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The selective Na^+–Ca^{2+}exchange inhibitor attenuates brain edema after radiofrequency lesion in rats

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Abstract

2-[4-[(2,5-Difluorophenyl)methoxy]phenoxy]-5-ethoxyaniline (SEA0400), a specific inhibitor of the Na^+–Ca^{2+} exchanger, exerts cytoprotective action and reduces brain infarct volume after cerebral ischemia. We examined the effect of SEA0400 on vasogenic brain edema in rats. Histological observations showed that radiofrequency current caused brain infarct and extravasation of endogenous albumin in the brain. SEA0400 (3 and 10 mg/kg, i.v.) significantly suppressed the increase in brain water content with attenuation of Evans blue dye and sodium fluorescein extravasation after radiofrequency lesion. The findings suggest that the Na^+–Ca^{2+} exchanger plays a role in vasogenic edema formation after radiofrequency lesion.

Keywords: SEA0400; Brain edema; Radiofrequency lesion; Blood–brain barrier; Na^+–Ca^{2+} exchanger

1. Introduction

Brain edema is a mortal pathological condition commonly observed in brain injuries including brain ischemia, trauma, tumor and infections. The pathogenesis of brain edema is classified into cytotoxic edema and vasogenic edema (Chan and Fishman, 1985). The former is characterized by cellular swelling, and the latter is accompanied by an increase in brain water content and extravasation of serum components through blood–brain barrier disruption. A large part of the blood–brain barrier is dependent on restricted intracellular transport systems constructed between brain capillary endothelial cells and astrocytes. Previous in vitro studies suggested that disruption of Ca^{2+} homeostasis in endothelial cells and astrocytes is responsible for dysfunction of blood–brain barrier permeability (Nishino et al., 1997; Abbruscato and Davis, 1999; Brown and Davis, 2002), although the exact modulation of blood–brain barrier integrity is not known.

The Na^+–Ca^{2+} exchanger is a transmembrane ion transporter and plays an important role in regulation of intracellular Ca^{2+} levels. We have developed 2-[4-[(2,5-difluorophenyl)methoxy]phenoxy]-5-ethoxyaniline (SEA0400) as a potent and selective inhibitor of the Na^+–Ca^{2+} exchanger and found that it protected astrocytes against Ca^{2+} reperfusion injury and reduced brain infarct volume after cerebral ischemia (Matsuda et al., 2001). In this study, we examined the effects of SEA0400 on brain edema generated by radiofrequency lesion to study the pathological role of the Na^+–Ca^{2+} exchanger in brain edema formation and blood–brain barrier disruption in rats. Radiofrequency lesion is widely used to cause denaturation of nerve tissue in targeted areas (Liu et al., 1997; Warburton et al., 1998; Pouzet et al., 1999).

2. Materials and methods

2.1. Radiofrequency lesion of rat brain

All experimental protocols conformed to the Guiding Principle for the Care and Use of Animals approved by the Japanese Pharmacological Society. All efforts were made to minimize the number of animals used. Male Wistar rats (250–300 g) were anaesthetized with sodium pentobarbital

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(50 mg/kg) and placed in a stereotaxic frame. Radiofrequency lesion was induced by a lesion generator (RFG-4A, Radionics, MA, USA) with a 0.7 mm diameter electrode. After the skull was exposed, a burr hole was made 3.0 mm posterior and 3.0 mm right lateral to the bregma, and the tip of the electrode was positioned at 6.0 mm below bregma. A reference electrode was fixed on the skull filled with saline. A radiofrequency current was applied to heat the tip of inserted electrode at 55 °C constantly for 3 min. For control rats, an electrode was inserted into brain but no radiofrequency current was applied. SEA0400 and vehicle (lipid emulsion) were synthesized by Taisho Pharmaceutical (Saitama, Japan). SEA0400 at 0.3, 1.0 and 10 mg/kg and its vehicle (a lipid emulsion containing 20% soybean oil) were administered at a volume of 0.7 ml/kg into the tail vein. In some experiments, mannitol at 1.0 g/kg was administered into the tail vein as a positive control of antiedema drug.

2.2. Histological observation

Each infarct area was visualized by 2,3,5-triphenyltetrazolium (TTC) staining. Rats were decapitated under pentobarbital anesthesia 24 h after the lesion, and brains were removed. Coronal brain sections (2 mm thick) were made on a brain slicer (Aster Industries, PA, USA) with a razor blade. Brain slices were incubated with 2% TTC in saline for 30 min. Extravasation of endogenous albumin was examined by an immunohistochemical method. Rat brains were fixed by perfusion of 3% paraformaldehyde and coronal sections (30 μm thick) were made as described previously (Ishikawa et al., 1997). Brain sections were labeled with antirat albumin rabbit antibody (Inter-Cell Tech., NJ, USA), and visualized with an ABC–DAB staining kit (Nichirei, Tokyo, Japan). Labeling of activated glial cells (anti-glial fibrillary acidic protein antibody for astrocytes and *Griffonia simplicifolia* BS-I isoletin-B4 for microglia/macrophage) was performed as described previously (Koyama et al., 2003).

2.3. Measurement of water content

Rat brains were mounted on the brain slicer and a coronal section (4 mm thick, between 2 mm posterior and 2 mm anterior to the electrode insert site) was cut out. The section was divided into hemispheres. The wet weight of each hemisphere was measured in a preweighted polypropylene tube. The section was then dried at 75 °C for 48 h and dry weight was measured. Water content of brain sections (%) was expressed as $100 \times (\text{wet weight} - \text{dry weight})/\text{wet weight}$.

2.4. Measurement of blood–brain barrier permeability

Blood–brain barrier permeability was evaluated by extravasation of two exogenous tracers (Evans blue-albumin as a high molecular weight marker, and sodium fluorescein as a low molecular weight marker). A mixture of 4% Evans blue, 10% bovine serum albumin and 4% sodium fluorescein was injected to each rat through the tail vein at 1.5 ml/kg 1 h before the experiment. A duration of 1 h was sufficient for the tracers to accumulate in the brain tissue with a high reproducibility. A small portion of blood was collected, and the tracers remaining in brain vessels were removed by perfusion of saline. The brain was removed and a coronal section (4 mm thick) was cut out as described above. The tracers in the brain section and the serum were extracted by 100% trichloroacetic acid. The content of Evans blue in the extract was determined by an absorbance at 655 nm and that of sodium fluorescein was by fluorescence intensity at 530 against 485 nm excitation. Extravasation of Evans blue and sodium fluorescein was expressed as a percentage of the tissue content (μg tracer/g tissue) to the serum content (μg tracer/ml serum).

2.5. Statistical analysis

Statistical analysis of the experimental data was carried out by Student's *t*-test or one-way analysis of variance (ANOVA) followed by Fisher's PLSD test. Values of $P < 0.05$ were considered to be significant.

3. Results

3.1. Characterization of radiofrequency lesion-induced brain edema

Radiofrequency current caused a brain infarct in a large area of the lesioned hemisphere, which was less stained with TTC (Fig. 1A, upper left). Extravasation of endogenous albumin, which plays a key role in induction of vasogenic brain edema, was also observed in the lesioned hemisphere (Fig. 1A, upper right). In the area where albumin was leaked, many glial fibrillary acidic protein-positive cells and *G. simplicifolia* BS-I isoletin-B4-stained cells were observed (Fig. 1A, lower parts), indicating the presence of activated astrocytes and microglia/macrophages around the lesioned area. Water content in the lesioned hemisphere increased significantly 1 h after the lesion and reached maximum at 24 h. In contrast, water content in the contralateral hemisphere has not changed until 24 h, although it showed a transient increase at 48 h (Fig. 1B). Evans blue and sodium fluorescein extravasation were increased 1, 6 and 24 h after the lesion in the lesioned hemisphere but it did not change until 6 h in the contralateral hemisphere (Fig. 1C).

3.2. Effect of SEA0400 on brain edema formation

Fig. 2 shows the effect of SEA0400 on the increase in brain water content and enhanced blood–brain barrier permeability after radiofrequency lesion. SEA0400 at 3 and 10 mg/kg significantly suppressed the increase in brain water content after the lesion (Fig. 2A). The effect of

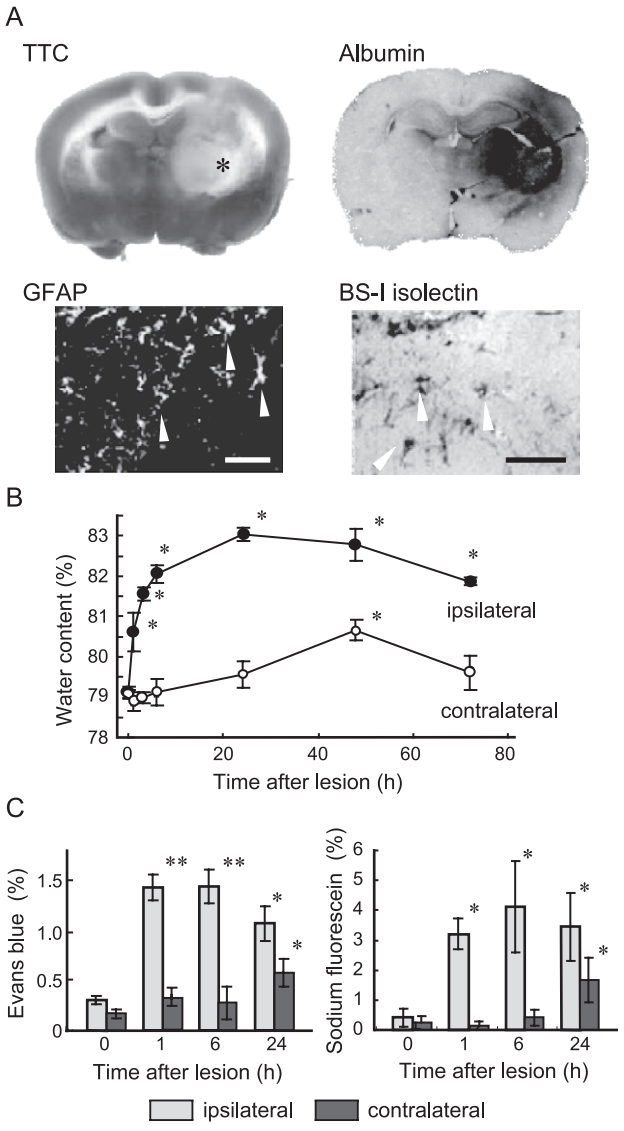

Fig. 1. Characterization of radiofrequency lesion-induced brain edema. (A) Histological observations. Brain sections were stained with TTC (upper left), antirat albumin antibodies (upper right), anti-glial fibrillary acidic protein antibody (GFAP, lower left) and *G. simplicifolia* BS-I isoletin-B4 (lower right) 24 h after the lesion. An asterisk in the upper left indicates the position where the tip of the electrode was located. Arrow heads in the lower parts indicate activated astrocytes and microglia/macrophages. Bar = 50 μm. (B) Brain water content. Water contents in the lesioned ipsilateral (●) and contralateral (○) hemispheres were measured at the indicated times after the lesion. The results shown are means ± S.E.M. of five to six experiments. *$P < 0.001$ vs. 0 time (no lesion), by one-way ANOVA followed by Fisher's PLSD test. (C) Extravasation of Evans blue and sodium fluorescein. Evans blue (left) and sodium fluorescein (right) contents in the ipsilateral and contralateral hemispheres were measured 1, 6 and 24 h after the lesion. Evans blue and sodium fluorescein were injected 1 h before the experiment. Results are means ± S.E.M. of four to six experiments and expressed as the percentage of those in serum. *$P < 0.05$, **$P < 0.001$ vs. 0 time, by one-way ANOVA followed by Fisher's PLSD test.

SEA0400 at 10 mg/kg is comparable with that of a serial administration of mannitol, an anti-brain edema drug. The increase in brain water content at 3 h after the lesion was also suppressed by SEA0400 at 10 mg/kg: the water contents (%, means ± S.E.M., $n = 5$) of sham, lesion without SEA0400 and lesion with SEA0400 were 79.06 ± 0.17, 81.21 ± 0.23, and 80.31 ± 0.06, respectively ($P < 0.05$, between lesion without SEA0400 and lesion with SEA0400, Student's *t*-test). SEA0400 at 1–10 mg/kg significantly reduced Evans blue extravasation (Fig. 2B) and, at 3 and 10 mg/kg, significantly reduced sodium fluorescein extravasation (Fig. 2C).

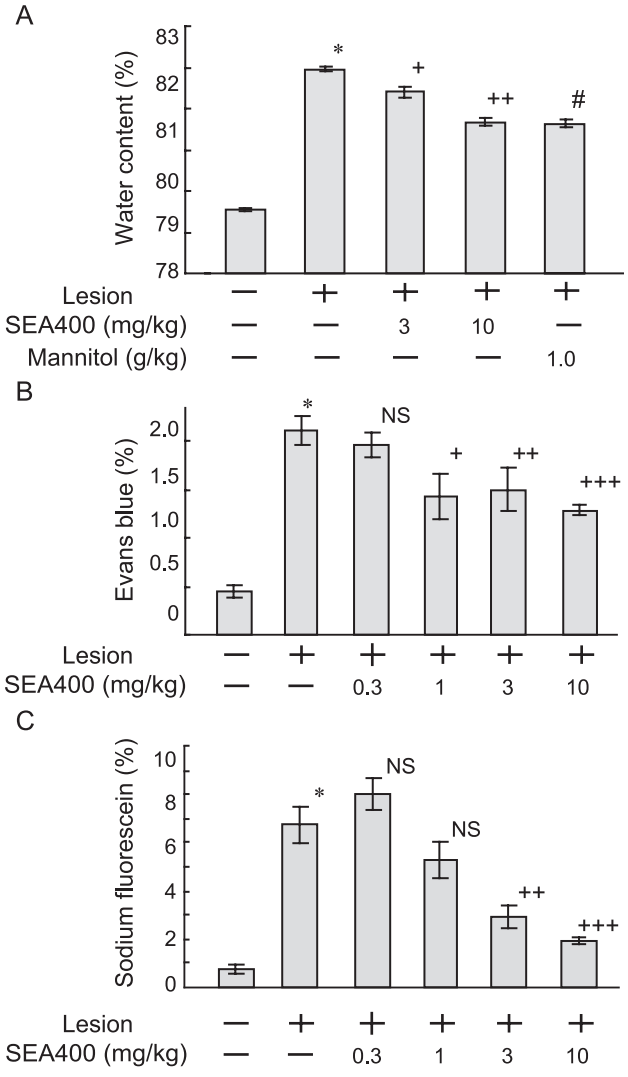

Fig. 2. Effect of SEA0400 on vasogenic edema after radiofrequency lesion. Water contents (A) in the lesioned hemisphere were measured 24 h after the lesion. SEA0400 at the indicated doses was administered within 5 min after the lesion. Mannitol was administered 4, 8, 12, 16 and 20 h after the lesion and the sum of the five bolus doses was 1.0 g/kg. Results are means ± S.E.M. of 9 to 11 experiments. Evans blue (B) and sodium fluorescein (C) contents in the ipsilateral and contralateral hemispheres were measured 3 h after the lesion. Evans blue and sodium fluorescein were injected 1 h before the experiment. SEA0400 was administered within 5 min after the lesion. Results are means ± S.E.M. of 6 to 11 experiments. *$P < 0.001$ vs. no lesion, by Student's *t*-test. NS; not significant, +$P < 0.05$, ++$P < 0.01$, +++$P < 0.001$ vs. lesion and no SEA0400, by one-way ANOVA followed by Fisher's PLSD test. #$P < 0.001$ vs. lesion and no SEA0400, by Student's *t*-test.

4. Discussion

The radiofrequency method used here could easily control the degree of damage: the brain edema was generated with higher reproducibility in the present method. The radiofrequency caused common histological signs of brain injury (brain infarct and glial activation) and significant changes in vasogenic edema parameters such as brain water content and extravasation of albumin, Evans blue and sodium fluorescein (Fig. 1). We found that SEA0400 significantly attenuated the increase in brain water content (Fig. 2A) and disruption of blood–brain barrier permeability (Fig. 2B and C) after radiofrequency lesion. These results indicate that SEA0400 has an inhibitory effect on vasogenic edema formation. In view of the evidence that SEA0400 is a specific inhibitor of the Na^+–Ca^{2+} exchanger (Matsuda et al., 2001), the present finding suggests that the Na^+–Ca^{2+} exchanger is involved in the generation of vasogenic edema accompanied by disruption of blood–brain barrier. To maintain the restricted intercellular transport systems responsible for blood–brain barrier permeability, brain capillary endothelial cells require the support of astrocytes (Abbott, 2002). Guerin et al. (2001) and Nishino et al. (1997) showed that selective destruction of astrocytes resulted in an increase in blood–brain barrier permeability. The Na^+–Ca^{2+} exchanger is present in astrocytes and regulates astrocytic intracellular Ca^{2+} levels (Matsuda et al., 1996; Smith et al., 2003). We previously showed that SEA0400 protected cultured astrocytes from Ca^{2+}-dependent cell damage (Matsuda et al., 2001). Although the present study does not clarify possible target cells of SEA0400, the protective effect of SEA0400 against edema may be due to protection of astrocyte injury.

In conclusion, the present findings show that SEA0400 attenuates brain edema formation by radiofrequency lesion and suggest that the Na^+–Ca^{2+} exchanger is involved in vasogenic edema formation and blood–brain barrier disruption.

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References

Abbott, N.J., 2002. Astrocyte–endothelial interactions and blood–brain barrier permeability. J. Anat. 200, 629–638.

Abbruscato, T.J., Davis, T.P., 1999. Combination of hypoxia/aglycemia compromises in vitro blood–brain barrier integrity. J. Pharmacol. Exp. Ther. 289, 668–675.

Brown, R.C., Davis, T.P., 2002. Calcium modulation of adherens and tight junction function: a potential mechanism for blood–brain barrier disruption after stroke. Stroke 33, 1706–1711.

Chan, P.H., Fishman, R.A., 1985. Brain edema. In: Lajtha, A. (Ed.), Handbook of Neurochemistry. Plenum, New York, pp. 153–174.

Guerin, C.J., Nolan, C.C., Mavroudis, G., Lister, T., Davidson, G.M., Holton, J.L., Ray, D.E., 2001. The dynamics of blood–brain barrier breakdown in an experimental model of glial cell degeneration. Neuroscience 103, 873–883.

Ishikawa, N., Takemura, M., Koyama, Y., Shigenaga, Y., Okada, T., Baba, A., 1997. Endothelins promote the activation of astrocytes in rat neostriatum through ETB receptors. Eur. J. Neurosci. 9, 895–901.

Koyama, Y., Tsujikawa, K., Matsuda, T., Baba, A., 2003. Intracerebroventricular administration of an endothelin ETB receptor agonist increases expressions of GDNF and BDNF in rat brain. Eur. J. Neurosci. 18, 887–894.

Liu, Y.C., Salamone, J.D., Sachs, B.D., 1997. Lesions in medial preoptic area and bed nucleus of stria terminalis: differential effects on copulatory behavior and noncontact erection in male rats. J. Neurosci. 17, 5245–5253.

Matsuda, T., Takuma, K., Nishiguchi, E., Hashimoto, H., Azuma, J., Baba, A., 1996. Involvement of Na^+–Ca^{2+} exchanger in reperfusion-induced delayed cell death of cultured rat astrocytes. Eur. J. Neurosci. 8, 951–958.

Matsuda, T., Arakawa, N., Takuma, K., Kishida, Y., Kawasaki, Y., Sakaue, M., Takahashi, K., Takahashi, T., Suzuki, T., Ota, T., Hamano-Takahashi, A., Onishi, M., Tanaka, Y., Kameo, K., Baba, A., 2001. SEA0400, a novel and selective inhibitor of the Na^+–Ca^{2+} exchanger, attenuates reperfusion injury in the in vitro and in vivo cerebral ischemic models. J. Pharmacol. Exp. Ther. 298, 249–256.

Nishino, H., Kumazaki, M., Fukuda, A., Fujimoto, I., Shimano, Y., Hida, H., Sakurai, T., Deshpande, S.B., Shimizu, H., Morikawa, S., Inubushi, T., 1997. Acute 3-nitropropionic acid intoxication induces striatal astrocytic cell death and dysfunction of the blood–brain barrier: involvement of dopamine toxicity. Neurosci. Res. 27, 343–355.

Pouzet, B., Veenman, C.L., Yee, B.K., Feldon, J., Weiner, I., 1999. The effects of radiofrequency lesion or transection of the fimbria–fornix on latent inhibition in the rat. Neuroscience 91, 1355–1368.

Smith, I.F., Boyle, J.P., Plant, L.D., Pearson, H.A., Peers, C., 2003. Hypoxic remodeling of Ca^{2+} stores in type I cortical astrocytes. J. Biol. Chem. 278, 4875–4881.

Warburton, E.C., Aggleton, J.P., Muir, J.L., 1998. Comparing the effects of selective cingulate cortex lesions and cingulum bundle lesions on water maze performance by rats. Eur. J. Neurosci. 10, 622–634.