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Arginine-vasopressin V_{1a} receptor inhibition improves neurologic outcomes following an intracerebral hemorrhagic brain injury

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ABSTRACT

Cerebral edema is a devastating consequence of brain injury leading to cerebral blood flow compromise and worsening parenchyma damage. In the present study, we investigated the effects of arginine-vasopressin (AVP) V_{1a} receptor inhibition following an intracerebral hemorrhagic (ICH) brain injury in mice and closely assessed the role it played in cerebral edema formation, neurobehavioral functioning, and blood–brain-barrier (BBB) disruption. To support our investigation, SR49059, an AVP V_{1a} receptor competitive antagonist, and NC1900, an arginine-vasopressin analogue, were used. Male CD1 mice ($n = 205$) were randomly assigned to the following groups: naïve, sham, ICH, ICH with SR49059 at 0.5 mg/kg, ICH with SR49059 at 2 mg/kg, ICH with NC1900 at 1 ng/kg, ICH with NC1900 at 10 ng/kg, and ICH with a combination of SR49059 at 2 mg/kg and NC1900 at 10 ng/kg. ICH was induced by using the collagenase injection model and treatment was given 1 h after surgery. Post assessment was conducted at 6, 12, 24, and 72 h after surgery and included brain water content, neurobehavioral testing, Evans Blue assay, western blotting, and hemoglobin assay. The study found that inhibition of the AVP V_{1a} receptor significantly reduced cerebral edema at 24 and 72 h post-ICH injury and improved neurobehavioral function while reducing BBB disruption at 72 h. Western blot analysis demonstrated increased protein expression of aquaporin 4 (AQP4) in vehicle, which was reduced with AVP V_{1a} receptor inhibition. Our study suggests that blockage of the AVP V_{1a} receptor, is a promising treatment target for improving ICH-induced brain injury. Further studies will be needed to confirm this relationship and determine future clinical direction.

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1. Introduction

Perihematomal cerebral edema is a serious life threatening consequence following an intracerebral hemorrhagic (ICH) brain injury (Xi et al., 1998). Two types of edema can present itself after such an event – namely vasogenic and cytotoxic edema. Vasogenic edema occurs predominately as a result of BBB disruption whereas, cytotoxic edema refers to the intracellular accumulation of water. Today, despite promising basic science research aimed at reducing and/or eliminating cerebral edema, bench work has not been able to translate into clinical improvements. This is partly due to the lack of clear understanding behind the pathophysiology of edema evolution.

Arginine vasopressin (AVP) is an antidiuretic non-peptide hormone responsible for regulating water and electrolyte homeostasis in the body (Shuaib et al., 2002). It is produced in the hypothalamus and released by the posterior pituitary into the blood stream, initiating a wide array of complex actions in the body. There is increasing evidence that AVP can be released directly into the brain by an alternate pathway, acting as a neurotransmitter regulating water permeability, ion homeostasis, and cerebrospinal fluid production (Vakili et al., 2005). Studies across a wide spectrum of brain injury paradigms including subarachnoid hemorrhage (Doczi et al., 1984), traumatic brain injury (Trabold et al., 2008), ischemic stroke (Liu et al., 2000), and global cerebral ischemia (Molnar et al., 2008) have speculated AVPs key role in brain edema formation. In an acute ischemic stroke model in gerbils, cerebral edema was exacerbated by intracerebroventricular administration of AVP while reduced by AVP antiserum injection (Liu et al., 1996).

Abbreviations: ICH, intracerebral hemorrhage; BBB, blood–brain-barrier; ICU, intensive care unit; AVP V1a, arginine–vasopressin V1a receptor; AQP, aquaporin.

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While in a middle cerebral artery occlusion model, AVP knock-out rats showed a reduction in cerebral edema 4 h after injury (Dickinson and Betz, 1992). However, even with previously published work acknowledging the role of AVP in brain edema formation, the exact mechanism has not been elucidated.

One possible mechanism that has been speculated is the role of brain aquaporins (AQP) in brain water homeostasis. Specifically AQP-4, the most abundant water channel in the brain, has been implicated in a number of brain injury-induced cerebral edema cases (Liu et al., 1996). Located in the blood–brain interface, AQP-4 is an integral membrane protein that regulates the flow of water in and out of the membrane (Yukutake and Yasui, 2009). Because there is an interaction between AVP and AQP in the kidneys where body water homeostasis is regulated, it begs the question if there is an interaction between these two proteins in the brain.

In the present study, we investigated the role of AVP V_{1a} receptor inhibition and its effects on cerebral edema, neurobehavioral functioning, and BBB disruption. We hypothesize that AVP activation is responsible for cerebral edema accumulation after injury and may do so, through opening of AQP4 channels. In order to test our hypothesis, two pharmacologic interventions were made to manipulate the AVP V_{1a} receptor – SR49059, a V_{1a} receptor competitive antagonist and NC1900, an AVP analogue. Both drugs were selected because of their high specificity to the V_{1a} receptor and marked affinity, selectivity, and efficacy towards both animal and human receptors (Serradeil-Le Gal et al., 1993).

2. Experimental procedures

2.1. Animal groups

This study was in accordance with the guidelines of the National Institute of Health for the treatment of animals and was approved by the Institutional Animal Care and Use Committee at Loma Linda University. Male CD1 mice (weight 35–45 g, Charles River, MA, USA) were housed in a 12-h light/dark cycle at a controlled temperature and humidity with free access to food and water. Mice were divided into the following groups: sham ($n = 31$), ICH ($n = 48$), ICH treated with low-dose antagonist (SR49059 at 0.5 mg/kg; $n = 6$), ICH treated with a high-dose antagonist (SR49059 at 2 mg/kg; $n = 50$), ICH treated with NC1900 at 1 ng/kg ($n = 6$), ICH treated with NC1900 at 10 ng/kg ($n = 30$), and ICH treated with a combination SR49059 at 2 mg/kg and NC1900 at 10 ng/kg ($n = 24$).

In order to test the potential systemic effects of SR49059, ten additional mice were used to evaluate blood pressure and blood gas analysis (5 naïve and 5 naïve animals treated with high-dose SR49059).

2.2. Operative procedure

The collagenase-induced intracerebral hemorrhage model (Rosenberg et al., 1990) was adapted as previously described in mice (Choudhri et al., 1997). Briefly, mice were anesthetized with a ketamine (100 mg/kg)/xylazine (10 mg/kg) combination intraperitoneal injection and positioned prone in a stereotaxic head frame (Stoelting, Wood Dale, IL, USA). An electronic thermostat-controlled warming blanket was used to maintain core temperature at 37 °C. The calvarium was exposed by a midline scalp incision from the nose to the superior nuchal line, and the skin was retracted laterally. With a variable speed drill (Fine Scientific Tools, Foster City, CA, USA) a 1.0 mm burr hole was made 0.9 mm posterior to the bregma and 1.45 mm right-lateral to the midline. A 26-G needle on a Hamilton syringe was inserted with stereotaxic guidance 4.0 mm into the right deep cortex/basal ganglia at a rate 1 mm/min. Collagenase (0.075 units in 0.5 μL saline; Sigma, St. Louis, MO, USA) was then infused into the brain at a rate of 0.25 μL/min over 2 min using an automatic infusion pump (Stoelting, Wood Dale, IL, USA). The needle was left in place for an additional 10 min after injection to prevent the possible leakage of collagenase solution. After removal of the needle, the incision was sutured closed and mice were allowed to recover. Sham operation was performed with needle insertion only.

In order to effectively measure potential systemic effects of our drugs, blood pressure and blood gas analysis were conducted on 10 separate mice. Animals were anesthetized first by ketamine (100 mg/kg IP) and xylazine (10 mg/kg IP). The left femoral artery was then cannulated for blood pressure recordings and withdrawal of blood samples. A blood pressure analyzer (Digi-Med BPA-100, Micro Med Inc. Louisville, USA) was used to measure blood pressure while the blood gas analysis was conducted using the GEM4000 premier (Instrumentation Laboratory Bedford USA). Baseline pO_2 and pCO_2 values were evaluated immediately after induction of anesthesia. Afterwards, SR49059 or vehicle was injected as described in Section 2.3. The effects of the drug were evaluated at 15, 30 and 60 min intervals following administration.

2.3. Treatment method

SR49059 (Tocris Bioscience, Ellisville, Missouri) was dissolved in 0.5% DMSO and administered one time intraperitoneally approximately 1 h after ICH induction. The agonist, NC-1900 was dissolved in saline and administrated as a single dose subcutaneously 1 h after ICH.

2.4. Brain water content

Brain water content was measured as previously described (Yang et al., 1994). Briefly, animals were sacrificed at 6 h, 12 h, 24 h and 72 h post ICH and brains were immediately removed and divided into five parts: ipsilateral and contralateral basal ganglia, ipsilateral and contralateral cortex, and cerebellum. The cerebellum was used as an internal control for brain water content. Tissue samples were then weighed on an electronic analytical balance (APX-60, Denver Instrument; Arvada, CO) to the nearest 0.1 mg to obtain the wet weight (WW). The tissue was then dried at 100 °C for 48 h to determine the dry weight (DW). The percent brain water content was calculated as $[(WW - DW)/WW] \times 100$.

2.5. Assessment of neurobehavioral deficits

Neurological outcomes were assessed by a blind observer at 6 h, 12 h, 24 h and 72 h post ICH using the Modified Garcia Score (Garcia et al., 1995).The Modified Garcia Score is an 21-point sensorimotor assessment system consisting of seven tests with scores of 0–3 for each test (max score = 21). These seven tests included: (i) spontaneous activity, (ii) side stroking, (iii) vibris touch, (iv) limb symmetry, (v) climbing (vi) lateral turning, and (vii) forelimb walking.

In the beam balance test, animals were placed at the middle of a beam, 590 cm in length by 51 cm in width, and positioned between two platforms horizontally. The ability of the animals to reach one of the platforms was recorded. Animals who reached one of the two platforms within 25 s were given a 5 point rating. Animals that moved on the platform between 25 and 40 s were given a 4 point rating. Animals that moved more than half the distance to the platform and stayed on the beam for at least 25 s were given a 3 point rating. Animals that stayed on the beam at least 25 s but moved less than half a distance to the platform were given a 2 point rating. Animals that were able to stay on the beam for at least 40 s without movement were given a 1point rating while those who fell off before 40 s got a 0 rating.

Wire hanging test was carried out similarly to what was described previously (Gerlai et al., 2002). Briefly mice were placed on a horizontal wire between two platforms. The animals were given an opportunity to grasp the wire with both forepaws before the blinded tester let go. Animals that were able to reach one of the platforms in 45 s or less were given a 5 point rating. Those animals that were able to hang on for more than 30 s and use more than their forelimbs (hind limps or tail) but did not reach the platform in <45 s were given a 4 point rating. Animals that were able to hang on for more than 30 s and use their forelimbs in a symmetrical manner for at least 10 s were given a 3 point rating. Mice that were able to hang on for more than 30 s in any manner were given a 2 point rating. Finally, animals that were able to hang on between 15 and 30 s were given a 1 point rating while those animals that fell off the wire within 15 s were given a 0 point rating.

The tests were repeated three times, and an average score was taken.

2.6. Western blotting of Aquaporin-4

Animals were perfused with cold 0.1 M PBS at 72 h post ICH. The peri-hematomal (right cortex) was isolated and snap-frozen in liquid nitrogen for analysis. Individual protein samples (50 μg each) were subjected to electrophoresis and then transferred to a nitrocellulose membrane for 80 min at 70 V (Bio-Rad). Blotting membranes were incubated for 2 h with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 and then incubated overnight at 4 °C with primary antibody (anti-aquaporin) (1:200; Santa Cruz Biotechnology, Santa Cruz, Calif). The membranes were incubated for 1 h with secondary antibodies (1:1000; Santa Cruz Biotechnology) and processed with an enhanced chemiluminescent reagent kit (Amersham Bioscience, Arlington Heights, Ill) on X-ray film (Kodak, Rochester, NY).

2.7. Evans blue assay

BBB permeability was measured as previously reported (Saria and Lundberg, 1983). Under general anesthesia Evans Blue dye (2%; 4 mL/kg) was injected intravenously into the jugular vein and allowed to circulate for 1 h. This was followed by perfusion with PBS (40 mL) via the aorta. The brains were subsequently removed and divided into right and left hemispheres frozen in liquid nitrogen and stored at −80 C. Brain samples were homogenized in 600 μL of PBS and centrifuged (30 min, 15,000 rcf, 4 °C). The supernatant was collected and equal amounts of 50% trichloroacetic acid was added followed by another centrifuge (30 min, 15,000 rcf, 4 °C). The amount of Evans Blue dye was measured by the spectrophotometer (Spectronix 3000, Milton-Roy, Rochester, NY, USA) and quantified according to a standard curve.

2.8. Hemoglobin assay

Initially, a standard curve was obtained using a 'virtual' model of hemorrhage. Hemispheric brain tissue was obtained from mice subjected to complete

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