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Lab resource

Cortical Aquaporin-4 in relation to brain oedema and neurological function of cortical cryo-injured mice

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ABSTRACT

To estimate the spatial and temporal expression of Aquaporin-4 (AQP-4) in a murine model of automated cerebral cryoinjury and correlate AQP-4 expression with development of brain oedema and neurological function. AQP-4 levels were determined quantitatively by Western blots at site of injury and at sites adjacent to and distant from injury in brains of cryoinjured (experimental) ($n = 18$), sham injured ($n = 18$) & normal mice at 24, 48, 72 h post injury. AQP-4 expression was correlated with percentage water content of brain, Neurological Severity Score (NSS) and rotarod scores. We found a 1.4-fold increase in expression of AQP-4 at the site of injury and at sites distant from injury at 24 h when compared to normal mice ($p = 0.05$). The increase in expression of AQP-4 24 h post injury was significantly higher in experimental group at the site of injury and at the site adjacent to the injury in the ipsilateral hemisphere when compared to the sham injured mice ($p = 0.05$). At 24 h post injury the median NSS score in the experimental group was 9 (interquartile range 7.25–10) and that in the sham group was 0.5 (interquartile range 0.0–1.0) ($p < 0.001$).

At 48 and 72 h, AQP-4 expression remained elevated in the experimental group when compared to normal brain, but the levels were not significantly different from that in sham group. AQP-4 expression was significantly elevated in the ipsilateral hemisphere in the first 24 h following cerebral cortical injury in mice and this could be correlated with worsening of neurological function. Over the next 48 h, there was a trend towards decrease in AQP-4 expression that was associated with partial recovery of neurological function.

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1. Introduction

Cerebral oedema following traumatic brain injury causes raised intracranial pressure, secondary neuronal damage, brain herniation and death [10]. Vasogenic oedema, the principal type of oedema seen in tumors, trauma, infections and ischemia, involves breakdown of the blood brain barrier and extravasation of the plasma into the brain [8,17,19,26]. This leads to increased brain volume, raised intracranial pressure, increased extracellular volume and brain displacement.

Aquaporin (AQP) belongs to family of transmembrane water channels present in plasma membranes of humans and higher mammals. The subtype aquaporin-4 (AQP-4), the main water channel in brain, is expressed in the astrocytic foot processes surrounding the capillaries and the glial limiting membrane of the brain [14]. It is also found in the astrocytes and ependymal cells throughout the brain and spinal cord at the pial and the ependymal surfaces that are in contact with CSF in subarachnoid spaces and ventricles [14,24].

AQP-4 is an important protein that plays a major role in maintaining the water homeostasis in the brain and a review of literature suggests that it has a role in early resolution of vasogenic oedema [1,6,13,15,20]. Several studies using AQP-4 knockout mice have demonstrated significant decrease in cerebral oedema following traumatic brain injury [24].

Although the spatial and temporal profile of AQP-4 following injury has been measured, its role in preserving neurological

Abbreviations: AQP-4, Aquaporin-4; NSS, neurology severity scale; RR, rotarod score.

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function has not been correlated with this spatio-temporal profile [6,13,21]. We proposed to validate the spatio-temporal profile of AQP-4 and study its relation to neurological function during the first 72 h following automated cerebral cortical cryoinjury. An improved understanding of the spatio-temporal profile of AQP-4 following brain injury could aid in determining the exact window period at which time AOP-4 modulation will be most effective after traumatic brain injury.

2. Materials and methods

The protocol was evaluated and approved by the Institutional Review Board and the Animal Ethics Committee of our institution.

2.1. Animals

Thirty-six young male adult (4–6 months of age) Swiss albino mice (18 each in the experimental and sham injury group) weighing 30–35 g were included. Twelve animals each were sacrificed at 24 h; 48 h and 72 h post procedure. Brains from six normal mice were obtained to determine the water content as well as AQP-4 distribution in the normal brain.

2.2. Creation of cold injury (Experimental group, n = 18)

Under general anesthesia (Inj. ketamine 100 mg/kg and Inj. xylazine 10 mg/kg), the mice were immobilized on a stereotactic frame (TMB Systems, Germany) after ensuring that the toe pinch withdrawal reflex was lost. The respiratory rate and color of the extremities were observed during the procedure.

The detailed protocol for the surgical procedure has been described previously by Turel et al. [22]. Briefly, a midline incision was made over the scalp to expose the skull from the coronal suture to the lambdoid suture under magnification using an operating microscope.

Using a high speed (35,000 rpm) electric dental drill, a 5 mm craniotomy was performed just right of midline and in between the coronal suture and lambdoid suture to expose the dura mater. A 3 mm tip cooled hollow copper cylinder (filled with dry ice-acetone mixture to attain a temperature of −50 °C to −55 °C) was placed on the intact dura for three minutes. The cylinder was connected to a force transducer to measure the amount of force delivered and this was standardized to 10 g weight, as has been previously described [22]. Postoperatively, the animals were provided adequate hydration and nutrition and housed in separate cages.

2.3. Sham injury (n = 18)

An equal number of animals were subjected to sham injury under the same anesthetic protocol wherein the craniotomy was performed and an empty (non pre-cooled) copper cylinder tip was placed on the intact dura.

2.4. Control group (n = 6)

These mice were not exposed to any anesthesia or injury. They were sacrificed to determine the expression of AQP-4 and percentage water content in normal brain.

2.5. Evaluation of functional outcome

Neurological status of the mice was evaluated using the Neurological Severity scale (NSS) [4] (Table 1) prior to injury and at 24, 48 and 72 h post injury by an independent observer blinded to whether the animal had received cryoinjury or sham surgery. For each parameter measured, the animal was scored 0 if it performed the test and 1 if it failed the test. The NSS score was the sum of the scores obtained for each of the parameters tested [4].

The animals were also subjected to a paradigm consisting of uniform acceleration at 4 rounds per min using the rotarod treadmill (TSE systems, Germany). They were pre-trained for 3 days before the injury to obtain their baseline scores, and serial measurements were performed thereafter at 24, 48, 72 h after the injury. The scores (in seconds) obtained on the rotarod treadmill were converted to percentage of the baseline scores for analysis.

2.6. Assessment of brain water content

Three mice from the experimental group and sham group each were sacrificed at 24 h, 48 h and 72 h post injury with an overdose of inhaled chloroform and their brains were removed and weighed immediately using an electronic weighing scale with a least count of 0.01 mg to determine the wet weight. The brains were exposed to a temperature of 105 °C for 48 h in a hot air oven and thus dehydrated. The dehydrated brains were then weighed to determine the dry weight. The water content of the injured brain was calculated as a percentage using the formula [22].

$$[(\text{wet weight-dry weight})/\text{wet weight}] \times 100$$

2.7. Determination of AQP-4 using Western blotting

Western blot analysis was performed as described previously [11,25]. Brain samples were obtained from the site of injury (A), contralateral hemisphere corresponding to the site of injury (B) and ipsilateral hemisphere adjacent to the injury (C) as shown in (Fig. 1). Brain tissue from normal control, sham and injured mice was weighed and rapidly frozen by dropping them into liquid nitrogen. Frozen tissues were ground into fine powder using pellet pestle motor with a pre-cooled pestle under liquid nitrogen.

To the frozen powder ice-cold Tris buffered saline (TBS) pH-7.4 was added and vortexed to get an even suspension.

Table 1
Neurological severity scale [4].

Task	Description	Points
Exit circle	Ability to exit the circle of 30 cm diameter within 3 min	0/1
Monoparesis/hemiparesis	Paresis of the upper/ lower limb on the contralateral side	0/1
Straight walk	Alertness initiative and motor ability to walk straight	0/1
Startle reflex	Innate reflex the mouse will bounce in response to loud sound	0/1
Seeking behaviour	Physiologic behavior as a sign of interest in surrounding	0/1
Beam balancing	Ability to balance on a beam of 7 × 7 mm for at least 10 s	0/1
Round stick balance	Ability to balance on a round stick of 5 mm for at least 10 s	0/1
Beam walk 3 cm	Ability to cross a beam of 30 cm length in 3 min	0/1
Beam walk 2 cm	Ability to cross a beam of 30 cm length in 3 min	0/1
Beam walk 1 cm	Ability to cross a beam of 30 cm length in 3 min	0/1
Maximal score		10

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