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Permeability of a Capsaicin Derivative, [^{14}C]DA-5018 to Blood-Brain Barrier Corrected with HPLC Method

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In the present work, the transport mechanism of a capsaicin derivative, DA-5018, through blood-brain barrier (BBB) has been investigated to evaluate the feasibility of potential drug development. The result of pharmacokinetic parameters obtained from the intravenous injection of plasma volume marker, [^{3}H]RSA and [^{14}C]DA-5018, indicated that both AUC, area under the plasma concentration curve and V_D, volume of distribution in brain of [^{3}H]RSA agreed with those reported (1620$\pm$10 percentage injected dose minute per milliliter (%IDmin/ml) and 12.0$\pm$0.1 μl/g, respectively). Elimination half-life and AUC of [^{14}C]DA-5018 is corrected by the HPLC analysis, 19.6$\pm$1.2 min and 7.69$\pm$0.85% IDmin/ml, respectively. The metabolic rate of [^{14}C]DA-5018 was very rapid. The blood-brain barrier permeability surface area (PS) product of [^{14}C]DA-5018 was calculated to be 0.24$\pm$0.05 μl/min/g. The result of internal carotid artery perfusion and capillary depletion suggested that [^{14}C]DA-5018 pass through BBB with the time increasingly. Investigation of transport mechanism of [^{14}C]DA-5018 using agonist and antagonist suggested that vanilloid (capsaicin) receptor did not exist in the BBB, and nutrient carrier system in the BBB has no effect on the transport of DA-5018. In conclusion, despite the fact that penetration of DA-5018 through BBB is significant, the intact drug found in the brain tissue is small because of a rapid metabolism. Therefore, for the central analgesic effect of DA-5018, the method to increase the metabolic stability in plasma and the brain permeability should be considered.

Key words : Blood-brain barrier, Capsaicin, DA-5018, Vanilloid receptor

INTRODUCTION

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), a pungent ingredient of red pepper, of which the structure comprises amide as basic unit with hydrophilic cyclic ring and hydrophobic chain shows an analgesic effect (Lundberg and Saria, 1987). Capsaicin is specific for primary afferent sensory nerves which consist of unmyelinated C-fiber and unmyelinated Aδ-fiber, and effects on the synthesis, storage, transport and release of a neurotransmitter, substance P (Hokfelt *et al.*, 1980). Exposure to the capsaicin induces initial excitatory effect and subsequently maintains a desensitization of long-lasting refractory state for chemical, mechanical and thermal stimuli (Saria *et al.*, 1988). The analgesic effect of capsaicin is not only superior to typical analgesic drug but also different from prostaglandin-mediated and opiate receptor-mediated analgesic effect (LaHann, 1983). However, this has been used only as topical agent due to the large side effect and toxicity (Holzer, 1991).

Recently, DA-5018 [N-{3-(3,4-dimethylphenyl)}-4-(2-aminoethoxy)-3-methoxyphenyl acetamide)], a non-narcotic analgesic drug, was reported (Park *et al.*, 1993). DA-5018 was first synthesized by Park *et al.* (Park *et al.*, 1993) at Korea Research Institute of Chemical Technology, and have been developed as analgesic drug for neuralgia (Bernstein *et al.*, 1991), diabetic neuropathy (Ross and Varipapa, 1989) and arthritis (McCarthy and McCarthy, 1992). DA-5018 which is non-narcotic and without mutagenicity, was considered to have excellent analgesic effect with much weaker toxicity than capsaicin (Lee *et al.*, 1994).

Drugs effect on the central nervous system, especially on the brain, must penetrate through the brain capillary endothelial wall, which makes up the blood-brain barrier (BBB). Therefore, it is very important to measure the extent of transport through the BBB. Capsaicin was suggested to transport through BBB because of high lipophilicity. However, the measurement of the transport appears unreliable (Reid and McCulloch, 1987).

The structural requirement to have a function like capsaicin suggested that capsaicin interacted on a specific receptor in order to show its analgesic effect. From the measurement of transport of a capsaicin analog, capsazepine, vanilloid (capsaicin) receptor was found to be present in rat or pig's spinal cord and dorsal

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root ganglion. A binding to this receptor was reported to show tissue, species and pharmacologic specificity (Szallasi and Goso, 1993). However, it is not known whether DA-5018 shows similar characteristics.

Therefore, in the present work, the brain uptake of a newly synthesized capsaicin derivative, DA-5018 was measured with HPLC method. In addition, potential inhibitory effect of capsaicin analogs on the transport of DA-5018 to elucidate the transport mechansim across BBB.

MATERIALS AND METHODS

Materials

N-succinimidyl [2, 3-^{3}H]-propionate ([^{3}H]NSP), 100 Ci/mmol, was purchased from Amersham Corp. (Arlington Heights, IL). Capsaicin and capsazepine were supplied by Korea Research Institute of Chemical Technology. DA-5018 and [^{14}C]DA-5018 (specific activity, 45 mCi/mmol) were provided by Dong-A Pharmaceutical Co. (Korea). DA-5018, [^{14}C]DA-5018, capsaicin and capsazepine were solubilized in dimethyl sulfoxide. Rat serum albumin (RSA, Fr. V), bovine serum albumin (BSA, Fr. V) and dextran were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of reagent grade. Male Sprague-Dawley rats (200~300 g) were purchased from Samyouk Co. (Korea). HPLC system consisted of Model 7725I injector (Rheodyne), and reverse-phase column (CLC-ODS, 5 μm, 4.6 mm I.D.×25 cm, Shimadzu, Japan), and guard column (CLC G-ODS, 5 μm, 4 mm I.D.×10 cm, Shimadzu, Japan) and fluorescence detector (excitation wavelength 270 nm. emission wavelength 330 nm).

Preparation of [^{3}H]RSA

Native RSA (nRSA) was tritiated by adding 1 mCi of [^{3}H]NSP (subsequent to evaporation of toluene diluent) to 100 μL of nRSA solution (0.1 M-NaBorate, pH 8.5), followed by capping and shaking at 4°C for 45 min. Glycine (0.1 M) was added to above solution and reacted for 30 min with an occasional stirring. Unlabeled protein was separated from [^{3}H]RSA by a Sephadex G-25 column (Pharmacia Biotech Co.) with 0.01 M phosphate buffered saline (0.01 M Na_2HPO_4/0.15 M NaCl containing 0.1% BSA, pH 7.4). Trichloracetic acid (TCA) precipitability was 99%. The result of the specific activity of [^{3}H]RSA (specific activity, 51 Ci/mmol) indicated that amino acid residue of RSA was ^{3}H-labeled.

Intravenous injection technique and pharmacokinetics

Male Sprague-Dawley rats were anesthetized with ketamine (100 mg/kg) and xylazine (2 mg/kg) by intramuscular injection. 200 μL Of Ringer's HEPES buffer (10 mM HEPES, 14 mM NaCl, 4 mM KCl and 2.8 mM $CaCl_2$, pH 7.4) with 0.1% nRSA containing 5 μCi of [^{14}C]DA-5018 and 15 μCi of [^{3}H]RSA were injected into the femoral vein via a PE-50 tube. Blood sample (0.3 ml) were collected from the femoral artery cannulae 0.25, 1, 2, 5, 15, 30 and 60 min after injection. After each blood sampling, the blood was replaced with the same volume of normal saline (containing 100 unit/ml of heparin). The plasma was separated by centrifugation (2,500 rpm, 4°C, 10 min). At 60 min after intravenous injection, rats were sacrificed, and the brain and the other organs (liver, kidney, lung and heart) were removed, homogenized and solubilized in Soluene 350 (Packard Instrument Co., Downers Grove, IL) for ^{3}H/^{14}C double isotope liquid scintillation counting.

Pharmacokinetic parameters were calculated by fitting plasma radioactivity data to a biexponential equation, i.e.

$$A(t)=A_1\, e^{-k_1 t}+A_2 e^{-k_2 t}$$

where A(t) is the percentage of injected dose (ID) per milliliter. The equation was fit to plasma data using a derivative free nonlinear regression analysis (PARBMDP, Biomedical Computer P Series Program developed at UCLA Health Sciences Computing Facilities). The data were weighed using weight=1/(concentration)^2, where concentration either disintegration per minute (dpm) per microliter (μL) or %ID per milliliter. The organ volume of distribution (V_D) of [^{14}C]DA-5018 at 60 min after intravenous injection was determined from ratio of dpm/g tissue divided by the dpm/μL of the corresponding terminal plasma. The plasma clearance (CL), the steady state volume of distribution (Vdss), the area under the plasma concentration curve (AUC) and the mean residence time (MRT) were calculated from the pharmacokinetic parameters, as described by Gibaldi and Perrier (Gibaldi and Perrier, 1982).

$$CL=\frac{D}{AUC}$$

$$AUC=\frac{A_1}{k_1}+\frac{A_2}{k_2}$$

$$MRT=\frac{A_1/k_1^2+A_2/k_2^2}{AUC}$$

$$Vdss=\frac{D(A_1/k_1^2+A_2/k_2^2)}{AUC^2}$$

where D is injected dose. The BBB permeability-surface area (PS) product of the [^{14}C]DA-5018 was determined as follows:

$$PS=\frac{[V_D-V_0]C_p(T)}{\int_0^T C_p(t)dt}$$

where Cp(T) is brain DA-5018 concentration at time T, and Vo is the organ volume of distribution of [^{3}H] RSA plasma volume marker.

The brain delivery or %ID/g brain was calculated as follows:

$$\%ID/g(t)=PS\times AUC(t)$$

The metabolic stability of [^{14}C]DA-5018 in plasma was examined by HPLC analysis of plasma radioactivity. At 60 min after intravenous injection, 50 μL of serum from three rats was pooled and [^{14}C]DA-5018 extracted by the previously reported method (Shim *et al.*, 1997). 100 μL Was injected onto the HPLC column at a flow rate of 1.0 ml/min for 30 min. Column fractions (1 ml) were counted for ^{14}C-radioactivity.

Internal carotid artery perfusion/capillary depletion method

In order to determine whether[^{14}C]DA-5018 undergoes transcytosis through the BBB, *in situ* internal carotid artery perfusion technique was used in ketamine-anesthetized rats as described previously (Takasato *et al.*, 1984). The perfusate consisted of Krebs-Henseleit buffer (119 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM D-glucose and 10 g/dl of BSA, pH 7.4) containing [^{14}C]DA-5018 (1 μCi) and [^{3}H]RSA (10 μCi), and infused at a rate of 4 ml/min for 0.75, 15 and 30 sec. At the end of the perfusion, the animal was killed by decapitation, and the brain was homogenized in 40% dextran and separated the vascular pellet from the postvascular supernatant as described previously (Triguero *et al.*, 1990). The brain volume of distribution (V_D) of the [^{14}C] DA-5018 was calculated from the ratio of dpm/g of tissue divided by the dpm/perfusate in the total brain homogenate, postvascular supernatant and vascular pellet. The postvascular supernatant V_D is a measure of the transcytosis of [^{14}C]DA-5018 through the brain vascular endothelium and into the brain interstitial space.

[^{14}C]DA-5018 transport through the BBB in the presence of structural analogs

To examine if vanilloid (capsaicin) receptor is present at the BBB, and if the transport mechanism of a capsaicin derivative, [^{14}C]DA-5018, is mediated by vanilloid (capsaicin) receptor, the *in situ* internal carotid artery perfusion technique of Takasato *et al.* was used (Takasato *et al.*, 1984). The perfusion solution consisted of Krebs-Henseleit buffer containing [^{14}C]DA-5018 (1 μCi) and various concentrations of unlabeled agonist and antagonist. After the 15 sec perfusion, the animal was decapitated, and the brain was removed and solubilized for measurement of total brain ^{14}C-radioactivity using liquid scintillation counting. Also,

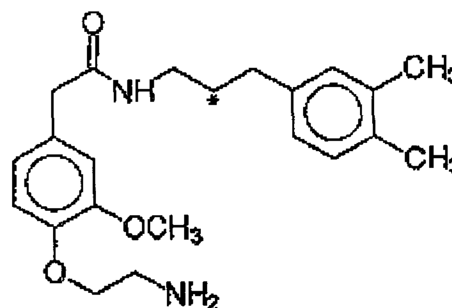

Fig. 1. Structure of DA-5018. The position of ^{14}C-label is marked with an asterisk.

D-glucose and a neutral amino acid, L-phenylalanine were co-injected with the same method to investigate that [^{14}C]DA-5018 is transported by mediation of the nutrient carrier systems which are present at the BBB.

RESULTS

%ID/ml of [^{14}C]DA-5018, corrected [^{14}C]DA-5018 by HPLC analysis and [^{3}H]RSA in peripheral plasma at 60 min after intravenous injection are shown in Fig. 2. Pharmacokinetic parameters for [^{14}C]DA-5018, corrected [^{14}C]DA-5018 by HPLC analysis and [^{3}H]RSA are listed in Table I. These data demonstrated that the [^{3}H]RSA, plasma volume marker, cleared from the plasma compartment monoexponentially with a elimination half-life of 110±1 min. In contrast to [^{3}H]RSA, [^{14}C]DA-5018 distributed with a half-life of 0.63±0.13 min and then was quickly eliminated from the plasma compartment biexponentially. CL of [^{14}C]DA-5018 was

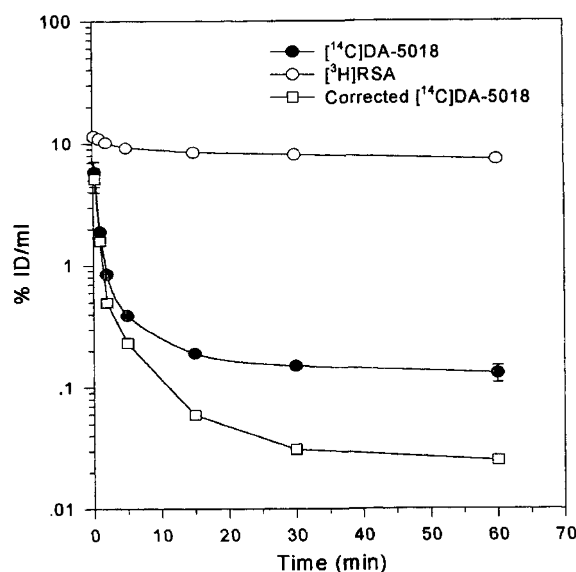

Fig. 2. The percentage injected dose (ID) per milliliter of plasma-time profile of [^{3}H]RSA, [^{14}C]DA-5018 and corrected [^{14}C]DA-5018 by HPLC analysis, after intravenous injection of the injectate for up to 60 min. Data are expressed as mean± S.E. (n=3).

over 68-fold faster than that of [^{3}H]RSA. This was reflected in the decreased AUC of [^{14}C]DA-5018 compared with [^{3}H]RSA (24.0±3.3 *vs.* 1620±10 %IDmin/ml). The organ clearance was small for organs such as brain or heart, was intermediate for kidney and was prominent for liver (Table II). Thus, [^{14}C]DA-5018 mainly distributed into the liver within 60 min following intravenous injection (3.02±0.90 %ID/g) as listed in Table II.

The chromatograms radioactivity of [^{14}C]DA-5018 in rat plasma are shown in Fig. 3. When 100 μl of standard solution of DA-5018, prepared by reported extraction method, was injected onto the column (Shim *et al.*, 1997), a intact peak of unlabeled DA-5018 appeared at 14 min and for 100 μl of standard solution of [^{14}C]DA-5018 at 14~17 min. As shown in Fig. 3, HPLC chromatograms of plasma obtained 0.25 and 60 min after intravenous injection of [^{14}C]DA-5018 represented a intact peak of [^{14}C]DA-5018 at 13~16 min, but the amount at 0.25 min was 89%. The amount of intact drug further decreased with the time, yielding the intact drug remaining at 60 min was 12% of the initial amount. The peak guessed that metabolite of [^{14}C]DA-5018 appeared at 1~5 min. HPLC corrected value of the %ID/ml in plasma of [^{14}C]DA-5018 within 60 min following intravenous injection is shown in Fig. 2 and Table III. As given in Table I, intact [^{14}C]DA-5018 was removed more rapidly than [^{14}C]DA-5018 with a elimination half-life of 19.6±1.2 min. The CL of intact [^{14}C]DA-5018 was also faster by 3-fold and the AUC of intact [^{14}C]DA-5018 was smaller. Relative recovery of [^{14}C]DA-5018 to standard of rat plasma sample spiked with extraction method was more than 90%.

Potential errors created by metabolism and by binding or endocytosis to the brain capillaries can be eliminated by intracarotid artery perfusion/capillary depletion method (ICAP/CDM) since it separates microvascular pellet from postvascular supernatant by density of dextran (Triguero *et al.*, 1990). Thus, this method was utilized to accurately determine the extent of DA-5018 penetration into the brain. The time course of [^{14}C]DA-5018 transport into the brain was examined by varying the length of the perfusion time from 7.5 sec to 30 sec. The brain homogenate V_D of [^{3}H]RSA and [^{14}C]DA-5018 at perfusion times is shown in Fig. 4. V_D of total homogenate, postvascular supernatant and vascular pellet for [^{3}H]RSA during 15 sec perfusion were 10.1±1.5, 9.4±1.7 and 0.020±0.003 μl/g, respectively (data not shown), and was comparable to previous estimates (Pardridge *et al.*, 1994). Approximately 90% of [^{14}C]DA-5018 entered from the brain capillary endothelial cells to the brain parenchymal cell as postvascular supernatant V_D was 85.1±11.4 μl/g and the vascular pellet V_D was 0.38±0.04 μl/g for [^{14}C]DA-5018 at 15 sec of perfusion. After [^{14}C]DA-5018 and [^{3}H]RSA were injected by ICAP/CDM, V_D of brain homogenate of [^{3}H]RSA was constant, whereas V_D of [^{14}C]DA-5018 showed no dose-dependency. Thus, the result of ICAP/CDM indicated the [^{14}C]DA-5018 passively penetrates through the BBB.

Brain V_D of [^{14}C]DA-5018 in the presence of agonists and antagonists at 15 sec by ICAP is given in Table V. First, when the mixture of 0.02 mM of [^{14}C]DA-5018 was co-injected with either 0.2 mM or 1 mM unlabeled DA-5018, the V_D value of [^{14}C]DA-5018 was increased significantly ($p<0.05$) relative to that of [^{14}C]DA-5018 alone. In order to investigate if [^{14}C]DA-

Table II. Organ clearance and delivery of [^{14}C]DA-5018

Organ	[^{3}H]RSA V_0 (μl/g)	[^{14}C]DA-5018	
		Organ clearance (μl/min/g)	Uptake (%ID/g)
Brain	12.0±0.1	0.94±0.24	0.015±0.004
Liver	110±1	189±57	3.02±0.90
Kidney	177±4	64.7±22.0	0.85±0.23
Lung	183±16	49.3±9.6	0.79±0.15
Heart	75.4±7.4	21.6±5.1	0.35±0.08

Mean±S.E. (n=3). Measurements made 60 min after intravenous injection. V_0; plasma volume of distribution.

Table I. Pharmacokinetic parameters for [^{3}H]RSA, [^{14}C]DA-5018 and corrected [^{14}C]DA-5018 by HPLC analysis

Parameters	[^{3}H]RSA	[^{14}C]DA-5018	Corrected [^{14}C]DA-5018
A_1 (%ID/ml)	10.2±0.2	6.62±1.73	6.40±1.72
A_2 (%ID/ml)	-	0.26±0.01	0.11±0.07
k_1 (min^{-1})	0.0061±0.0010	1.19±0.20	1.31±0.21
k_2 (min^{-1})	-	0.015±0.002	0.036±0.002
$t^1_{1/2}$ (min)	110±1	0.63±0.13	0.56±0.10
$t^2_{1/2}$ (min)	-	48.6±6.2	19.6±1.2
AUC (%IDmin/ml)	1620±10	24.0±3.3	7.69±0.85
V_{dss} (ml/kg)	-	889±14	603±73
CLss (ml/min/kg)	0.25±0.01	17.0±2.6	52.0±6.4
MRT (min)	-	54.6±7.2	11.6±0.1

Plasma measurements were extended up to 60 min for [^{3}H]RSA, [^{14}C]DA-5018 and corrected [^{14}C]DA-5018 by HPLC analysis. Mean±S.E. (n=3 rats).

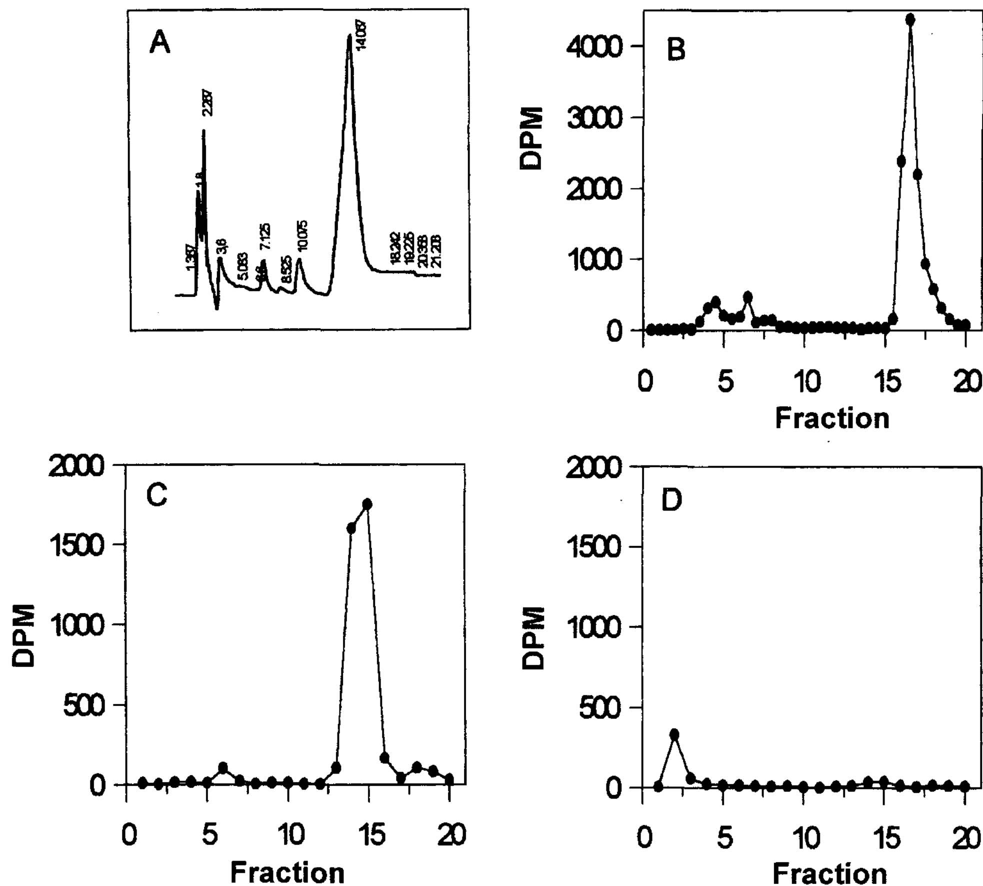

Fig. 3. (A) Chromatograms of DA-5018 extracts (10 μl/ml) from control rat plasma. The column (ODS, 5 μm, 4.6 mm×25 cm) was eluted at flow rate 1.0 ml/min. Injectate size was 100 μl. Mobile phase - 5 mM methanesulfonic acid/10 mM NaH_2PO_4 (pH 2.5)-acetonitrile, 70:30 (v/v), Detection-Fluorescence detector (excitation wavelength 270 nm, emission wavelength 330 nm). (B) Elution profile of HPLC of standard [^{14}C]DA-5018 before injection of tracer into rats. The column was eluted at 0.5 ml/min and 0.5 ml fractions were collected and counted for ^{14}C-radioactivity (DPM=disintegration per minute). HPLC chromatogram of plasma obtained 0.25 min (C) and 60 min (D) after intravenous injection of [^{14}C]DA-5018. Elution of plasma were pooled from three rats. Each fraction=1 ml.

Table III. The data of HPLC analysis of [^{14}C]DA-5018 in plasma

Time (min)	%ID/ml	Intact (%)	Corrected %ID/ml
0.25	5.80±1.33	89	5.14±1.18
1	1.90±0.11	83	1.59±0.10
2	0.85±0.02	59	0.50±0.01
5	0.39±0.01	59	0.23±0.01
15	0.19±0.01	30	0.059±0.001
30	0.15±0.01	20	0.031±0.003
60	0.13±0.02	12	0.025±0.002

HPLC corrected value for the percentage injected dose (ID) per milliliter of plasma-time profile of [^{14}C]DA-5018, after intravenous injection. Data are expressed as mean ±S.E. (n=3).

5018 transport through the identical receptor, capsaicin, a vanilloid (capsaicin) receptor agonist, which has the structure similarity to [^{14}C]DA-5018, was co-administered intravenously at 0.5 mM along with [^{14}C]DA-5018, the V_D of [^{14}C]DA-5018 was not significantly different from V_D of [^{14}C]DA-5018 alone. Higher concentrations of agonist and antagonist could not be used, owing to the relative insolubility in dimethyl sulfoxide. The result of ICAP of 0.02 mM of [^{14}C]DA-5018 and 0.2 mM of capsazepine, a competitive inhibitor of vanilloid (capsaicin) receptor, does not show a significant decrease compared to injection of [^{14}C]DA-5018 alone. These results reflected that [^{14}C]DA-5018 was not transported through the BBB by vanilloid (capsaicin) receptor mediated mechanism.

Co-injection with D-glucose and L-phenylalnine, 10 mM and 12.5 mM, respectively, did not also inhibit transport of [^{14}C]DA-5018. Therefore, the transport mechanism of [^{14}C]DA-5018 has no relation with glucose and neutral amino acid transport system.

DISCUSSION

In this work, an accurate brain uptake of capsaicin

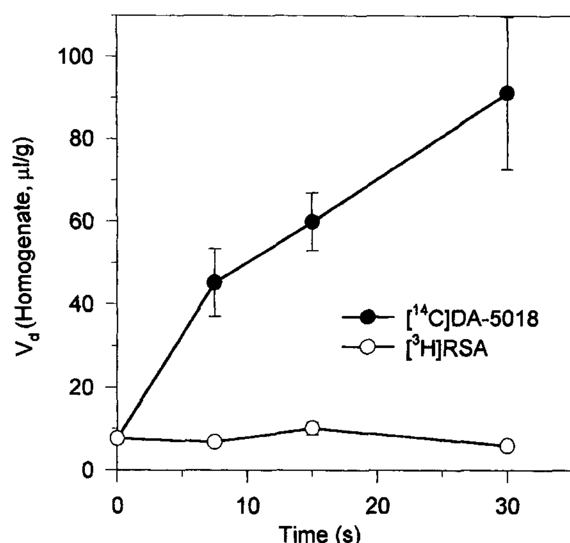

Fig. 4. Brain distribution volume (V_d) of [^{3}H]RSA (○) and [^{14}C]DA-5018 (●) is plotted against time determined by the internal carotid artery perfusion followed by capillary depletion method. Data are expressed as mean ±S.E. (n=3). The V_d of RSA represents the blood volume.

derivative, DA-5018 was measured. The degree of inhibition for the transport in the presence of agonist and antagonist was also determined to elucidate if the transport of DA-5018 through BBB was mediated by vanilloid (capsaicin) receptor.

In this study, we measured the extent of BBB permeability of the capsaicin derivative, DA-5018. DA-5018 was solubilized in dimethyl sulfoxide not exceeding 0.15%.

In 1982, Broadwell *et al.* reported that, in mice, dimethyl sulfoxide changes BBB reversibly so that the brain uptake of anticancer drug and enzyme like horseradish peroxidase increased (Broadwell *et al.*, 1982). Since then, the use of dimethyl sulfoxide and solvents like ethanol which destroy BBB integrity is regulated because the abnormality like neurotoxicity, serum osmolarity are accompanied by the use of the solvents. However, these disorders by dimethyl sulfoxide occur due to a large dose and small dose had no appreciable effect on the integrity of BBB (Michael Scheld, 1989). Therefore, the increase of the transport through BBB of [^{14}C]DA-5018 with time was not affected by the presence of dimethyl sulfoxide.

The brain uptake parameters of [^{14}C]DA-5018 and [^{3}H]morphine are compared in Table VI. After the correction for the metabolites, AUC of [^{14}C]DA-5018 was much smaller than [^{3}H]morphine. The corrected brain uptake expressed in %ID/g of [^{14}C]DA-5018 was 5.4 times lower than that of [^{3}H]morphine. The difference before and after HPLC correction is due to the fact that PS value of [^{14}C]DA-5018 was 44 times lower than that of [^{3}H]morphine. The brain uptake of

Table IV. Internal carotid artery perfusion/Capillary depletion method analysis of [^{14}C]DA-5018 distribution in brain

Time (s)	V_D (µl/g)		
	Homogenate	Supernatant	Pellet
7.5	45.2±8.2	36.0±2.7	0.28±0.05
15	59.9±7.0	35.7±4.6	0.22±0.06
30	91.2±18.6	85.1±11.4	0.38±0.04

Brain volume of distribution (V_D) of [^{14}C]DA-5018 following 7.5 sec, 15 sec, and 30 sec internal carotid artery perfusion followed by capillary depletion method of brain homogenate. Data are corrected for the V_0 of [^{3}H]RSA at each time. Data are expressed as mean±S.E. (n=3).

[^{14}C]DA-5018 was smaller than 0.02 %ID/g though molecular weight of [^{14}C]DA-5018 was smaller than that of dermorphin derivative, DALDA ([D-Arg^2, Lys^4] dermorphin analogue) which shows metabolic stability (Samii *et al.*, 1994). Thus, [^{14}C]DA-5018 showed similar analgesic effect to [^{3}H]morphine but the amount delivered to brain is so small that the method to increase the delivery to brain should be devised to enhance a central function of DA-5018.

The results of HPLC analysis for the stability of [^{14}C] DA-5018 in plasma after the intravenous injection showed that 85% [^{14}C]DA-5018 was intact after 0.25 min while only 12% [^{14}C]DA-5018 remained unchanged after 60 min, suggesting a rapid metabolism for [^{14}C]DA-5018. (Fig. 3 and Table III) Though the metabolite of DA-5018 was not clarified, the formation of DA-5018 metabolite was referred from the experiment. Also, the metabolism between 15 min and 60 min was found to be much rapid based on comparison of a corrected % ID/ml value in peripheral plasma and of the intact [^{14}C]DA-5018 concentration in 60 min plasma samples. Similar retention time of unlabeled DA-5018 and [^{14}C] DA-5018 indicated the same materials (Fig. 2).

As explained above, vanilloid (capsaicin) receptor is known to exist in the central (spinal cord and dorsal root ganglion) and peripheral (bladder, airway) etc. (Szallasi and Goso, 1993). In the present study, the presence of this receptor in BBB was examined. Brain distribution volume (V_D) did not decrease upon ICAP injection of unlabeled DA-5018 and capsaicin as agonists about 50 times and 20 times, respectively. Further, the co-injection of DA-5018 increased V_D of [^{14}C]DA-5018 significantly (Table V). This indicated that the transport of [^{14}C]DA-5018 through BBB was not saturated in the presence of unlabeled DA-5018. Capsazepine, a competitive inhibitor of vanilloid (capsaicin) receptor, did not decrease V_D upon the co-injection with [^{14}C]DA-5018. Thus, capsazepine did not inhibit the transport of [^{14}C]DA-5018 through BBB. The concentration of capsazepine can not increase above 1 mM due to the solubility limit.

The above results suggested that the transport mech-

Table V. Volume distribution in brain of [^{14}C]DA-5018 with additives at 15 sec by internal carotid artery perfusion method

Additive	Conc.	V_D (μl/g)
[^{14}C]DA-5018[a]	0.02 mM	90.5±6.1
DA-5018	0.2 mM	124±7[b]
	1 mM	132±12[b]
capsaicin	0.5 mM	121±17
capsazepine	0.2 mM	95.7±2.5
L-phenylalanine	12.5 mM	103±2
D-glucose	10 mM	96.5±8.4

[a]Volume percentage of dimethyl sulfoxide in the solution was 0.15%.
[b]$p<0.05$.

Table VI. The brain uptake parameters and lipid solubility for [^{14}C]DA-5018, corrected [^{14}C]DA-5018 by HPLC analysis and [^{3}H]Morphine at 60 min after an i.v. injection

Parameters	[^{14}C]DA-5018	Corrected [^{14}C]DA-5018	[^{3}H]Morphine*
PS (μl/min/kd)	0.91±0.20	0.24±0.05	8.03±0.28
%ID/g	0.015±0.004	0.0018±0.0004	0.081±0.001
AUC_{0-60}	16.0±1.3	7.33±0.79	10.1±0.4
lipid solubility (P)	68.8	68.8	0.21

*Data were taken from ref. 27.
P: partition coefficient.
Mean±S.E. (n=3 rats).

anism of [^{14}C]DA-5018 through BBB did not occur via vanilliod (capsaicin) receptor, indicating that vaniloid (capsaicin) receptor may not present in BBB.

The specific carrier transport systems are known to be present in BBB to deliver L-dopa, α-methyldopa etc. to brain (Nutt *et al.*, 1984; Sved *et al.*, 1980). Since glucose is an energy source in brain, its transport mechanism across BBB has been investigated extensively (Pardridge, 1983). It has been demonstrated that glucose uptake into the brain is saturable, energy-dependent, sodium-independent and stereospecific. The transport was suppressed with phlorein and cytochalasin (Betz *et al.*, 1979). A large neutral amino acid such as L-phenylalanine passed normal BBB in bidirectional ways. At least 10 amino acids competes in BBB for the same carrier protein (Pardridge, 1983). Such transport having mutual competition is sodium-independent L-system suppressed with specific analogue and partly affected with hormone (Brust, 1986). A small amino acid such as glycine is known to show unidirectional transport from brain to plasma on the antiluminal membrane of brain capillary (Goldstein and Betz, 1986).

In this work, the effect of glucose transporter and neutral amino acids transporter were investigated under the assumption that [^{14}C]DA-5018 may pass BBB using such carrier transport systems. V_D of [^{14}C]DA-5018 did not change upon the co-injection with D-glucose and L-phenylalanine of sufficient concentration which is likely to inhibit the transport (Table V). Therefore, the transport of [^{14}C]DA-5018 through BBB seemed to have no relationship with glucose transporter and neutral amino acids transporter.

The transport of [^{14}C]DA-5018 through BBB seemed to occur via simple diffusion, consistent with the relatively high lipophilicity of the drug. The log P of DA-5018 was 1.84 at pH 7 which was larger than -0.69 of morphine (Wu *et al.*, 1997). V_D of [^{14}C]DA-5018 increased significantly most likely due to a large lipophilicity of unlabeled DA-5018 compared to capsaicin and capsazepine (Table VI).

As indicated above, DA-5018 passed through BBB with simple diffusion due to high lipophilicity but the amount delivered to brain was small after the peripheral injection. Therefore, the stability of DA-5018 should be increased and its transport should be improved in order to enhance the central analgesic effect.

From the present study, the following three conclusions can be drawn. The first is that a significantly rapid clearance and metabolism occurs after the systemic administration of [^{14}C]DA-5018. The second is that [^{14}C]DA-5018 had a high permeability through BBB and showed a linear dependence up to 30 sec. The last is that the vanilloid (capsaicin) receptor was not present in BBB and nutrient carrier system was not participated when [^{14}C]DA-5018 passed through BBB. Therefore, the capsaicin derivative, DA-5018 passed well through BBB with simple diffusion due to high lipophilicity. However, the amount of [^{14}C]DA-5018 delivered to brain was small due to rapid metabolism in plasma after a peripheral administration.

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