Novel modified method for injection into the cerebrospinal fluid *via* the cerebellomedullary cistern in mice

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A modified method of injection into the cerebellomedullary (CM) cistern of mice was developed based on fixation of the mouse with a special mask under inhalation anesthesia, and exposure of the sagittal suture of the cranium and midline of the nape to allow us to visualize injection point directly. The accuracy of the modified method was evaluated using the temporal and spatial intracranial distribution of dye by intracisternal injection of 6-μl methylene blue aqueous solution. A high concentration of dye was found in the CM cistern, the ventral cisterns, and intracranial proximal portion of the main cranial nerves at 1 hour after injection. The color of the dye in the CM cistern and the ventral cisterns was lighter, and the dye had reached the intracranial distal portion of the main cranial nerves at 6 hours after injection. The dye was completely eliminated by cerebrospinal fluid (CSF) circulation at 24 hours after injection. No severe brain injury was found in any of the 20 mice. Intracisternal injection was successful in all 14 mice sacrificed 1 hour or 6 hours after injection according to the confirmation of dye distribution. The effects of central administration of endothelin-1 (ET1) were evaluated on cerebral blood supply, constriction of cerebral arteries, and change of respiration in mice. Three doses of ET1 were studied: 2 μg (0.8 nmol), 4 μg (1.6 nmol), and 6 μg (2.4 nmol). Cerebral blood flow (CBF) was monitored for 60 minutes following injection using a laser Doppler probe. Intracisternal ET1 injection induced dose-dependent reduction of CBF, constriction of cerebral arteries, and respiratory depression in mice. This modified method of injection into the CM cistern under direct visualization provides accurate and reproducible injection into the CSF, and can be used to investigate the effects of various chemical substances on the central nervous system in mice.

Key words: intracisternal injection, mouse, endothelin, cerebral blood flow, ischemia

INTRODUCTION

Sustained cerebrospinal fluid (CSF) concentrations of agents may be required to achieve efficacy, especially in dealing with stroke, pain, or other neurodegenerative diseases (Ueda et al. 1979, Li et al. 2005, Tang 2008). However, the blood-brain barrier (BBB) markedly restricts the transport of chemical substances between the circulation and the central nervous system. Central administration of a chemical substance allows the pharmacological effects and mechanisms of action of the substance to function without hindrance by its permeability through the BBB (Anderson and Sorenson 1994, Muehlschlegel and Sims 2009). Central

administration methods include direct intrathecal, intraventricular, intraparenchymal, and intracisternal injection, and are used to deliver drugs and chemotherapeutic agents for the treatment of patients (Grabow et al. 2001, Rainov and Kramm 2001).

Intracisternal injection is widely applied in various animal species such as the rat, rabbit, dog, and monkey (Koedel et al. 1996, Johnston et al. 2005, van der Flier et al. 2005, Shirao et al. 2008) for basic research because of the inherent advantages of minimal invasiveness and simplicity without the need for complicated stereotactic instruments and techniques as required for intraventricular administration. However, intracisternal injection is rarely performed in mice because of the uncertainty and technical demands related to the small size of mice. A novel method of cerebellomedullary (CM) intracisternal administration in mice using a hand-made

curved needle was originally developed in 1979 (Ueda et al. 1979). The curved needle controls the puncture distance and helps to control the puncture direction. However, specialized manipulations such as transcutaneous injection without visual guidance and selection of the head position of the mouse by hand are very difficult to achieve, so have restricted the use of this technique. Injection into the CM cistern is still uncertain and sometimes the needle caused damage to the brain parenchyma (Ueda et al. 1979). Consequently, the original method of intracisternal injection in mice is used rarely so far.

The present study describes a substantial modification of Ueda's method to allow accurate and replicable injection into the CM cistern in mice through direct visualization of the puncture place by the skin incision instead of transcutaneous blind injection. The accuracy of the modified method was assessed using the temporal and spatial intracranial distribution of methylene blue dye. The modified method was used to confirm that intracisternal endothelin-1(ET1) injection induced dose-dependent reduction of cerebral blood flow (CBF), constriction of cerebral arteries, and respiratory depression in mice.

METHODS

Animals

All animal-related procedures were performed in accordance with the guidelines of the University of Tokyo. Male C57BL/6 mice aged 10–14 weeks (Charles River Japan, Yokohama, Kanagawa, Japan) and weighing 22–26 g at the time of experiments were housed under a 12:12 light/dark cycle with *ad libitum* access to water and rodent chow.

Preparation of the puncture needle and injection apparatus

The long segment of a 27-gauge dental needle was curved (40°) at 2.5 mm from the tip, with the inclined plane of the tip facing laterally (Fig. 1C). The short segment of the needle was attached to a microsyringe (10 μl; Hamilton Company, Reno, NV) *via* PE20 plastic tubing. The microsyringe was controlled by an infusion pump (KD Scientific, Holliston, MA).

Injection into the CM cistern

Anesthesia was maintained during the procedure with 1.5% isoflurane in a 30% O_2/70% N_2O gas mixture delivered *via* a specially made facemask (Fig. 1D). Anesthetized mice were placed in the prone position. The upper front teeth and anterior craniofacial part of the mouse were fixed with wires incorporated in the facemask, and the head was bent (angle between head and body of about 120°), resulting in nape elevation and distention (Fig. 1A). The nape of the neck was incised at the midline, and the sagittal suture of the cranium and midline of the nape were exposed. These two anatomical landmarks were used to determine the midline during puncture (Fig. 1B). The needle was held parallel to the external surface of occipital bone just before puncture. The tip of the needle was run along the external surface of the occiput and inserted into the cleft between
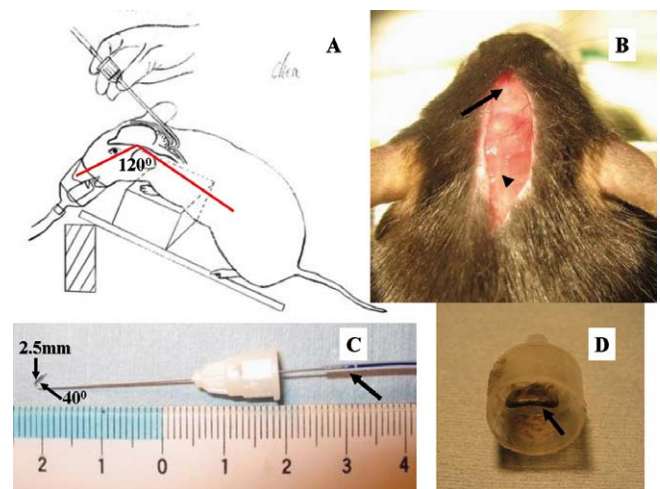

Fig. 1. Illustrations of the modified method of injection into the CM cistern. (A) Schematic diagram showing the prone position with nape elevation and extension under inhalational anesthesia (angle between head and body is about 120°). The hand-made curved tip of the needle was inserted into the CM cistern, keeping the needle on the midline. (B) Photograph taken after incision of the skin of the nape on the midline, showing exposure of the sagittal suture of the cranium (arrow) and midline of the nape (arrowhead), which are used to recognize the midline during puncture. (C) Photograph showing the long segment of a 27-gauge dental needle curved (40°) at 2.5 mm from the tip, with the inclined plane of the tip facing laterally. The short segment of the needle was connected to PE20 plastic tubing (arrow). (D) Photograph of the special facemask prepared from a 5-ml syringe with a wire (arrow) fixed in the inner cavity of the facemask. Grid width is 1 mm.
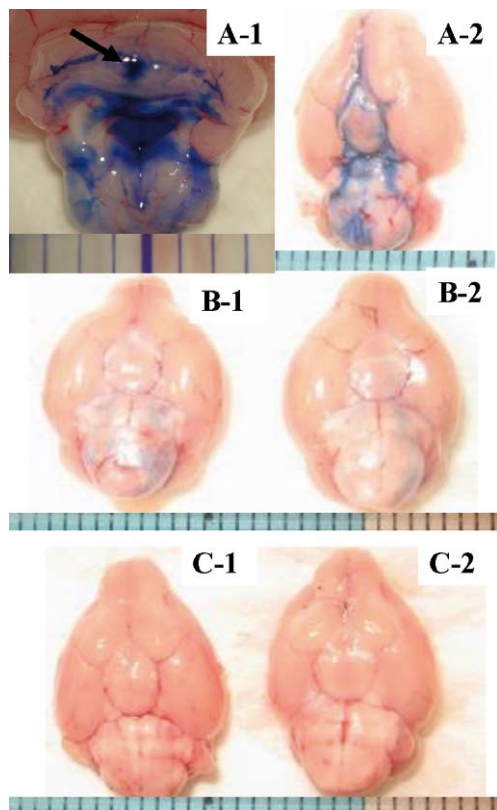
Fig. 2. Dye distributions after injection. Two brains of each
time point were shown. A high concentration of dye was
found in the CM cistern (A-1) and the ventral cisterns (A-2)
1 hour after injection. The puncture path in the vermis of the
cerebellum (arrow in A-1) could be recognized in about half
of the mice. The color of the dye in the ventral cisterns (B-1,
B-2) was lighter 6 hours after injection. No dye was observed
in the ventral cisterns 24 hours after injection. Grid width is
1 mm.

the occiput and the atlas vertebra through the muscles
and ligaments in the midline. The needle was kept in
close contact with the external surface of the occiput
and on the midline during the entire period of injection
(from puncture through injection until withdrawal). A
6-µl volume of solution was injected slowly (3 µl/min)
into the CM cistern, and the needle was then with-
drawn. The incision was sutured. The mice was resus-
citated from anesthesia by maintaing a 30% O_2/70%
N_2O gas mixture but withdrawing 1.5% isoflurane.

Checking the intracranial distribution of the dye following intracisternal injection

Twenty mice underwent intracisternal injection of 6
µl 0.4% methylene blue aqueous solution. Mice were
sacrificed 1 hour (n=8), 6 hours (n=6), or 24 hours
(n=6) after injection. The whole brain was removed
and examined to assess the intracranial distribution of
the dye and to check for any evidence of brain tissue
injury.

Evaluation of the central effects of intracisternal ET1 injection

Prior to intracisternal injection, a laser Doppler
probe (Advance Co., Ltd., Tokyo, Japan) was fixed on
the left temporal skull surface as described previously
(Chen et al. 2008). ET1 (AnaSpec, Inc., San Jose, CA)
was dissolved in sterile normal saline (NS) at various
concentrations (2 µg/6 µl, 4 µg/6 µl, 6 µg/6 µl). A 6-µl
portion of these three doses [2 µg (0.8 nmol), 4 µg (1.6
nmol), or 6 µg (2.4 nmol)] of ET1 or NS (n=6 for each
group) was delivered into the CM cistern. CBF was
monitored for 60 minutes following injection.
Respiratory condition was also recorded. Two mice
from each group were sacrificed and the whole brain
examined to check intracranial vasculature changes
following intracisternal injection.

Statistical analysis

Values were compared using one-way analysis of
variance followed by a *post hoc t*-test. Values are pre-
sented as mean ± standard error of the mean, with
findings of $P<0.05$ considered significant and
$0.05<P<0.07$ considered as indicative of significance.
All statistical analyses were performed on a personal
computer using the JMP program (Version 8; SAS
Institute Inc., Cary, NC).

RESULTS

Intracisternal injection of methylene blue aqueous solution

Temporal and spatial changes in dye distribution

Table I summarizes the findings of dye distribu-
tion. At 1 hour after injection (n=8), high concentra-
tions of dye were found in the CM cistern (Fig. 2 A-1)
and the ventral cisterns (Fig. 2 A-2), and low concen-
trations in the proximal two-thirds of the trigeminal
nerve roots and the proximal segment of the optic
nerves between the optic chiasm and brainstem in all

mice. At 6 hours after injection (*n*=6), the concentration of the dye was reduced in the CM cistern and the ventral cisterns (Fig. 2 B-1, B-2). Dye had disappeared around the trigeminal nerve roots, but a low concentration persisted around the optic nerves beyond the optic chiasm. At 24 hours after injection (*n*=6), no dye was observed in the intracranial space in any of the mice (Fig. 2 C-1, C-2).

Success rate and brain tissue injury

Dye present in the cerebral cisterns and main intracranial nerve roots within 6 hours of injection, showed that intracisternal injection had been performed correctly. Therefore, the puncture needle was successfully inserted into the CM cistern in all 14 mice sacrificed 1 hour or 6 hours after injection. The brainstem, upper segment of the cervical spinal cord, and cerebellum were carefully examined to check for brain tissue injury in all 20 mice. The puncture path could be recognized in the vermis of the cerebellum in about half of the mice (Fig. 2 A-1). A pinpoint region of hemorrhage was observed in the ventromedial vermis of the cerebellum in some mice, but no subarachnoid hemorrhage and no brainstem or spinal cord injury was detected in any of the 20 mice.

Effects on vital signs and neurological status

Respiratory impairment occurred in three mice, at the end of injection in one and just after withdrawal of the puncture needle in two. Recovery from respiratory impairment was observed within several minutes without additional management. All mice were smoothly resuscitated from anesthesia. No neurological deficits were observed.

Intracisternal injection of ET1

CBF values

The initial CBF reading, just before injection, was considered to represent the 100% baseline, and subsequent flow changes are expressed relative to this baseline. CBF was recorded at fixed time points after injection (at 5 minutes and every 10 minutes thereafter). Details are shown in Table II and Figure 3. CBF in the 2-μg group varied over time and did not significantly differ from the control group values. CBF in the 4-μg

group was significantly decreased at 10 and 40 minutes, and tended to decrease at 30, 50, and 60 minutes compared with the control group. CBF in the 6-μg group showed the severest reduction, as all mice in this group died or suffered severe respiratory depression 20 minutes after injection, so the CBF values of this group are only shown at 5 and 10 minutes.

Intracranial vasculature changes

Two mice from each of the NS, 2-μg ET1, and 4-μg ET1 groups were sacrificed 60 minutes after injection, just following the CBF monitoring period. Since most of the mice in the 6-μg group died of respiratory insufficiency 20–30 minutes after injection, 2 mice in the 6-μg group were sacrificed immediately after respiratory arrest occurred at 20 minutes after ET1 injection. No significant changes in vasculature were found on the dorsal side of the brain in all groups. However, significant differences in vasculature were found on the ventral side of the brain (Fig. 4). Clear vasculature was found in the NS group. Constriction of arteries was unclear in the 2-μg group: mild artery constriction was observed in one mouse, but no change in the other mouse compared to the NS group. Constriction of arteries was obviously observed in the 4-μg group: larger arteries, such as the proximal segment of the middle cerebral artery and basilar artery, were thinner, vasculature was sparser, and some small arteries had even disappeared compared to the NS group. Constriction of arteries was most severe on the ventral side of the brains in the 6-μg group.
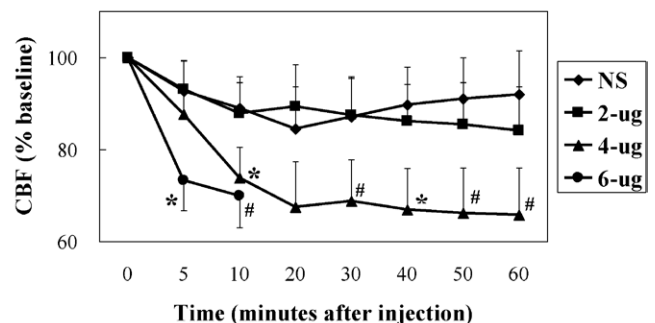

Fig. 3. CBF changes during the 60-minute period following intracisternal NS or 2-μg (0.8 nmol), 4-μg (1.6 nmol), or 6-μg (2.4 nmol) ET1 injection. Significant decrease in CBF (*P<0.05) and tendency toward significant decrease (#0.05<P<0.07), compared to the control group (NS group), are seen at the same time point.

Table I

Distributions of dye at 1 hour, 6 hours, and 24 hours after injection

	1 hour	6 hours	24 hours
CM cistern	++	+	−
Ventral cisterns	++	+	−
Trigeminal nerve (2/3 portion near brainstem)	+	−	−
Optic nerve (between optic chiasm and brainstem)	+	−	−
Optic nerve (beyond optic chiasm)	−	+	−

Staining was assessed as strong (++), weak (+), or not detectable (−)

Respiratory impairment

No mouse in the NS group suffered respiratory impairment. Mild sob-like respiration appeared immediately after ET1 injection in most mice (5/6) in the 2-µg group, but recovery was observed within 60 minutes. Obvious sob-like respiration appeared in all mice in the 4-µg group, but disappeared after resuscitation from anesthesia, except in 1 mouse that died during the 60-minute CBF monitoring period. All mice in the 6-µg group died of respiratory insufficiency within 60 minutes after injection (4/6 died within 20–30 minutes, and 2/6 within 40–50 minutes after injection).

DISCUSSION

Intraventricular injection is the most common method for the study of central pharmacological action, but requires complicated stereotactic instruments and training to accomplish. Moreover, models of pathological conditions of the cerebrum such as focal cerebral ischemia result in swelling of the forebrain and narrowing and shifting of the small lateral ventricles to the collateral side, so intraventricular injection is very difficult to accomplish, especially in small animals such as rats or mice. In contrast, the location and size of the CM cistern remains unchanged or only mildly altered even under pathological conditions. Therefore, injection into CM cistern is an important method of central administration in small animals. The mouse is the most appropriate animal model for the study of genetic modifications, so is largely used in transgenic technology concerning studies in molecular pathophysiology of central nervous systems (Chan et al. 1995, Chen et al. 2005). The increased use of mice in experimental study also requires for an accurate and repro-

Table II

CBF changes at fixed time points (5, 10, 20, 30, 40, 50, and 60 minutes) after intracisternal NS or 2-µg (0.8 nmol), 4-µg (1.6 nmol), or 6-µg (2.4 nmol) ET1 injection

Group	5 min	10 min	20 min	30 min	40 min	50 min	60 min
NS	92.7 ± 6.5	89.0 ± 6.8	84.4 ± 9.1	87.1 ± 8.3	89.7 ± 8.1	90.9 ± 9.0	91.9 ± 9.5
2 µg	92.9 ± 6.5	87.7 ± 6.8	89.2 ± 9.1	87.5 ± 8.3	86.1 ± 8.1	85.4 ± 9.0	84.1 ± 9.5
4 µg	87.6 ± 6.5	73.8 ± 6.8*	67.5 ± 9.9	68.7 ± 9.1#	67.0 ± 8.9*	66.2 ± 9.8#	65.7 ± 10.4#
6 µg	73.2 ± 6.5*	69.9 ± 6.8#					

Values (mean ± standard error of the mean) are percentages of baseline. Significant decrease in CBF (*$P<0.05$) or tendency toward significant decrease (#$0.05<P<0.07$), compared with the control group (NS group), are shown. Since most of the mice in the 6-µg group died of respiratory insufficiency 20–30 minutes after injection, CBF values were recorded at only two time points (5 and 10 minutes) for the 6-µg group.

ducible method for injection into the CM cistern in mice.

The original intracisternal method was described by (Ueda et al. 1979), but had various disadvantages. The atlanto-occipital space is very narrow in mice, so unguided transcutaneous injection cannot guarantee correct needle penetration. The unfixed position interfered with the accuracy and reproducibility. The head of the mouse was bent and the position was maintained by hand, so that the individual position varied case by case. Mild movement of the hands could disturb the direction of injection or cause the displacement of needle. Therefore, Ueda's method required experience, and the results varied among investigators. Moreover, failure of injection into the CM cistern or even damage to the brain tissues could not be avoided completely (Ueda et al. 1979). We have recognized the substantial causes of the failure of original intracisternal method based on the our previous experiments (data not shown). Although atlanto-occipital space is relatively easy to find by touch, accurate position (direction and depth) of the CM cistern is very difficult to confirm due to the narrow space of the particular small animals such as mice by the blind injection method. Given the needle is inserted to the appropriate space, there is no robust guarantee of the injection of the dye or drug into the cistern because of the unstability of the needle. Then we have introduced the modified method for CM cistern focusing on fixing the position and the direct visualization of the injection point. Our modification of Ueda's method fixed the mouse in position instead of hand holding. Under inhalational anesthesia, the upper foreteeth and anterior craniofacial part of the mouse were fixed by wires in the inner cavity of the facemask and the inner wall of the facemask, and then the nape was elevated and extended to widen the atlanto-occipital space and facilitate puncture. This fixed position also helped to maintain a free airway and decreased the possibility of needle displacement during injection. The skin of the nape was incised instead of percutaneous puncture, so that the location of the puncture could always be observed and accurately determined. The atlanto-occipital portion (puncture target) could be seen directly, so that extracranial injection was avoided. Furthermore, the exposure of the sagittal suture of the cranium and midline of the nape could locate the midline accurately, thus increasing the targeting injection of the CM cistern. This method also used the long segment of a 27-gauge dental needle curved at 2.5 mm

from the tip instead of 3.5 mm, which was too long according to our pilot study (data not shown), with the inclined plane of the needle tip facing laterally because this direction facilitated outflow of the injected solution into the small and narrow CM cistern.

The accuracy and reproducibility of our modified method were verified by intracisternal injection of methylene blue aqueous solution. Success was achieved in all 14 mice according to the dye distribution in the cisterns examined at sacrifice 1 hour or 6 hours after injection. The dye color in the cisterns was consistent
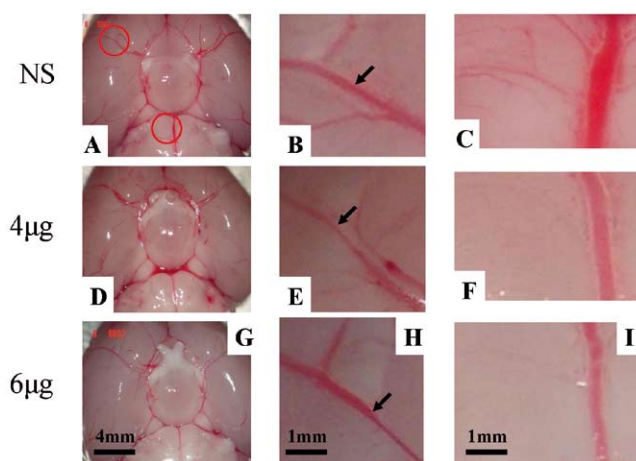

Fig. 4. Constriction of the cerebral arteries in mice following intracisternal ET1 injection. General appearances of the vasculature on the ventral side of the brain are shown in A (NS), D [4 µg (1.6 nmol)], and G [6 µg (2.4 nmol)]. The magnified views B and C (NS), E and F (4 µg), and H and I (6 µg) near the margin of the right sylvian cistern and ventral side of the pons are shown in A by the red circles. The margin of the right sylvian cistern is marked with an arrow in B, E, and H. Clear vasculature was found on the ventral side of the brain extracted 60 minutes after NS injection (A–C). The arteries were obviously constricted 60 minutes after 4-µg ET1 injection: larger arteries within cisterns were thinner (proximal segment of middle cerebral artery in E; basilar artery in F), vasculature was sparser, and some small arteries had even disappeared compared to the NS group (D–F). The severest constriction of arteries was observed on the ventral side of the brain extracted 20 minutes after 6-µg ET1 injection (just after respiratory arrest) (G–I). The proximal segment of middle cerebral artery within the sylvian cistern was very thin, although the diameter of the distal segment of the middle cerebral artery beyond the sylvian cistern exhibited no clear change after 4-µg (E) and 6-µg (H) injection. Dose-dependent constriction was found in the proximal segment of the middle cerebral artery, basilar artery, and small arteries on the ventral side of the brain.

within all mice at both time points, indicating the high accuracy and replicability of this novel modified method. Moreover, the experiment showed new findings in terms of the temporal and spatial distribution of the dye after CM injection in mice. The dye was eliminated by the CSF circulation gradually and the locations of the dye also changed with time. Distribution of the dye was confirmed within 6 hours after injection and complete elimination of the dye by the CSF circulation was confirmed at 24 hours after injection. Most previous experiments have required dye injection together with drug administration to confirm the distribution (Ueda et al. 1979, Balerio and Rubio 2002, Ro et al. 2007). The present findings suggest that indications of distribution by dye tracer are only accurate within 6 hours in terms of the temporal distribution of the dye after CM injection in mice. The advantage of this modified method is that dye injection together with drug administration was not needed because of its high accuracy and reproducibility. Therefore, this method allows us to assess the pure efficacy of the drug without the effects of dye tracer.

This study also examined the central effect of ET1 in the CSF. ET1 is a 21-amino-acid peptide that acts through specific receptors (ETA and ETB) which are widely distributed throughout the central nervous system on the vascular endothelium, smooth muscle cells, neurons, astrocytes, and microglia (Hughes et al. 2003). ET1 induces profound constriction of both large and small cerebral arteries, and intracerebral injection of ET1 produces reproducible infarction in the rat (Fuxe et al. 1989, Sharkey and Butcher 1995, Windle et al. 2006). ET1 also potently constricts isolated mouse cerebral arteries (Bai et al. 2004, Salomone et al. 2008), but whether ET1 can induce cerebral ischemia in mice remains unclear (Wang et al. 2007, Horie et al. 2008). Therefore, the central pharmacological effects of ET1 in mice require careful evaluation. The effects of ET1 in the CBF circulation have been evaluated in the rat (Macrae et al. 1991, 1993), but not in the mouse. This study found that intracisternal injection of 4-µg ET1 induced significant reduction of CBF compared with the NS or 2-µg ET1 injection. Administration of 6-µg ET1 yielded the severest reduction of CBF. Morphological change of cerebral vessels was also confirmed, which showed dose-dependent constriction effects of ET1 on the cerebral arteries consistent with the effects on CBF. This study also showed that ET1 had vasoconstriction effects on the cerebral circulation in mice. However, the sensitivity of the mice to ET1 was different to that of the rat, because no significant reduction of CBF was induced by administration of 2 µg ET1 in mice, in contrast to the potent effect in rats (Robinson et al. 1990, Nikolova et al. 2009).

CONCLUSION

The present modified method of injection into the CM cistern is accurate, replicable, and safe. Using this method, we found that central intracerebral administration of ET1 induced dose-dependent constriction of the cerebral arteries in mice. The new modified intracisternal injection method is generally applicable for assessing the pharmacological effects and mechanisms of the central action of drugs on the nervous system in mice.

REFERENCES

Anderson PM, Sorenson MA (1994) Effects of route and formulation on clinical pharmacokinetics of interleukin-2. Clin Pharmacokinet 27: 19–31.

Bai N, Moien-Afshari F, Washio H, Min A, Laher I (2004) Pharmacology of the mouse-isolated cerebral artery. Vascul Pharmacol 41: 97–106.

Balerio GN, Rubio MC (2002) Baclofen analgesia: involvement of the GABAergic system. Pharmacol Res 46: 281–286.

Chan PH, Epstein CJ, Li Y, Huang TT, Carlson E, Kinouchi H, Yang G, Kamii H, Mikawa S, Kondo T (1995) Transgenic mice and knockout mutants in the study of oxidative stress in brain injury. J Neurotrauma 12: 815–824.

Chen J, Zacharek A, Zhang C, Jiang H, Li Y, Roberts C, Lu M, Kapke A, Chopp M (2005) Endothelial nitric oxide synthase regulates brain-derived neurotrophic factor expression and neurogenesis after stroke in mice. J Neurosci 25: 2366–2375.

Chen Y, Ito A, Takai K, Saito N (2008) Blocking pterygopalatine arterial blood flow decreases infarct volume variability in a mouse model of intraluminal suture middle cerebral artery occlusion. J Neurosci Methods 174: 18–24.

Fuxe K, Cintra A, Andbjer B, Anggard E, Goldstein M, Agnati LF (1989) Centrally administered endothelin-1 produces lesions in the brain of the male rat. Acta Physiol Scand 137: 155–156.

Grabow TS, Derdzinski D, Staats PS (2001) Spinal drug delivery. Curr Pain Headache Rep 5: 510–516.

Horie N, Maag AL, Hamilton SA, Shichinohe H, Bliss TM,

Steinberg GK (2008) Mouse model of focal cerebral ischemia using endothelin-1. J Neurosci Methods 173: 286–290.

Hughes PM, Anthony DC, Ruddin M, Botham MS, Rankine EL, Sablone M, Baumann D, Mir AK, Perry VH (2003) Focal lesions in the rat central nervous system induced by endothelin-1. J Neuropathol Exp Neurol 62: 1276–1286.

Ide K, Yamakawa K, Nakagomi T, Sasaki T, Saito I, Kurihara H, Yosizumi M, Yazaki Y, Takakura K (1989) The role of endothelin in the pathogenesis of vasospasm following subarachnoid haemorrhage. Neurol Res 11: 101–104.

Johnson ND, Duysen EG, Lockridge O (2009) Intrathecal delivery of fluorescent labeled butyrylcholinesterase to the brains of butyrylcholinesterase knock-out mice: visualization and quantification of enzyme distribution in the brain. Neurotoxicology 30: 386–392.

Johnston M, Zakharov A, Koh L, Armstrong D (2005) Subarachnoid injection of Microfil reveals connections between cerebrospinal fluid and nasal lymphatics in the non-human primate. Neuropathol Appl Neurobiol 31: 632–640.

Koedel U, Bernatowicz A, Frei K, Fontana A, Pfister HW (1996) Systemically (but not intrathecally) administered IL-10 attenuates pathophysiologic alterations in experimental pneumococcal meningitis. J Immunol 157: 5185–5191.

Li F, Hayashi T, Jin G, Deguchi K, Nagotani S, Nagano I, Shoji M, Chan PH, Abe K (2005) The protective effect of dantrolene on ischemic neuronal cell death is associated with reduced expression of endoplasmic reticulum stress markers. Brain Res 1048: 59–68.

Macrae I, Robinson, M, McAuley M, Reid J, McCulloch J (1991) Effects of intracisternal endothelin-1 injection on blood flow to the lower brain stem. Eur J Pharmacol 203: 85–91.

Macrae IM, Robinson MJ, Graham DI, Reid JL, McCulloch J (1993) Endothelin-1-induced reductions in cerebral blood flow: dose dependency, time course, and neuropathological consequences. J Cereb Blood Flow Metab 13: 276–284.

Muehlschlegel S, Sims JR (2009) Dantrolene: mechanisms of neuroprotection and possible clinical applications in the neurointensive care unit. Neurocrit Care 10: 103–115.

Nikolova S, Moyanova S, Hughes S, Bellyou-Camilleri M, Lee TY, Bartha R (2009) Endothelin-1 induced MCAO: dose dependency of cerebral blood flow. J Neurosci Methods 179: 22–28.

Rainov NG, Kramm CM (2001) Vector delivery methods and targeting strategies for gene therapy of brain tumors. Curr Gene Ther 1: 367–383.

Ro JY, Lee J, Capra NF, Zhang Y (2007) Role of soluble guanylate cyclase in the trigeminal subnucleus caudalis in capsaicin-induced muscle hypersensitivity. Brain Res 1184: 141–148.

Robinson M, Macrae I, Todd M, Reid J, McCulloch J (1990) Reduction of local cerebral blood flow to pathological levels by endothelin-1 applied to the middle cerebral artery in the rat. Neurosci Lett 118: 269–272.

Salomone S, Potts EM, Tyndall S, Ip PC, Chun J, Brinkmann V, Waeber C (2008) Analysis of sphingosine 1-phosphate receptors involved in constriction of isolated cerebral arteries with receptor null mice and pharmacological tools. Br J Pharmacol 153: 140–147.

Sharkey J, Butcher SP (1995) Characterisation of an experimental model of stroke produced by intracerebral microinjection of endothelin-1 adjacent to the rat middle cerebral artery. J Neurosci Methods 60: 125–131.

Shirao S, Fujisawa H, Kudo A, Kurokawa T, Yoneda H, Kunitsugu I, Ogasawara K, Soma M, Kobayashi S, Ogawa A, Suzuki M (2008) Inhibitory effects of eicosapentaenoic acid on chronic cerebral vasospasm after subarachnoid hemorrhage: possible involvement of a sphingosylphosphorylcholine-rho-kinase pathway. Cerebrovasc Dis 26: 30–37.

Tang BL (2008) Leptin as a neuroprotective agent. Biochem Biophys Res Commun 368: 181–185.

Ueda H, Amano H, Shiomi H, Takagi H (1979) Comparison of the analgesic effects of various opioid peptides by a newly devised intracisternal injection technique in conscious mice. Eur J Pharmacol 56: 265–268.

van der Flier M, Coenjaerts FE, Mwinzi PN, Rijkers E, Ruyken M, Scharringa J, Kimpen JL, Hoepelman AI, Geelen SP (2005) Antibody neutralization of vascular endothelial growth factor (VEGF) fails to attenuate vascular permeability and brain edema in experimental pneumococcal meningitis. J Neuroimmunol 160: 170–177.

Wang Y, Jin K, Greenberg DA (2007) Neurogenesis associated with endothelin-induced cortical infarction in the mouse. Brain Res 1167: 118–122.

Windle V, Szymanska A, Granter-Button S, White C, Buist R, Peeling J, Corbett D (2006) Analysis of four different methods of producing focal cerebral ischemia with endothelin-1 in the rat. Exp Neurol 201: 324–334.