Video Article

Continuous IV Infusion is the Choice Treatment Route for Arginine-vasopressin Receptor Blocker Conivaptan in Mice to Study Stroke-evoked Brain Edema

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Abstract

Stroke is one of the major causes of morbidity and mortality in the world. Stroke is complicated by brain edema and other pathophysiological events. Among the most important players in the development and evolution of stroke-evoked brain edema is the hormone arginine-vasopressin and its receptors, V1a and V2. Recently, the V1a and V2 receptor blocker conivaptan has been attracting attention as a potential drug to reduce brain edema after stroke. However, animal models which involve conivaptan applications in stroke research need to be modified based on feasible routes of administration. Here the outcomes of 48 hr continuous intravenous (IV) are compared with intraperitoneal (IP) conivaptan treatments after experimental stroke in mice. We developed a protocol in which middle cerebral artery occlusion was combined with catheter installation into the jugular vein for IV treatment of conivaptan (0.2 mg) or vehicle. Different cohorts of animals were treated with 0.2 mg bolus of conivaptan or vehicle IP daily. Experimental stroke-evoked brain edema was evaluated in mice after continuous IV and IP treatments. Comparison of the results revealed that the continuous IV administration of conivaptan alleviates post-ischemic brain edema in mice, unlike the IP administration of conivaptan. We conclude that our model can be used for future studies of conivaptan applications in the context of stroke and brain edema.

Video Link

The video component of this article can be found at https://www.jove.com/video/54170/

Introduction

Stroke continues to be an enormous burden for patients and clinicians. Animal stroke models have been used in the laboratory setting for nearly two decades.[1] Nevertheless, experimental treatments that work in animals often fail in humans.[2] This discrepancy in treatment outcomes may be due to various factors, such as administration routes for drugs used in animal research, drug metabolism and elimination rate, and many other aspects. One of the major complications of stroke, brain edema, is a focus of current research in neuroscience. Mechanisms of brain edema formation involve disturbances in water and electrolyte balance triggered by the arginine-vasopressin (AVP) response to ischemic brain injury.[3] Therefore, AVP and its receptors (V1a and V2) are a major focus of research studies of post-ischemic brain edema formation.[3]

We have developed a methodology to study the effects of mixed arginine-vasopressin (V1a and V2) receptor blocker conivaptan on post-ischemic brain edema in mice.[4] Molecular targets of conivaptan[5] make the drug a suitable candidate for exploration of its properties in alleviation of brain edema. Furthermore, conivaptan needs to be studied in the context of pathophysiological events of stroke.[6] In designing this study, we considered comparing treatment outcomes using two different routes of administration for conivaptan: intravenous (IV)[4] and intraperitoneal (IP).[7] Effects of the treatments on stroke-induced brain edema were evaluated. Here detailed protocols are described for surgical induction of experimental stroke by middle cerebral artery occlusion (MCAO), and followed by continuous conivaptan treatment using the installation of a jugular IV catheter. After induction of stroke, animals were randomly allocated into the following groups: vehicle or conivaptan (0.2 mg/day) treated IV or IP.

Protocol

Experiments were carried out in accordance with the guidelines of the National Institutes of Health for the care and use of animals in research and were approved by the Swedish Medical Center Animal Care and Use Committee. All procedures were performed with appropriate aseptic techniques. Experimental animals utilized for the study were male, 3 months old, wild type C57 mice with body weight from 25 to 27 g.

1. *In Vivo* Stroke Induction

1. Pre-coat the Filament with Dental Resin Prior to the Surgery.
    1. Cut a 12 mm long piece of filament from a 7-0 nylon suture. Mix 2 parts of the resin with 1 part of the hardener, and then immediately dip the tip of the filament into the mixture to cover about 1/3 of the entire length. Remove it quickly and verify that it is covered with a smooth-surfaced coat of dental resin.
    2. Air dry the filament for 2 hr before use.

2. Place the mouse into an anesthesia induction chamber (2 L volume), and set the flow of 1.5% isoflurane in 25% oxygen-enriched room air at 2 L/min going into the chamber.
3. Allow the mouse to remain in the induction chamber until fully anesthetized,[8] as determined by lack of response to tail pinch.
4. Place the mouse onto the operating table with a heated pad, and set the isoflurane concentration on 1% for maintenance of anesthesia delivered spontaneously through the nose cone.
5. Clip hair on the front and the back of the neck, and spray the neck with rubbing alcohol. Apply veterinary ophthalmic ointment on both eyes.
6. To continuously monitor body temperature, use a rectal temperature probe connected to digital display during the surgery.
7. Place the mouse in the supine position, apply povidone-iodine 10% scrub solution for disinfection, cover with a sterile drape, and then make a midline incision along the neck with a size 10 surgical scalpel blade.
8. Temporarily ligate the left common carotid artery (CCA) with 6.0 silk suture.
9. Place another tie on the left external carotid artery (ECA).
10. Place a microvascular clip on the internal carotid artery (ICA).
11. Cut a small hole on the external carotid artery with microvascular scissors. Insert a filament made from 7-0 nylon suture coated with dental resin into the opening in the ECA, and advance it cephalically, while removing the clip, until resistance is felt (about 5-7 mm from the bifurcation point of the CCA).
12. Place a temporary tie onto the internal carotid artery to secure the filament in position. Refer to the diagram, **Figure 1A**. Close the skin with silk suture, and infiltrate the wound on the front of the neck with 0.2 ml of 0.5% bupivacaine, as in the step 2.6.
13. Allow the mouse to awaken from anesthesia and regain consciousness in a recovery chamber for 60 min. Do not leave the animal unattended until it has regained sufficient consciousness to maintain sternal recumbence.
14. Test the mouse for neurological deficit scoring (NDS) as follows: 0 = normal motor function, 1 = flexion of torso and of contralateral forelimb on tail lift, 2 = circling to the contralateral side but normal posture at rest, 3 = leaning to contralateral side at rest, 4 = no spontaneous motor activity.[4,9]
    1. For the NDS testing, lift mouse by the tail and place on a flat surface for observation (2-3 min). Alternatively, observe mice in the recovery chamber. Exclude mice that exhibit NDS lower than 2 from the experiment due to insufficient occlusion of the MCA.
    Note: Laser Doppler flowmetry technique can be used to confirm MCA occlusion.[9-11]

15. Re-anesthetize the animal after 60 min of recovery in the induction chamber as described above, re-sterilize the previous skin incision with 10% povidone-iodine scrub solution, and reopen the surgical wound on the front of neck by cutting and removing the silk suture.
16. To remove the filament from the ECA perform the following steps:
    1. Place a microvascular clip on the ICA. Untie the suture on the ICA and pull the filament out while holding clip open.
    2. Close the clip and place a tie onto the ECA proximally to the cut.
    3. Remove the clip and remove the ligature from the CCA to restore blood flow to the brain. Refer to the diagram, **Figure 1B**.

2. Installation of IV Catheter into the Jugular Vein for Continuous 48 hr Treatment

Note: Proceed to installation of the catheter immediately after the step 1.16.3 without awakening of the mouse.

1. Use two different sizes of flexible tubing: insert the inner tubing (0.94 mm outside diameter; to contain the drug being infused) into the outer tubing (3.18 mm outside diameter; for protection of the inner tubing).
2. Place the mouse in the prone position, apply 10% povidone-iodine scrub solution for disinfection, and make a 1 cm incision on the back of the neck with a size 10 surgical scalpel blade. Turn the mouse into the supine position, re-sterilize the front of the neck with 10% povidone-iodine scrub solution, and reopen the surgical wound on the front of the neck. Tunnel the tubing under the skin from the incision made on the back to the front of the neck, and exteriorize it from the open wound 1 cm.
3. Remove subcutaneous adipose tissue from the left side of the front of the neck about 1 cm laterally from the midline. Locate the left jugular vein, place two ties along the vein 5 mm apart, and slightly stretch the vein. Make a small hole on the jugular vein between the two ties with microvascular scissors; insert the tip of the inner tubing 5 mm deep caudally (towards the heart).
4. Secure the tubing with both ties to the jugular vein. Close the skin on the front of the neck with a 3-0 silk suture. Refer to the diagram, **Figure 1C**.
5. Secure the tubing to the skin on the back of the neck with a suture, and connect the tubing to a microinfusion IV pump through a swivel. The swivel allows free movement of mice inside the cage with full access to the food and water.
6. Infiltrate the skin incisions on the front and the back of the neck with 0.2 ml of 0.5% bupivacaine to prevent post-operative pain.
    Note: Depending on institutional specific animal protocols, animals may be treated with opioids, NSAIDs, or antibiotics pre- and post-operatively. However, mentioned treatments may alter results of ischemic stroke outcome (see **Discussion Section**).[11-14]
7. Allow the animal to awaken in the recovery chamber, and then house the animal in a separate cage until the end point of the experiment. Do not leave the animal unattended until it has regained sufficient consciousness to maintain sternal recumbence.
8. Set the infusion rate for continuous 48 hr IV treatment of conivaptan (0.14 mg/ml in 5% dextrose) or vehicle at 1.5 ml/kg/hr,[4] and start the infusion.
    Note: The total amount of IV infused conivaptan should be approximately 0.2 mg/mouse/day in a total volume of 1.44 ml.

Note: Perform Intraperitoneal (IP) injection of conivaptan twice daily with total amount of 0.2 mg in 1.44 ml of 5% dextrose. Implement IP injection was as previously described.[15] Restrain mice by gripping the skin of the posterior surface of the body and the tail. Inject conivaptan or vehicle into the left lower quadrant of the abdomen using a 26 G/1/2 inch needle.[15]

9. Observe the animals twice a day for the entire duration of the experiment (48 hr). Allow the animals to have full access to food and water. If the animal exhibits signs of distress or dyspnea consult a veterinarian. If euthanasia is necessary for the animal in severe distress refer to the step 3.1 below, and exclude the animal from the study.

3. Evaluation of Stroke-evoked Brain Edema at the end Point

1. Sacrifice the mouse by over-anesthetizing with 5% isoflurane.
2. Use a scalpel to make a skin incision over the skull bone along the midline, and retract the skin to expose the entire skull over the brain. Beginning at the foramen magnum, cut the skull bone laterally along the perimeter of the brain on each side, and lift it carefully without touching the brain beneath it. Sever the brain from the spine and the nerves attached on the base of the skull.
3. Remove the brain, separate it from the olfactory bulb and the cerebellum, and dissect the cerebrum along the interhemispheric fissure into the ischemic and non-ischemic cerebral hemispheres, as previously described.[9]
4. Assess brain edema by comparing wet-to-dry ratios (WDR) as described previously[4,9]. Weigh tissues before and after drying for 3 days at 100 °C in an oven. Calculate brain water content (BWC) as % H_2O = (1-dry wt. /wet wt.) x 100%.

Representative Results

Body temperature of the animals was within the physiological range and stable throughout the surgical procedure of stroke induction. Two mice that exhibited NDS lower than 2 immediately after MCAO were excluded from the study.

MCAO in mice produces infarct volume in the ipsilateral hemisphere at 48 hr. Evaluation of the TTC-stained slices shows that about 50% of the hemisphere is affected by infarct after MCAO (**Figure 1D**), as published by Zeynalov, E. and Dore, S., 2009.[10] The onset of brain ischemic stroke causes severe deprivation of brain tissue in oxygen and nutrients. As a result of this local brain tissue hypoxia, neurons and glial cells die, which form the infarcted part of the brain. **Figure 1D** shows representative mouse brain slices affected by MCAO-induced infarct, and gives an estimation of the size of an injury.[10]

Evaluation of brain water content (BWC) at 48 hr after 60 min MCAO induction revealed that conivaptan administered IV (0.2 mg/day) significantly reduces brain edema in both ipsilateral and contralateral hemispheres, (**Figure 2A**). In contrast, IP-treatment of conivaptan by 0.2 mg bolus daily failed to produce similar positive effects on stroke-evoked brain edema (**Figure 2B**).

Confirmation of conivaptan effect on plasma and urine osmolality was achieved in all experimental animals at the end point of the experiment. Conivaptan treated IV or IP increased plasma and decreased urine osmolality in all mice.[4] Plasma osmolality values in mice were as follows: 313 ± 4.4 mOsm/kg (vehicle, IV) vs. 355 ± 7.5* mOsm/kg (conivaptan, IV),as previously published,[4] and 340 ± 7.7 mOsm/kg (vehicle, IP) vs. 383 ± 8.8* mOsm/kg (conivaptan, IP). Urine osmolality values in mice were as follows: 1210.6 ± 13.4 mOsm/kg (vehicle, IV) vs. 595.0 ± 57.4* mOsm/kg (conivaptan, IV), as previously published,[4] and 1535.0 ± 80.9 mOsm/kg (vehicle, IP) vs. 242.0 ± 23.2* mOsm/kg (conivaptan, IP). All data presented as mean ± SEM, *p ≤0.05 vs. corresponding control.

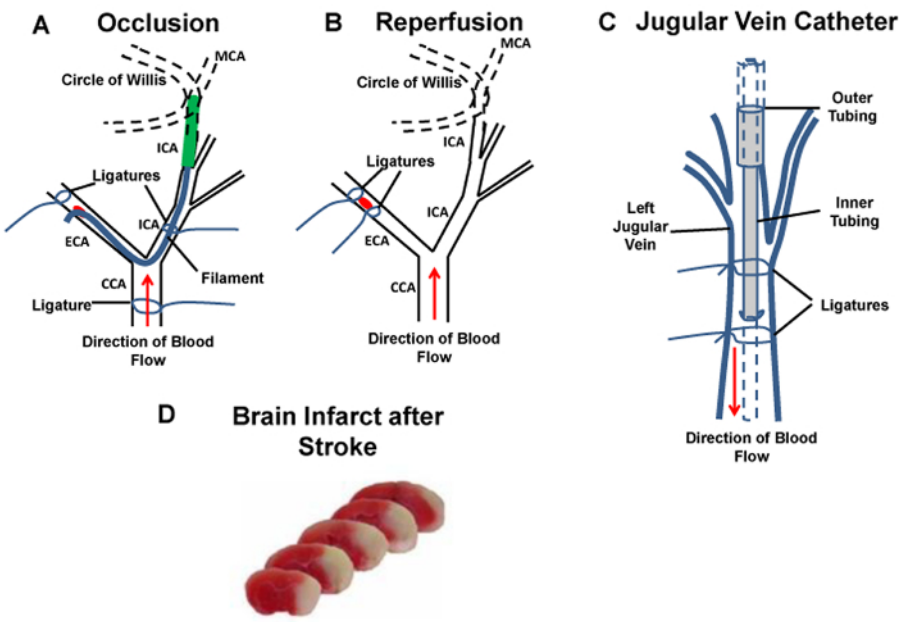

Figure 1: Diagram of the experimental stroke model, IV catheter installation, and representative infarct volume at 48 hr after stroke. Position of the filament (shown in blue) pre-coated with resin (green) in the left internal carotid artery (ICA) during the occlusion (**A**) and the reperfusion time (**B**). Correct position of the flexible tubing tip in the left jugular vein (**C**). Experimental stroke in mice produces infarct volume in the ipsilateral hemisphere at 48 hr. Mice were sacrificed at 48 hr after MCAO, and brains were removed, as described in the protocol (steps 3.1-3.3). Brains were cut in 2 mm thick sections, and stained with TTC (**D**). Figure 1D has been modified from the original article by Zeynalov, E. and Dore, S., 2009.[10] Please click here to view a larger version of this figure.

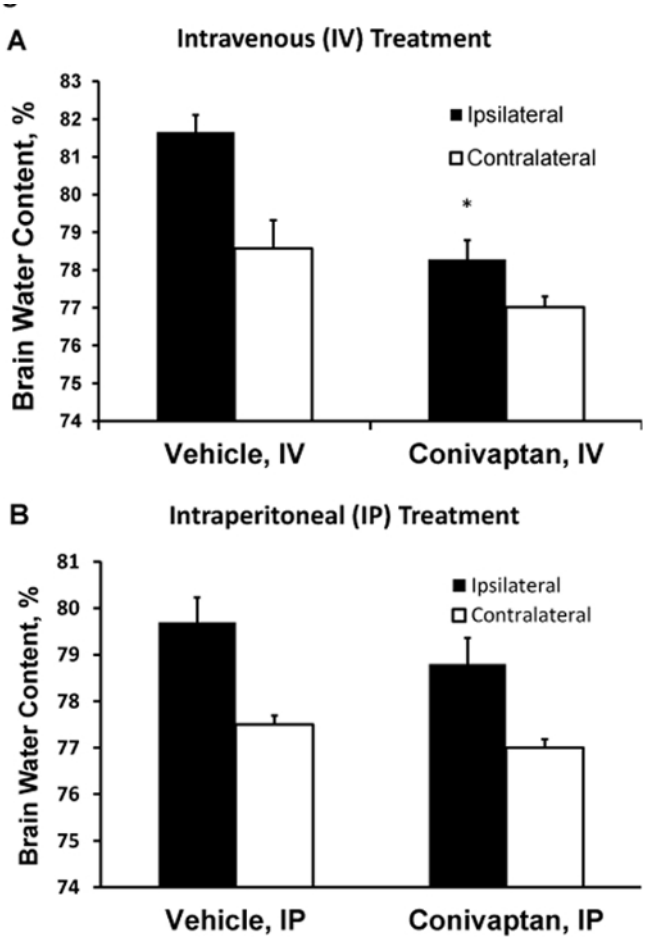

Figure 2: Effects of continuous intravenous (IV) and intraperitoneal (IP) conivaptan (0.2 mg/day) treatments on stroke-evoked brain edema. Conivaptan IV treatment was administered for 48 hr. Conivaptan treatment administered IV reduced post-ischemic brain edema in the ipsilateral and contralateral hemisphere (**A**). Figure 2A has been modified from the original article by Zeynalov *et al*., 2015.[4] Conivaptan IP treatment for 48 hr failed to reduce brain edema caused by experimental stroke, n = 10 in each group. Mice underwent MCAO with reperfusion as described in the protocol above (steps 1.1.1-1.15.3). Then IP injections of conivaptan (0.2 mg) were administered immediately, at 22 hr and 46 hr of reperfusion. Mice were sacrificed; the brains were removed and dried in the oven, as described in the protocol (steps 3.1-3.4). (B) All data is presented as mean ± SEM, *p <0.05 vs corresponding control. Please click here to view a larger version of this figure.

Discussion

This study has important value for preclinical stroke research. This study reveals that continuous IV infusion of conivaptan (0.2 mg/day) after experimental stroke in mice efficiently reduces brain edema after 48 hr of treatment. The effect of IP injection of the same dose of conivaptan on brain edema was also investigated. Conivaptan treatment by both IV and IP routes produces aquaresis in mice as indicated by: 1) increase in plasma osmolality slightly above physiological levels; and 2) decrease in urine osmolality due to blockage of water reabsorption in the kidney. Therefore, based on the treatment outcomes, we report that the IV delivery method has additional important beneficial effects on stroke-evoked edema formation.

Our major finding is that IV treatment produced better effect on brain edema alleviation than the IP route for conivaptan. Conivaptan is a combined V1a and V2 receptor antagonist, available for clinical use to correct hyponatremia, blood volume and osmolality.[16] Conivaptan has been shown to correct hyponatremia in patients with various conditions.[17] Clinical availability of conivaptan suggests that its exploration in the context of brain edema can result in fast bench-to-bedside translation. Use of experimental selective V1a receptor blockers delivered intracerebroventricularly has been proven to reduce post-ischemic brain edema formation described by other researchers.[3,18] However, neither the drug used in those studies nor the treatment route for the drugs suggests possible clinical application in the near future.

Mechanisms by which brain ischemia and AVP cause brain edema are complex. However, some important molecular players are sodium and chloride co-transporters[19,20] and V1a receptors which are localized in vascular walls at the BBB interface. The V2 blocking effect of conivaptan is responsible for excretion of excess water by the kidney and elevation of blood osmolality, which may also help prevent development of brain edema.[21] These mechanisms may still be in play when conivaptan is delivered directly into the circulation to avoid tissue barriers that may reduce conivaptan availability. However, justification of the dose and the treatment duration was based on previously published observations.[4] It remains to be determined whether conivaptan reaches the ischemic site of the brain after injury-induced decreases of regional blood flow. It also needs to be investigated whether the brain receives sufficient amount of conivaptan to produce the desirable local effects, such as prevention of vasoconstriction by blocking V1a receptors in cerebral arteries.

The experimental protocol was designed to address the question of choice for the treatment route of conivaptan. Based on the results, the study provides strong support for the IV route of conivaptan administration. However, most critical steps of protocol needed to be established and fine-tuned in order to detect significant differences in treatment outcomes after stroke in mice. Among those steps are: 1) the duration of the MCAO (60 min); 2) the daily dose (0.2 mg), and the infusion rate (1.5 ml/kg/hr); and 3) the length of the treatment (48 hr). Duration of MCAO determines the degree of the ischemic insult which is extremely crucial for the protocol because it irreversibly damages the brain tissue. Too severe of an injury would make it difficult for the animal to survive for 48 hr, or the animals could become less responsive to conivaptan treatment against brain edema. On the other hand, duration of MCAO less than 60 min may be insufficient to induce an ischemic insult in the mouse, and, therefore, would not produce brain edema. The dose and the length of the treatment were chosen based on the FDA recommendations for conivaptan use.[16] However, it has been reported that the human dose for conivaptan was not efficient in mice to reduce brain edema after stroke. Therefore, the dose had to be increased 10 times for mice, which induced significant protection against post-ischemic brain edema.[4] The importance of choosing the correct infusion rate is that it can affect the total blood volume, blood pressure and interstitial fluid accumulation if the rate greatly exceeds the suggested rate. However, measurements of total blood volume and blood pressure would be very informative.

Surgical induction of the MCAO is an invasive procedure, and requires precise attention to all the steps of the protocol, and it is important to know the troubleshooting tips for the technique. Some unexpected surgical complications, such as excessive arterial bleeding due to an accidentally torn artery reduce the success rate for the experiment and increase the mortality rate. However, venous bleeding can be stopped by applying light pressure with a sterile sponge.

This protocol describes a unilateral induction of brain ischemia, which implies that the MCA occlusion is produced either on the right or the left MCA. However, the protocol can be modified depending on the surgeon's preferred side of MCA occlusion and the IV catheter installation. **Figure 1** diagram depicts a drawing of the most critical parts of the MCAO procedure, **Figure 1A** and **B**, the IV catheter installation protocol, **Figure 1C**, and the representative brain slices of MCAO-induced brain infarct at the end-point of the experiment (48 hr), as previously published,[10] **Figure 1D**. Although the diagram and the representative brain slices imply that the MCAO was induced on the left side of the brain, we don't anticipate dramatic differences in results if MCAO was induced on the right side of the brain as long as consistency throughout the entire study is maintained.

During the recovery period after the surgery and anesthesia, the animals may experience distress due to post-operative pain and severe neurological deficits. In many cases, the postoperative pain management may be limited to using only local anesthetics such as 0.5% bupivacaine. Stroke due to cerebrovascular accident in humans as well as experimental stroke in mice involves pathophysiological factors such as inflammation, brain infarct, and brain edema.[9] These pathophysiological events are engaged by key molecular players such as cyclooxygenases (COX)[13] and G-protein related receptors.[22] Interference with the molecular pathways that involve COX may be caused by the NSAID drugs,[13] if they were used in these settings. Opioids act on their receptors which are linked to G-protein and second messengers[12] and can alter pathophysiological outcomes of stroke,[11] and the results of this study. These factors limit usage of pain medication in mice postoperatively.

In summary, our study explores the possibility of repurposing conivaptan as a far reaching goal. Our animal model of experimental stroke induction followed by continuous IV infusion of conivaptan has been shown to produce consistent results which suggest its potential use in stroke patients. More specifically, we have demonstrated that the model of continuous IV treatment of conivaptan after stroke to prevent secondary brain injury can be used for preclinical study in mice. This study was intended to offer an alternative application of conivaptan treatment as an additional tool to study brain edema in mice after stroke because fast translation into clinical settings may be lifesaving for ICU patients.

Disclosures

The authors have nothing to disclose.

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