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Modulation of AQP4 expression by the selective V1a receptor antagonist, SR49059, decreases trauma-induced brain edema

Keisuke Taya · Salih Gulsen · Kenji Okuno · Ruth Prieto · Christina R. Marmarou · Anthony Marmarou

Abstract
Background Currently, there are no pharmacological treatments available for traumatically induced brain edema and the subsequent rise of ICP. Evidence indicates that Aquaporin-4 (AQP4) plays a significant role in the pathophysiology of brain edema. Previously we have reported that SR49059 reduced brain edema secondary to ischemia. We, therefore, examined whether the selective V1a receptor antagonist, SR49059, reduces brain edema by modulating AQP4 expression following cortical contusion injury (CCI).
Methods Traumatic brain injury (TBI) was produced in thirty-two adult male Sprague-Dawley rats by lateral CCI (6.0 m/sec, 3 mm depth). Animals were randomly assigned to vehicle (n=16) or SR49059 treatment (n=16) groups and administered drug (960 μl/hr i.v.) immediately after injury over a 5 hr period. Animals were sacrificed for assessment of brain water content by Wet/Dry method and AQP4 protein expression by immunoblotting expressed as the ratio of AQP4 and Cyclophilin-A densitometries.
Findings Elevated AQP4 expression levels and water content were observed on the right injured side in both the right anterior (RA) and right posterior (RP) section compared to the left non-injured side inclusive of the left anterior (LA) and right anterior (RA) sections. The average AQP4 expression levels in contused areas for animals receiving SR drug treatment (RA: 1.313±0.172, RP: 1.308±0.175) were significantly decreased from vehicle-treated animals (RA: 2.181±0.232, RP: 2.303±0.370, $p=0.001$, $p=0.003$). Water content levels on SR treatment (78.89±0.14) was also significantly decreased from vehicle levels (80.38±0.38, $p<0.01$) in the traumatized hemisphere.
Conclusions SR49059 significantly reduced trauma-induced AQP4 up-regulation in the contused hemisphere. Moreover, brain water content was also significantly reduced paralleling the AQP4 suppression. These data provide further support that vasopressin (AVP) and V1a receptors can control water flux through astrocytic plasma membranes by regulating AQP4 expression. Taken in concert, these results affirm our laboratories contention that AQP4 can be effectively modulated pharmacologically.

Keywords AQP4 · AVP · V1a receptor · SR49059 · Brain edema

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Introduction

Brain edema, the infiltration and accumulation of excess fluid in the brain, leads to an increase in brain tissue

volume, a key determinant of morbidity and mortality following TBI [16, 21, 25]. The cellular and molecular mechanism contributing to the development and resolution of TBI-associated brain edema are not well understood and current treatments are unsatisfactory.

Aquaporins (AQPs), a family of water channel proteins ubiquitously distributed throughout the body, comprises at least 12 members in mammals [1, 34] and mediate rapid trans-membrane movement of water. One member of this family, aquaporin-4(AQP4), is abundantly expressed in brain, specifically located in astrocytes and ependymal cells, the cells facing capillaries and pia mater [22]. Recently, many groups have proposed that AQP4 plays a significant role in the pathophysiology of brain edema [1, 2, 25, 33]. Manley et al. demonstrated that AQP4 deletion protected mice from brain swelling in two models of primarily cytotoxic edema: water intoxication and permanent focal cerebral ischemia [20].

It has long been known that the brain contains an intrinsic arginine vasopressin (AVP) fiber system [4, 9, 18]. Many authors have described that centrally released AVP plays an important role in the brain capillary water permeability [12, 26] and ionic homeostasis [6, 8]. Of great theoretical and practical importance, there exists some possibility that of AVP may be mediated by modulation of a specialized water channel, AQP4 [17, 23]. AVP regulates AQP4 expression and translocation in kidney collecting ducts, modulating water re-absorption and maintaining water homeostasis. In brain, in-vitro studies suggest that AQP4-mediated water flux is facilitated by vasopressin V1a receptor agonist [23]. The non-peptide V1 receptor antagonist OPC-21268 significantly reduced brain edema after cold brain injury [3].

Recently, our laboratory has explored the hypothesis that modulation of AQP4 channels may represent a potential avenue for therapeutic intervention. Kleindienst et al. (2006) demonstrated that the selective vasopressin receptor (V1a) antagonist, SR49059, reduced AQP4 expression and brain edema following middle cerebral artery occlusion (MCA-O) [17]. The results of these studies suggest that AQP4 modulation may be linked to AVP release and receptor activation and be part of the mechanisms controlling brain capillary water permeability and ionic homeostasis. Therefore, the objective of this study is to examine whether the selective V1a receptor antagonist, SR49059, reduces brain edema by modulating AQP4 expression following TBI.

Materials and methods

Animals

All experimental procedures involving animals were approved by the Virginia Commonwealth University (VCU) Institutional Animal Care and Use Committee (IACUC) and were conducted in accordance with the recommendations provided in the National Institutes of Health (NIH) guide for the Care and Use of Laboratory Animals. Experiments were carried out on 350 to 430 g adult male Sprague- Dawley rats (Harlan, Indianapolis IN). Rats were housed at 22±1°C with 60% humidity, 12-hour light/12-hour dark cycles, and pellet food and water ad libitum.

Controlled cortical impact injury and surgical procedure

A well-established controlled cortical impact injury models as previously described was used to cause TBI [11]. Rats were initially anesthetized with isoflurane (4.0%), intubated and then artificially ventilated with a gas mixture of nitrous oxide (70%), oxygen (30%) and isoflurane (0.4–2.0%). Rectal temperature was maintained at 37±0.5°C using a heat lamp. Catheters (P.E.50, Becton Dickenson and Company, Sparks, MD) were placed into the femoral artery and femoral vein. Mean arterial blood pressure (MABP), arterial blood gas levels, and brain temperature were monitored and recorded continuously using a data acquisition system (ADInstruments, Colorado Springs, CO). Animals were mounted on stereotaxic frame and secured by two ear bars and an incisor bar. A midline scalp incision was made, and, the skin and periosteum were retracted from the skull surface. A 10-mm-diameter craniotomy was made midway between bregma and lambda on the right side, with the medial edge of the craniotomy 1 mm lateral to midline. Injury was produced using a pneumatic impactor mounted at an angle of 10° from the vertical plane. A single impact at a velocity of 6 m/sec with a deformation depth of 3.0 mm was delivered. After injury, the removed skull section was replaced and sealed with bone wax and the incision was closed with 4-0 silk suture.

Study protocol and drug preparation

The objective of these experiments was to assess the effect of intravenous SR49059 administration on brain swelling and AQP4 expression following CCI. The animals were randomly assigned to vehicle infusion (n=16), or SR49059 infusion (n=16). SR49059 (Sanofi Recherche, Montpellier, France) was dissolved in 1.5% dimethyl sulfoxide (DMSO) as vehicle solution (Sigma-Aldrich, St Louis, MO). SR49059 was injected intravenously immediately after injury and then used in amount of 2.76 mg/kg at the same speed (960 μL/hr) on the basis of information obtained from the literature [29, 30] and our studies [17]. The drug was intravenously administered for 5 hours by using a continuous infusion pump (sp210w syringe pump, KD Scientific, Holliston, MA). Five hours after TBI, animals were

sacrificed by an overdose of isoflurane (5%) and decapitated in order to remove the brain. Cerebral tissue was used to measure brain water content by wet /dry weight method and AQP4 expression was assessed by immunoblotting.

Brain edema measurement

Percent brain water content was determined using the wet/dry weight method. After animals were killed by decapitation, brains were quickly removed and the cerebellum discarded. The right and the left hemispheres along the anatomic midline were separated, and the wet weight of each hemisphere was measured. The tissue was then completely dried in a desiccating oven at 95°C for 5 days, and dry weight of each hemisphere was recorded. The percentage water content (%water) was calculated for each hemisphere as follows: %water=[(wet weight−dry weight)/wet weight] ×100. Water content was reported in the left uninjured hemisphere and the right injured hemisphere comparing vehicle and SR treated groups.

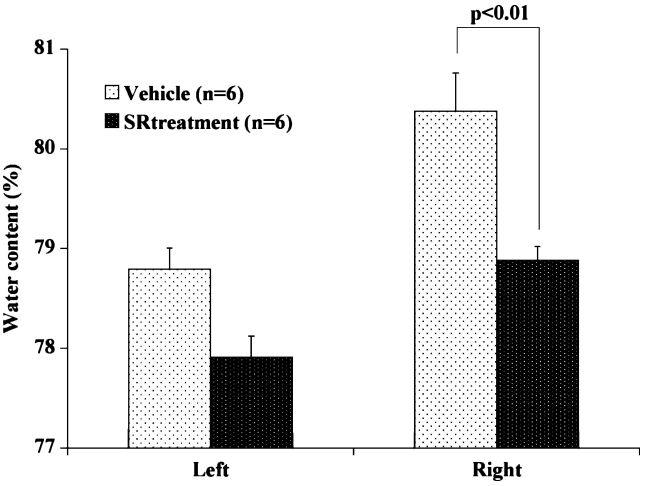

Fig. 2 Graph demonstrates that SR49059 treatment significantly reduced brain water content in the traumatized hemisphere. Both sections in the traumatized hemispheres (Right) showed increased water content compared to those in the non-traumatized hemispheres (Left)

Immunoblotting

Cerebral tissue was immediately cut into four consecutive 4 mm coronal sections excluding the most rostral and caudal sections from further analysis. The central 4 mm coronal section was then bisected into anterior and posterior sections and then bisected at the midline. This yielded 4 sections of tissue for analysis, two on the uninjured side designated left, anterior (LA) and right, posterior (RP) and two sections on the contused, or injured side designated, right anterior (RA) and right posterior (RP). Each of the four sections was homogenized on ice with a tissue homogenizer in 1200 μL of radioimmunoprecipitation buffer (50 mM Tris, 150 mM NaCl, 1% Igepal, 0.5% sodium *n*-dodecyl sulfate (SDS), 1%, pH 7.2) containing proteolysis inhibitors (Aprotinin 1.5 nM, E-64 Protease Inhibitor 0.01 μM, Leupeptin 0.01 μM). Homogenates were centrifuged at 13500 g at 4°C for 30 minutes to obtain supernatants and remove nuclei and mitochondria. Each protein concentration of supernatants was determined using protein assay kit (Bio-Rad Laboratories, Hercules, CA) and loading samples were adjusted to the same concentration (0.6 μg/μL) using sample buffer (Invitrogen, Carlsbad, CA). Protein (15 μg) from each sample was loaded for electrophoresis into 4–12% Bis-Tris polyacrylamide gels (Invitrogen, Carlsbad, CA), and subsequently transferred to a nitrocellulose membrane (Invitrogen, Carlsbad, CA). After the transfer, membranes were blocked for 45 minutes at room temperature in tris buffered saline plus Tween-20 (TBS-T)(10 mM Tris, 150 mM NaCl, 0.05% Tween-20, pH 7.5) with 3% milk powder, then incubated overnight at 4°C in AQP4 monoclonal antibody (Abcam, Inc., Cambridge, MA) diluted 1:750. The next morning, the membrane in primary antibody was incubated at room temperature for 20 minutes, washed three times for 10 minutes in TBS, blocked for 30 minutes, and subsequently incubated for 2 hours in secondary goat anti-mouse antibody (Rockland Gilbertsville, PA) diluted 1:5000. After two washes in TBS-T and three in TBS, immunodetection of AQP4 proteins was accomplished using an enhanced chemiluminescence system (Amersham, Buckinghamshire UK). Densitometric analysis was used to quantify AQP4 protein expression levels by determining intensity values for each band relative to cyclophilin-A (used as an internal control for lane loading).

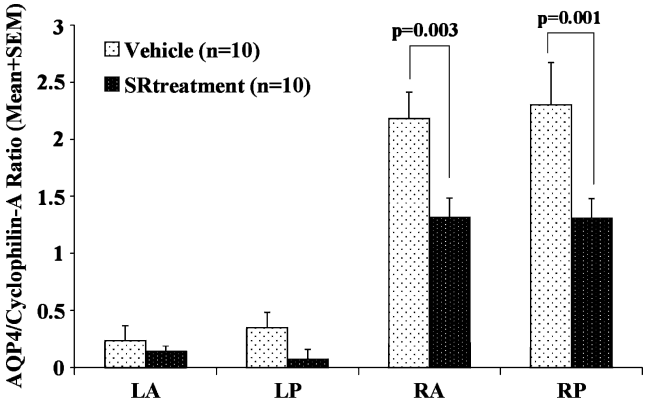

Fig. 1 Graph demonstrates that selective V1a receptor antagonist, SR49059, significantly reduced trauma-induced AQP4 up-regulation in the contusional areas. Both traumatized areas (RA, RP) showed the strong up-regulation of AQP4 compared to non-traumatize areas (LA, LP)

Statistical analysis

All data are given as means ± SEM. Statistical analyses were performed by Student's unpaired two-tail t-test. Values of $p<0.05$ were considered significant.

Result

All experiments were carried out without injury-induced morality. MABP and arterial blood gases were kept within physiological limits throughout the experimental procedure, requiring few adjustments in the isoflurane concentration and ventilation parameters. Elevated AQP4 expression levels and water content were observed on traumatized side (RA and RP) compared to the non-traumatized side (RA and RP). In brain sections bearing the contusion, the average AQP4 expression level in rats subjected to SR treatment (RA:1.313±0.172, RP:1.308±0.175) was significantly decreased from those treated with vehicle (RA:2.181±0.232, RP: 2.303±0.370, $p=0.001$, $p=0.003$) (Fig. 1). Water content levels on SR treatment (78.89± 0.14) was also significantly decreased from vehicle levels (80.38±0.38, $p<0.01$) in the traumatized hemisphere (Fig. 2).

Discussion

Arginine vasopressin (AVP) is a neuropeptide that is synthesized in the hypothalamus and transported via the axonal fiber system to the neurohypophysis, which releases the hormone into the blood stream [4]. AVP has been implicated in numerous central functions, including influence on brain water permeability [12, 26], regulation of intracranial pressure [24] and cerebrospinal fluid production [14]. The intraventricular injection of AVP aggravated cold injury edema and ischemic brain edema in mammals [10, 13, 27]. In vitro, AVP has also been shown to regulate glial cell volume [5, 7, 19]. Moreover, treatment with AVP receptor antagonist prevented cold-induced vasogenic edema [3] and hemorrhagic brain edema [28]. Accordingly, the increase of AVP is strongly associated with brain edema formation and facilitating transport of water from blood to brain.

It is generally assumed that V1a receptors mediate the central effects of AVP. The compound SR49059 has been characterized recently as the most potent and selective non-peptide AVP V1a antagonist described thus far, with marked affinity, selectivity and efficacy in both animal and human receptors [29, 32]. This compound inhibits AVP-induced vascular smooth cell contraction, blood pressure elevation and platelet aggregation [29, 31].

In this study, we investigated the effects of intravenously administered SR49059, a non-peptide selective AVP V1a receptor antagonist, on trauma-induced brain edema in rats. Our results showed that the increase in brain water content induced by CCI was significantly suppressed by treatment with SR49059. Recently, in our laboratory we have also found that treatment with SR49059 significantly reduced brain water content following middle cerebral artery occlusion model [17]. Collectively, these studies provide further experimental confirmation that the V1a receptor antagonist has protective effects on brain edema.

Water can cross cell membranes through different pathways: specific water channels (aquaporins), the lipid bilayer [15], or ion-water co-transport proteins [35, 36]. Specifically, in the central nervous system, AQP4 has been suggested to play a crucial role in cerebral water balance because of its anatomical and cellular localization. One possible explanation for the protective effects of SR49059 on brain edema formation found in this study is that AQP4 expression or activity may be regulated by AVP through V1a receptor. In these experiments, we found that trauma-induced AQP4 up-regulation was prevented by SR49059. To the best of our knowledge, this study is the first to demonstrate that following CCI, AQP4 expression is reduced by treatment with SR49059, the selective AVP V1a receptor antagonist. Although the precise molecular mechanisms of AQP4 regulation are not yet known, taken in concert, our study strengthens the basic concept that AQP4 expression can be regulated pharmacologically to effect a reduction in brain edema. Further experiments are needed to elucidate the fundamental mechanisms of AQP4 regulation and the relationship of AVP to V1a to provide novel treatments for reducing brain edema following TBI.

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Conflict of interest statement We declare that we have no conflict of interest.

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