rats were plotted (Fig. 5), and CL_{up} was calculated as the proportionality constant (the slope) from the relationship between AUC_t and C_2 using linear regression analysis.

2.6. Analytical procedure

Plasma samples were obtained by centrifugation of the blood at 5000 rpm for 5 min. The plasma (100 μL) was transferred to a counting vial and treated with 0.5 mL of Soluene-350® (PerkinElmer, Waltham, MA, USA). Each sample was neutralized with 50 μL of 5 N HCl and then 10 mL of Clear-sol I® (Nacalai Tesque, Kyoto, Japan) was added. CSF (70 μL) was transferred to a counting vial and 10 mL of Clear-sol I® was added. Following decapitation of the rat, the brain was removed from the skull. The brain was washed with physiological saline and the meninges were carefully removed. Since the drug concentration in the CSF around the cerebral cortex was expected to be high, the concentration in the cerebral cortex was determined. The dissected cerebral cortex was transferred to a counting vial and its weight was measured. In order to solubilize the brain tissue, 1 mL of Soluene-350® was added. After the tissue was completely solubilized, 100 μL of 5 N HCl and then 10 mL of Clear-sol I® were added. All the concentration in the cerebral cortex was corrected for the intravascular drug with vascular volume (1.487%, v/w), which was obtained from another experiment using ^{3}H-inulin (Sakane et al., 1999). The radioactivity in the plasma, CSF and brain tissue were counted by a liquid scintillation counter (LSC3500, Aloka, Tokyo, Japan).

2.7. Statistical analysis

Tukey ANOVA multiple comparison was tested by a statistical analysis software Dr. SPSS II (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. CSF concentration of 5FU after intravenous infusion and nasal perfusion

The effect of AZA on the nasal delivery of 5FU to the CSF was confirmed. Fig. 1 shows plasma concentration–time curves up to 20 min and the concentration of 5FU in the CSF 20 min after starting the intravenous infusion or nasal perfusion. Dosing concentrations and infusion rate were adjusted so as for the plasma concentration following i.v. infusion and nasal perfusion to show the similar profiles. Although the plasma concentrations of the three groups were similar, nasal perfusion(+AZA) showed significantly higher concentrations in the CSF compared to the intravenous infusion group (i.v. infusion) and perfusion(−AZA) group.

The epithelial cells of the choroid plexus secrete CSF by a process that involves the movement of Na^+, Cl^- and HCO_3^- from the blood to the ventricles of the brain. This creates the osmotic gradient, which drives the secretion of H_2O (Speake et al., 2001), and AZA is an inhibitor of carbonic anhydrase and is known to reduce the CSF secretion by blocking that process (Carrion et al., 2001). It is noteworthy that the CSF concentration of 5FU after nasal perfusion(+AZA) is significantly higher than that after nasal perfusion(−AZA), showing that AZA enhances drug delivery from the nasal cavity to the brain parenchyma. This enhancement is due to the fact that AZA inhibits the secretion of CSF from the choroid plexus, which increases the concentration of 5FU in the CSF. Thus, the lower volume of secreted CSF, which normally flows into the systemic circulation, produces a slower rate of removal of the CSF (containing 5FU) from arachnoids villi to the blood. Finally, the longer residence time of 5FU in the subarachnoid space permits the diffusion of a higher amount of the drug from the CSF to the brain. On the other hand, the enhanced delivery of 5FU to the CSF is caused by a reduction of CSF secretion from the Choroid plexus, which is followed by an increase in the concentration and residence time of 5FU in the subarachnoid space. Most likely, higher concentrations of 5FU, together with a prolonged residence in the subarachnoid space, would result in lower efflux into the systemic blood and higher diffusion into the CNS.

Thorne et al. (2004) also suggested that the rapid nature of drug delivery (i.e. insulin-like growth factor-I) into the CNS from the nasal passages was most consistent with absorption via an extracellular route along components of the peripheral olfactory and trigeminal systems by using high-resolution phosphor imaging autoradiography. Our result of nasal perfusion(−AZA) also indicates that 5FU is directly transported from the nasal cavity to the CSF by the transepithelial pathway since the appearance of drug in the CSF was very rapid and CSF concentration should be similar to plasma concentration if it is transported by the systemic pathway (Dhanda et al., 2005).

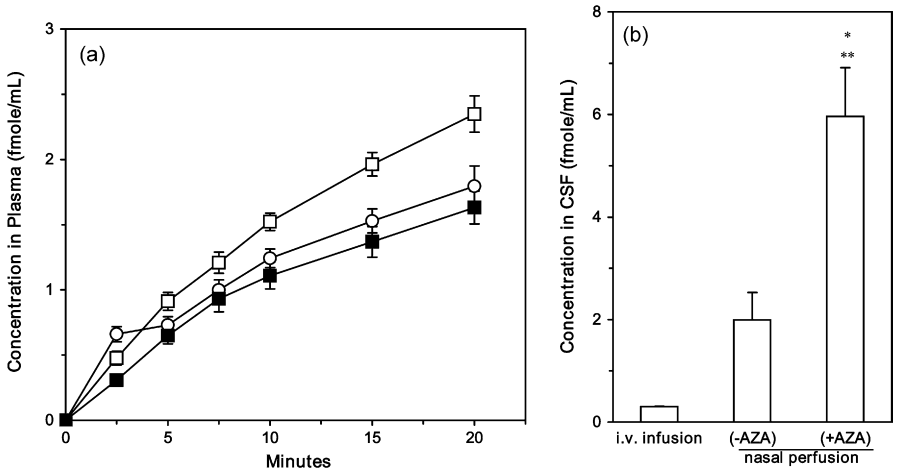

Fig. 1. The concentration–time profiles of 5FU in the plasma up to 20 min (a) and the concentration of 5FU in the CSF 20 min (b) following the start of intravenous infusion or nasal perfusion. After starting the intravenous infusion or nasal perfusion of 5FU, blood samples were taken at 2.5, 5, 7.5, 10, 15, and 20 min to obtain the plasma concentration time courses. After 20 min of i.v. infusion or nasal perfusions, sampling of the CSF (>150 μL) was performed by cisternal puncture. Each point represents mean ± S.E. *Key*: ○; i.v. infusion ($n = 5$), □; perfusion(−AZA) ($n = 6$), ■; perfusion(+AZA) ($n = 6$). Differences of the mean values were tested by Tukey ANOVA multiple comparison test. $^{*}p < 0.01$ (vs. i.v. infusion); $^{**}p < 0.01$ (vs. perfusion(−AZA)).

There is a possibility that radiolabeled 5FU might be metabolized in the nasal cavity and in the compartments where its concentration is measured, and this would cause that the measured radioactivity might be associated not only with the parent drug, but also with metabolites. However, this possibility appears negligible for various reasons. First, the duration of the experiment was very short (20 min). Second, 5FU is mainly metabolized by the liver (Calabresi and Chabner, 1996). Third, the plasma concentrations in the nasally perfused and intravenously infused groups were similar. Similar plasma concentrations of a drug should be associated with the same fraction of metabolites. Therefore, the similarity in plasma drug concentrations between the nasally perfused and intravenously infused groups appears to indicate that the extent to which associated metabolites contribute to the measured plasma radioactivity, in the two treatment groups, is the same.

The CSF is produced mainly by the choroid plexus in the lateral and third cerebral ventricules. The CSF begins its flow inside the brain, flows out on the brain surface at cisterna magna and finally joins the venous blood at superior sagittal sinus through archnoid villus. The nasal drug directly reaches the subarachnoid CSF and is taken up from the surface of the cerebral cortex. The flow of CSF is considered much faster than the uptake by the brain. Consequently, the CSF concentration of directly transported drugs is increased by the reduction of CSF bulk flow. According to Carrion et al. (2001) secretion of CSF is reduced by 39% and 49% in child patients at doses of 50 mg/kg/day and 75 mg/kg/day of AZA, respectively. If drug disappearance from the CSF is dependent on the bulk flow of CSF and assuming that drug absorption from the nasal cavity to the CSF is not changed by the administration of AZA, drug concentration in the CSF can be expected to achieve levels two to three-fold higher because the bulk flow of CSF is reduced to approximately 50–60%. In our result, intravenous AZA enhanced the concentration of 5FU in the CSF by 200–300% compared with nasal perfusion(−AZA). Taking the data from the published report (Carrion et al., 2001) and our results together, a 200–300% increase in the CSF concentration is reasonable.

3.2. Change of concentration of 5FU in the plasma and the cerebral cortex during intravenous infusion and nasal perfusion

Fig. 2 shows a typical change of the concentration of 5FU in the plasma as a function of time. The plasma concentration increased linearly with time. Nasal perfusion(−AZA) and nasal perfusion(+AZA) showed similar plasma concentrations since dosing concentrations and infusion rate were adjusted so that the plasma concentration following i.v. infusion and nasal perfusion show the similar profiles. Fig. 3 shows time courses of the concentration of 5FU in the cerebral cortex. The brain concentration of nasal perfusion(+AZA) at 60 min is higher than nasal perfusion(−AZA), while no marked difference was observed in the concentration up to 30 min. Higher brain concentrations, together with similar plasma concentration as indicated in Fig. 2, demonstrate the feasibility of nasal delivery of 5FU to the brain through the reduction by AZA of the secretion of CSF from the Choroid plexus.

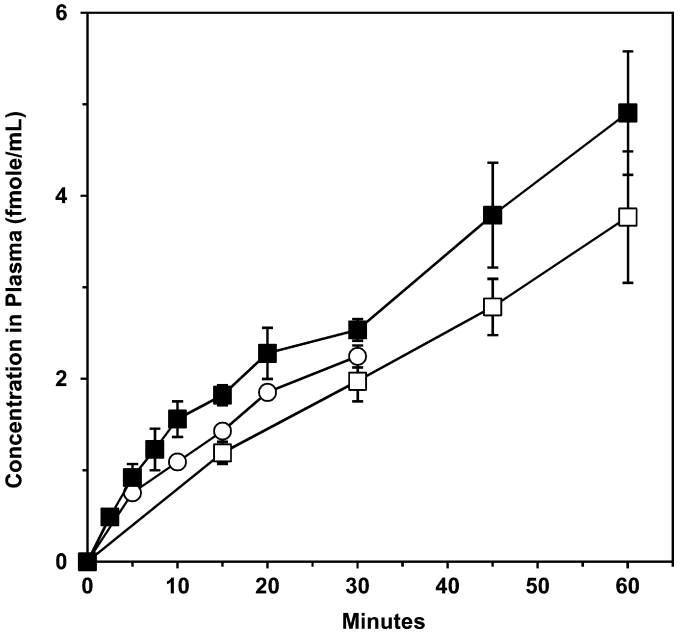

Fig. 2. Typical plasma concentration–time profiles of 5FU during intravenous infusion or nasal perfusions. Brain tissue (cerebral cortex) samples were taken at the end of the infusion or perfusion period. To obtain a representative plasma concentration–time course, blood samples were taken at 5, 10, 15, 20, and 30 min in the 30 min i.v. infusion experiment, at 15, 30, 45, and 60 min in the 60 min nasal perfusion(−AZA) experiment, and at 2.5, 5, 7.5, 10, 15, 20, 30, 45, and 60 min in the 60 min nasal perfusion(+AZA) experiment. Each point represents mean ± S.E. ($n = 4$). *Key*: ○; i.v. infusion, □; perfusion(−AZA), ■; perfusion(+AZA).

Table 1
Area under the concentration–time curve up to designated time and the concentration in the cerebral cortex following intravenous infusion and nasal perfusions.

Time (min)	I.v. infusion		Perfusion(−AZA)		Perfusion(+AZA)	
	AUC (fmol min/mL)	Concentration in the cerebral cortex (fmol/g tissue)	AUC (fmol min/mL)	Concentration in the cerebral cortex (fmol/g tissue)	AUC (fmol min/mL)	Concentration in the cerebral cortex (fmol/g tissue)
5	3.4 ± 0.4	0.022 ± 0.001	2.0 ± 0.2	0.053 ± 0.005	n.d.	n.d.
10	7.9 ± 0.6	0.048 ± 0.003	5.0 ± 0.5	0.067 ± 0.009	7.5 ± 0.3	0.115 ± 0.019
15	15.5 ± 1.5	0.090 ± 0.010	10.7 ± 1.2	0.114 ± 0.016	n.d.	n.d.
20	17.5 ± 0.6	0.097 ± 0.002	17.7 ± 2.2	0.193 ± 0.019	20.1 ± 2.8	0.229 ± 0.027
30	41.5 ± 1.6	0.264 ± 0.017	37.6 ± 4.1	0.302 ± 0.035	44.0 ± 1.3	0.488 ± 0.065
45	n.d.	n.d.	n.d.	n.d.	94.8 ± 10.8	1.370 ± 0.079
60	n.d.	n.d.	117.5 ± 14.2	1.039 ± 0.061	170.8 ± 20.7	2.122 ± 0.485

Data represent mean ± SEM of 3–4 rats. AUC: area under the plasma concentration–time curve; n.d.: not determined.

The change of the concentration in the nasal perfusion fluid is indicated in Fig. 4. The disappearance of 5FU from the perfusate was less than 10% even 60 min after starting the nasal perfusion. Therefore, the concentration in the nasal perfusion fluid was almost constant during the experiment.

3.3. Relationship between the plasma AUC and the concentration in the cerebral cortex

In order to quantitatively examine the nasal delivery of 5FU to the brain (Ohno et al., 1978), the brain 5FU concentrations at discrete time points were plotted against the corresponding plasma AUC (from time zero to the time at which the brain 5FU concentration was measured) to evaluate the brain uptake clearance (Table 1 and Fig. 5). Significant correlations were observed between plasma AUC and brain concentrations at corresponding time points in each group. Correlation coefficients were 0.983 for i.v. infusion, 0.988 for nasal perfusion(−AZA) and 0.928 for nasal perfusion(+AZA). Slopes of the lines, which represent the apparent brain uptake clearance, CL_{up}, were determined by linear regression analysis (Table 2). CL_{up} for the nasal perfusion(−AZA) and nasal perfusion(+AZA) showed 40% and 104% increases, respectively, compared with that of the i.v. infusion. The difference of CL_{up} between nasal perfusions are significant (Table 2). These findings clearly indicate that nasal drug delivery to the brain was significantly improved in the presence of AZA.

Since CL_{up} is based upon the assumption that the degree of uptake is proportional to the concentration difference between the brain and the blood as shown in the experimental section, it gives the same value irrespective of both the application site and the dose if the drug is passively taken up by the brain only from systemic circulation. Consequently, a large difference in nasal and intravenous doses has no influence on CL_{up}. At the same time, the difference in CL_{up} between nasal and intravenous administrations represents

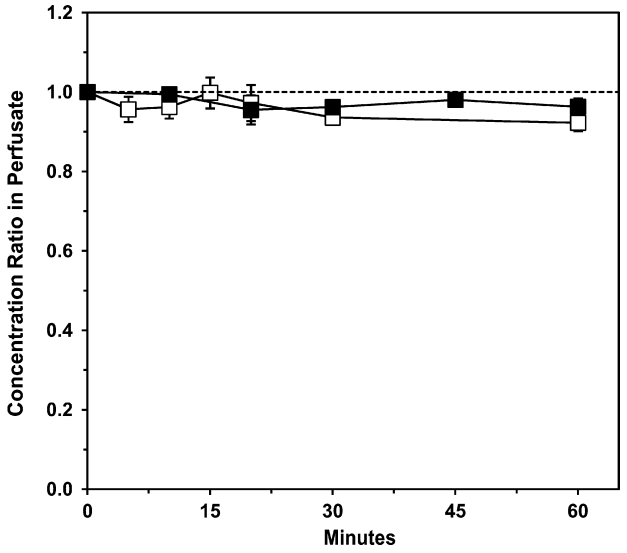

Fig. 4. Change of 5FU concentration in the nasal perfusion fluid as a function of time. Perfusate samples (10 μL) were taken at various times to determine the change in the 5FU concentration in the nasal perfusion fluid. The data represent % of initial concentration. Each point is the mean with S.E. (n = 3–4). *Key*: □; perfusion(−AZA), ■; perfusion(+AZA).

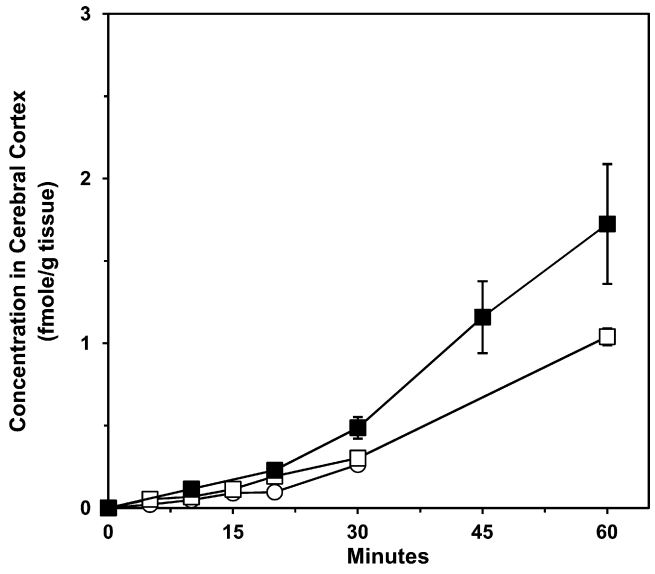

Fig. 3. Concentration–time profiles of 5FU in the cerebral cortex during intravenous infusion or nasal perfusions. For the i.v. infusion group, brain tissue (cerebral cortex) samples were taken at the end of the following infusion periods: 5 (n = 3), 10 (n = 4), 15 (n = 3), 20 (n = 3), or 30 (n = 4) min, and the total volumes of dosing solution infused were 75, 150, 225, 300, and 450 μL, respectively. For the nasal perfusion(−AZA) and perfusion(+AZA) groups, respectively, brain tissue (cerebral cortex) samples were taken at the end of the following perfusion periods: 5 (n = 3), 10 (n = 3), 15 (n = 4), 20 (n = 4), 30 (n = 4), or 60 (n = 4) min and 10 (n = 4), 20 (n = 4), 30 (n = 4), 45 (n = 3), or 60 (n = 4) min. Each point represents mean ± S.E. *Key*: ○; i.v. infusion, □; perfusion(−AZA), ■; perfusion(+AZA).

Table 2
Apparent brain uptake clearance of 5FU following intravenous infusion and nasal perfusions.

	CL_{up} (μL/min/g tissue)	95% confidence intervals
I.v. infusion	6.20	5.80 < S < 6.59
Perfusion(−AZA)	8.65	8.07 < S < 9.23
Perfusion(+AZA)	12.62*	10.8 < S < 14.5

Student's t-test was applied on the analysis on the brain uptake clearance of nasal perfusion(−AZA) and nasal perfusion(+AZA).
* $p < 0.001$.

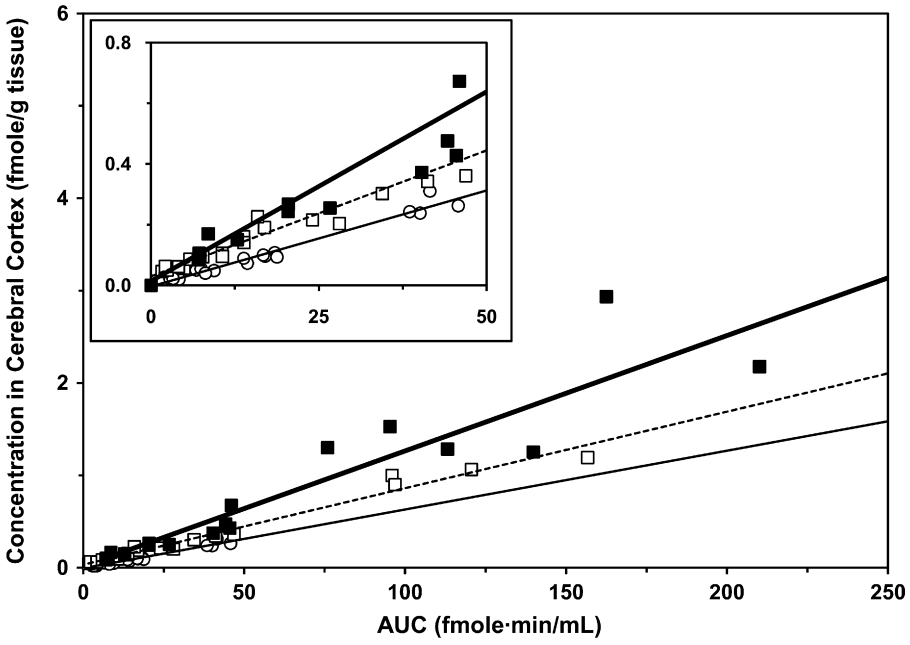

Fig. 5. The relationship between the area under the plasma concentration–time curve (*x*-axis) and the concentration in the cerebral cortex (*y*-axis) after intravenous infusion and nasal perfusions. Inset figure shows data points of which AUC values are smaller than 50 fmol min/mL. Each point represents the data from a single animal. *Key*: ○; i.v. infusion, □; perfusion(−AZA), ■; perfusion(+AZA). Kruskal–Wallis non-parametric test were applied on brain uptake clearances derived from individual rats. The results indicated the significant difference among three groups ($p < 0.001$). Tukey ANOVA multiple comparison test was also done on the individual brain uptake clearances. According to the test result, differences between all the combinations of the groups were statistically significant ($p < 0.01$).

the degree of transport other than from systemic circulation, that is, from the nasal cavity to the brain through the CSF (Sakane et al., 1999).

4. Conclusions

Intravenous AZA enhanced the concentration of nasally applied 5FU that reached the CSF by 200–300% compared to nasal perfusion(−AZA). The drug concentration–time profiles in the plasma and the brain indicate the direct nose-to-brain transport of 5FU. CL_{up} of nasal perfusion(+AZA) and nasal perfusion(−AZA), which were determined from the relationship between the plasma AUC and the brain concentration of 5FU, were 200% and 140% of that after i.v. infusion, respectively. The difference in CL_{up} between nasal perfusion(−AZA) and nasal perfusion(+AZA) was twice that between i.v. infusion and nasal perfusion(−AZA). AZA enhances drug delivery to the brain through the nose-to-brain pathway by decreasing the CSF secretion in the choroid plexus and thus increasing the concentration of the nasally applied drug in the CSF.

Acknowledgements

This work was supported in part by grants from the Uehara Memorial Foundation and the Ministry of Education, Culture, Sciences and Technology, Japan. Professor N. Yata, T. Nadai and H. Sezaki are gratefully acknowledged for valuable advices and comments.

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