302

Available online at www.sciencedirect.com

SCIENCE DIRECT

ELSEVIER

JNMU

Journal of Nanjing Medical University, 2007, 21(5):302–306

www.elsevier.com/locate/jnmu

Research Paper

Effects of dexamthasone with different doses on aquaporin-4 in brain of intracerebral hemorrhage rats ☆

Jixiang Chen*, Hui Li

Department of Neurology, the Affiliated Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei Province, China

Received 17 March 2007

Abstract

Objective: To determine the relationship between the expression of aquaporin-4(AQP4) after intracerebral hemorrhage and dexamethasone treated. **Methods:**Collagenase Ⅶ was injected in caudate nucleus in a stereotaxis frame to establish the intracerebral hemorrhage(ICH) animal models. The intracerebral hemorrhage(ICH) rats were randomly divided into four groups: the sham group (group A), the ICH group(group B), low dose-treated group(group C), moderate dose group(group D) and high dose group(group E). The groups were respectively received an intraperitoneal dexamethasone injection with 1 mg/kg, 15 mg/kg, 30 mg/kg, twice a day for three days. The brain water content(BWC), the permeability of blood-brain barrier(BBB) and the expression of AQP4 were observed. **Results:**Both the BBB disruption and AQP4 expression decreased in treated groups, and the AQP4 expression had a dose-dependent manner in the dexamethasone treatment. And it seemed that low dose dexamethasone was in favor of brain swelling elimination, but the higher dosage had not similar effect. **Conclusion:**Dexamethesone may play a critical role on expression of AQP4 in the physiopathology of hemorrhagic edema.

Keywords: auqaporin-4; brain edema; dexamethasone; intracerebral hemorrhage

INTRODUCTION

The aquaporins(APQs) are a family of at least 11 homologous transmembrane water channel proteins, which provide the major pathway for water movement in various tissues. In nervous system, the aquaporin-4 (AQP4) is the most abundant, which principally assembles in gial limitans and facilitates bi-directional water transport across membranes, especially in cerebral edema[1,2]. Cerebral edema is an important contributor to morbidity and mortality resulted from intracerebral hemorrhage(ICH). The control of the brain edema is a key treatment for improving the prognosis of intracerebral hemorrhage. It is generally demonstrated that AQP4 plays a significant auto-regulatory role in the brain edema induced by many disorders[3]. Considering its significant present in gial cells, it might be a suitable target for drug discovery regarding cerebral edema. There has been a paucity of laboratory research focused effects of glucocorticoid for controlling edema management on AQP4. In our study we tried to elucidate the changes of AQP4 occurring in the hematoma-induced edema in brain and its influence to the edema.

MATERIALS AND METHODS

Experimental animal and group

A total of 75 healthy male Wistar rats, each weighing 250-350 g, which were provided by the laboratory animal center of the Tongji Medical College, were used for experiments. The ICH animals were randomly divided into the following groups: the sham group(group A, $n = 5$), the experimental control(group B, $n = 5$), the low dose treated group(group C, $n = 5$), the moderate

☆ The research was supported by science research foundation in Hubei province (2004AA30C97)

*Corresponding author.

E-mail address: cz283895@yahoo.com.cn

dose treated group(group D, $n = 5$) and the high dose treated group(group E, $n = 5$).

The preparation of the ICH model[4]

Animals were anesthetized with 10% chloral hydrate (300 mg/kg, IP), and then were positioned in a stereotaxis frame. A sagittal skin incision was made and a cranial hole about 1mm in diameter was drilled near the right coronal suture 3.5 mm lateral to the midline. 0.5U collagenase dissolved in 2 μl saline was injected stereotaxically into the right basal ganglia (coordinates: 0.2 mm anterior, 5.5 mm ventral, and 3.5 mm lateral to the bregma) by a microsyringe(5 μl). The needle was remained for 5 minutes to prevent reflux after injection. Bone wax was used to seal the cranial hole. Then the skin incision was closed. The animals in the treated groups received dexamethasone treatment(1 mg/kg, 15 mg/kg, 30 mg/kg, IP) twice a day for three days after surgery respectively. The sham group received 2ml saline stereotaxically, while the experimental control group received no treatment after surgery.

Brain water content

Brains were quickly removed after anesthetized by chloral hydrate(300 mg/kg, IP). Hemispheres were separated and cut coronally. The slices around the hematoma sites in thickness of 4mm were weight before and after drying foe 24 h in an oven at 100℃. Brain water content was expressed as the percentage change between wet weight(WW) and dry weight(DW) using the formula: WW-DW/WW×100% for evaluating the edema.

Blood brain barrier permeability

For the blood-brain integrity assessment, Evans blue extravasation was measured[5]. Rats were injected with Evans blue(2%in saline,4 ml/kg, IV) through the tail vein. One hour after injection, rats were anesthetized with chloral hydrate (300 mg/kg, IP), and intracardialy perfused with 100 ml isotonic saline. Brains were quickly removed and placed on a frozen plate. Tissue samples were cut coronally with the thickness of 4 mm centered the hematoma lesion. The slices were immersed in the formamide(1 ml/100 mg) at 56℃ for 24 h, then Evans blue was extracted from the incubation. The dry was quantified by measuring the optical density at 630 nm. Absorbance was compared with a standard curve of 1~10 μg/ml of Evans blue in formamide. Extravasation was expressed as microgram of Evans blue per gram of wet weight.

Immunohistochemistry

The animals were anaesthetized with chloral hydrate (300 mg/kg, IP) followed by intracardiac perfusion with 4% paraformaldehyde in phosphate buffer. Rats were decapitated and brains were removed. A 2mm thick coronal brain around the hematoma was sliced, post-fixed in 4% paraformaldehyde for 8 hours to immunocytochemical studies. Sections(7 μm) were successively incubated in 3% hydrogen for 5~10 minutes and in goat serum for 10 minutes at room temperature and primary antibody at 1:200 dilution for 3 hours at 37℃. Following washing with PBS, sections were incubated in biotinylated goat anti-rat secondary antibody for 2 hours at room temperature, and then washed and placed in ABC for 2 h. After washing with PBS again, the sections were reacted for peroxidase enzyme activity by using diaminobenzidine(DAB) and H_2O_2, and the reaction was stopped by 0.01 mol/L PBS and mounted into gelatinized slides.

Reverse transcriptase polymerase chain reaction

Total RNA was prepared from brain sample using the Trizol reagent and cDNA was generated from 5 μg of total RNA using M-MLV reverse transcriptase and the random primer olig-dT. The cDNA were used to amplify a 213 bp fragment by PCR using specific primers for the AQP4 sequence(forward 5'-TTG GAC-CAA TCA TAG GCG C-3';reverse5'-GTC AAT GTC GAT CAC ATG C- 3') and a 318 bp fragment using primers for β-actin sequence(forward 5'-ATC ATG TTT GAG ACC TTC AAC-3'; reverse 5'-CAT CTC TTG CTC GAA GTC CA-3'). The reaction was carried out for 30 cycles, with 95℃,30 s, denaturing; 53℃, 30 s, annealing; and 72℃, 45 s, extension. For quantification, the PCR products were subjected to electrophoresis in a 2% agarose gel and stained with ethidium bromide. Photographs were taken with a Polaroid camera. Densitometric analysis was performed and presented as the AQP4/ β-actin ratio.

Statistical analysis

Data are presented as the mean±S.E. The statistical significant difference between the experimental groups was assessed by student *t* test and *q*-test. Differences were considered significant at a level of $P < 0.05$ using SPSS12.0 software.

RESULTS

Brain water content

ICH rats received low intraperitoneal dose of dexamethasone(1 mg/kg) showed a significant decrease in water content. However, no significant difference was seen between higher dose(15 mg/kg, 30 mg/kg) of dexamethasone and the control(***Tab 1***).

Evens blue extravasation

Integrity of BBB was evaluated on day 3 after ICH. Concentration of Evans blue in brain parenchyma was detected. EB extravasation in treated groups was significantly decreased vs. the control(***Tab 1***).

Tab 1 **The brain water content(BWC) and Evens blue(EB) extravasation in ICH rats**

	Group A	Group B	Group C	Group D	Group E
BWC	77.67 ± 0.91	84.81 ± 0.48	80.38 ± 1.18*	83.85 ± 0.48	83.71 ± 0.21
EB	7.57 ± 0.28	13.55 ± 0.61	12.10 ± 1.08*	11.38 ± 1.08**	10.88 ± 0.79**

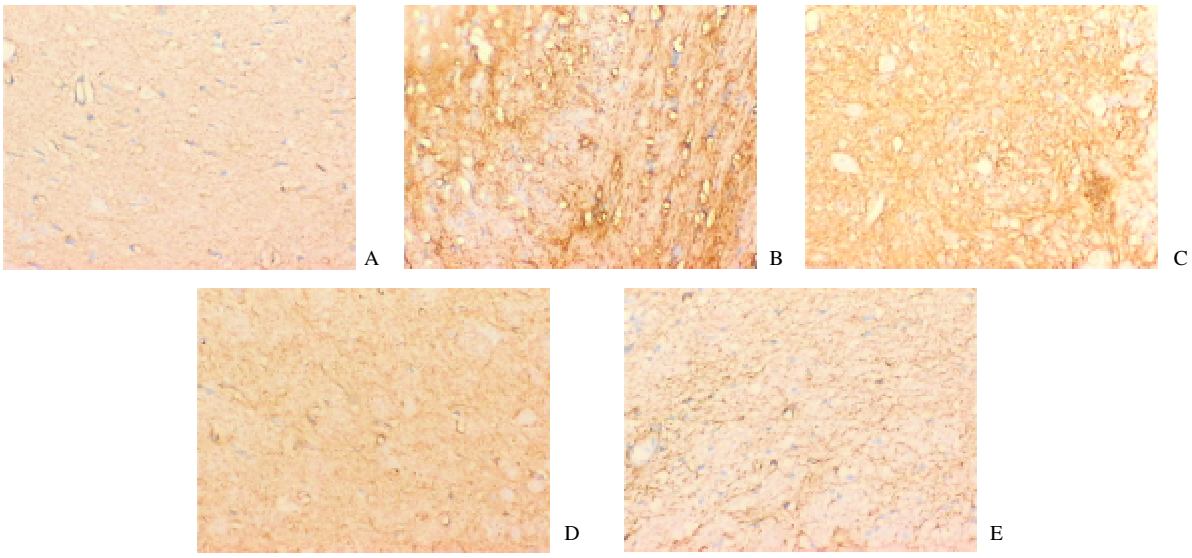
Compared with group B,** $P < 0.01$, * $P < 0.05$. Group A:sham group; Group B:experimental control group; Group C:low dose-treated group(1 mg/kg); Group D:moderated dose-treated group(15 mg/kg); Group E:High dose-trented group(30 mg/kg)

Immunohistochemistry

AQP4 immunolabelling appeared as a brown deposit over cell processes surrounding miscrovessels(***Fig 1***). The optical density was detected to assess AQP4 expression. Significant differences were also found in AQP4 protein expression between treated groups and controls(***Fig 2***).

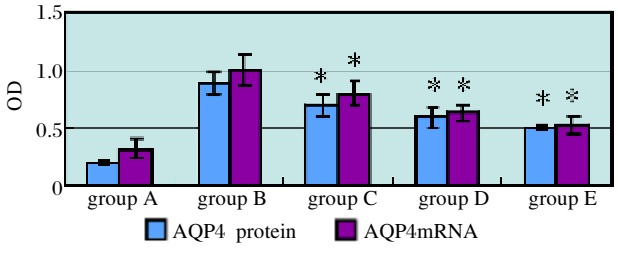
RT-PCR

The semi-qualitative analysis of aquaporin-4 mRNA was investigated by RT-PCR. The statistical results showed that aquaporin-4 was downregulated in dexamethasone groups(***Fig 3***). There was statistical significant difference between all dexamethasone groups and control group in mRNA level of AQP4. There was statistical significant difference between group B and group C, between group B and group D, between group C and group D(***Fig 2***).



A:sham group; B:experimental control group; C:low dose-treated group(1mg/kg); D: moderated dose-treated group(15 mg/kg); E: high dose-treated group(30 mg/kg). AQP4 immunolabeling was shown as brown deposit over astrocyte membrane and its expression was evaluated according to the optical density(OD).

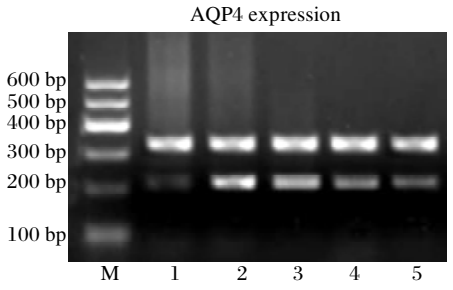
Fig 1 The result of AQP4 expression in each group(Immunohistochemistry, × 200)



Compared with group B, * $P < 0.01$.

Group A: sham group; Group B: experimental control group; Group C: low dose-treated group(1mg/kg); Group D: moderated dose-treated group(15 mg/kg); Group E: high dose-treated group(30 mg/kg).

Fig 2 Expression of AQP4 mRNA in dexamethasone groups and the control group



M:Marker; 1:sham group; 2:experimental control group; 3: low dose-treated group(1 mg/kg); 4:moderated dose-treated group(15 mg/kg); 5: high dose–treated group(30 mg/kg).

Fig 3 PCR-amplified product of AQP4 in brain samples

DISCUSSION

Glucocorticoid used for brain edema therapy is controversial until now[6,7]. This therapy is known to be mediated by mechanisms, such as protecting blood-brain barrier(BBB), decreasing production and enhancing reabsorption of cerebrospinal fluid, inhibiting lipid peroxidation, reducing intracellular calcium accumulation and so on[8].

Desaiod et al[9] investigated 26 patients with ICH randomized to dexamethasone vs.placebo. The number of patients with a good neurologic outcome was the same in both group, and no difference was detected in the rate of adverse effects[9]. And recently in collagenase models of intracerebral hemorrhage, administration of low dose of dexamethasone was associated with decreased brain edema, smaller hematoma volume, decreased number of necrotic cortical neurons, and improved neurological scores[10.11] .

AQPs are a family of water channel proteins distributed in various tissues. In the brain AQP4 is the major species expressed, in a polarized way by ependymogial cells and by perivascular processes of astrocytes. Regarding brain water homeostasis AQP4 in the perivascular astrocyte membranes has been shown to be critical involved in the formation and dissolution of brain edema[12,13]. The current study revealed that the expression level of AQP4 underwent major changes after intracerebral hemorrhage, which may contain the molecular mechanisms underlying the generation and resolution of edema after ICH. There is no significant innovation about the brain swelling therapy since the 1980's. Until finding the AQP4, it may be of potential value if better management of brain edema could be achieved through rational way of regulating AQP4 expression.

Glucocorticoid using for brain edema is tightly related with AQPs (including AQP4[14],AQP3[15], AQP1[16,17,18]). Both beta-adrenergic agonist and glucocorticoid hormone which believed to be the factors accelerated the clearance of fetal lung liquid induced fetal lung AQP4 mRNA, which was found by Yasui[14].

Aimed at determining the correlation between glucocorticoid and AQP4 further, brain water content, blood-brain barrier permeability, and AQP4 expression were observed using ICH rat models treated with dexamethasone at different doses. The results indicate dexamethasone is found to have a protective effect on the BBB, and reveal a downregulation of AQP4 expression induced by dexamethasone in dose-dependent manner by means of immunohistochemistry in protein level and RT-PCR in mRNA level. The brain water content coinciding with that low dose dexamethasone seems to be beneficial for the treatment of brain edema. We hypothesize that dexamethasone has double-side action on brain swelling, especially vasogenic edema which is dominant at day 3 after intracerebral hemorrhage. It ameliorates vasogenic edema following protecting BBB from injury, and concurrently attenuates the AQP4 function of clearance of excess fluid in brain while it accelerates cytotoxic brain edema. In conclusion low dose of dexamethasone found to be more beneficial for treatment of cerebral edema after ICH, whereas high dose seems to be no obviously beneficial effect. In addition to some factors such as α-syntrophin, hypoxia, osmotic pressure, ammine, vasopressin et al[19,20,22], the glucocorticoid may be considered to be ranged in modulators of aquaporin-4 expression regarding the molecular mechanism about cerebral swelling.

References

[1] Bloch O, Papadopoulos MC, Manley GT, Verkman AS.Aquaporin-4 gene deletion in mice increases focal edema associated with staphylococcal brain abscess. *J Neurochem* 2005 ;95:254-62.

[2] Liu H, Wintour EM. Aquaporins in development -a review. Reprod *Biol Endocrinol* 2005; 11;3-18.

[3] Papadopoulos MC, Manley GT, Krishna S, Verkman AS. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *FASEB J* 2004 ;18:1291-3.

[4] Chesney JA, Kondoh T, Conrad JA, Low WC. Collagenase-induced intrastriatal hemorrhage in rats results in long-term locomotor deficits. *Stroke* 1995 ;26:312-6; discussion 317.

[5] Hellal F, Bonnefont-Rousselot D, Croci N, Palmier B, Plotkine M, Marchand-Verrecchia C.Pattern of cerebral edema and hemorrhage in a mice model of diffuse brain injury. *Neurosci Lett* 2004;357:21-4.

[6] Norris JW. Steroids may have a role in stroke therapy. *Stroke* 2004; 35:228-9.

[7] Poungvarin N. Steroids have no role in stroke therapy. *Stroke* 2004; 35:229-30.

[8] Gomes JA, Stevens RD, Lewin JJ 3rd, Mirski MA, Bhardwaj A. Glucocorticoid therapy in neurologic critical care. *Crit Care Med* 2005 ; 33:1214-24.

[9] Desai P, Prasad K. Dexamethasone is not necessarily unsafe in primary supratentorial intracerebral haemorrhage. *Journal of Neurology, Neurosurgery & Psychiatry* 1998; 65:799-800.

[10] Lema PP, Girard C, Vachon P. Evaluation of dexamethasone for the treatment of intracerebral hemorrhage using a collagenase-induced intracerebral hematoma model in rats. *J Vet Pharmacol Ther* 2004 ;27 :321-8.

[11] Vachon P, Moreau JP. Low doses of dexamethasone decrease brain water content of collagenase-induced cerebral hematoma. *Can J Vet Res* 2003 ;67 :157-9.

[12] Griesdale DE, Honey CR.Aquaporins and brain edema. *Surg Neurol* 2004 ;61:418-21.

[13] Verkman AS, Binder DK, Bloch O, Auguste K, Papadopoulos MC. Three distinct roles of aquaporin-4 in brain function revealed by knockout mice. *Biochim Biophys Acta* 2006;1758:1085-93.

[14] Yasui M, Serlachius E, Lofgren M, Belusa R, Nielsen S, Aperia A. Perinatal changes in expression of aquaporin-4 and other water and ion transporters in rat lung. *J Physiol* 1997; 505:3-11.

[15] Kitahara T, Fukushima M, Uno Y, Mishiro Y, Kubo T. Up-regula-

tion of cochlear aquaporin-3 mRNA expression after intra-endolymphatic sac application of dexamethasone. *Neurol Res* 2003; 25:865-70.

[16] Peng J, Zhang H, Li T, Li Z, Wu Y. Effect of dexamethasone and aquaporin-1 antisense oligonucleotides on the aquaporin-1 expression in cultured human trabecular meshwork cells. *J Huazhong Univ Sci Technolog Med Sci* 2006;26:137-40.

[17] Fukushima M, Kitahara T,Uno Y, Fuse Y, Doi K, Kubo T. Effects of intratympanic injection of steroids on changes in rat inner ear aquaporin expression. *Acta Otolaryngol* 2002;122:600-6.

[18] Kobayashi H, Yokoo H, Yanagita T, Satoh S, Kis B, Deli M,et al. Induction of aquaporin 1 by dexamethasone in lipid rafts in immortalized brain microvascular endothelial cells. *Brain Res* 2006; 1123:12-9.

[19] Amiry-Moghaddam M, Xue R, Haug FM, Neely JD, Bhardwaj A, Agre P, et al. Alpha-syntrophin deletion removes the perivascular but not endothelial pool of aquaporin-4 at the blood-brain barrier and delays the development of brain edema in an experimental model of acute hyponatremia. *FASEB J* 2004;18:542-4.

[20] Kaur C, Sivakumar V, Zhang Y, Ling EA. Hypoxia-induced astrocytic reaction and increased vascular permeability in the rat cerebellum. *Glia* 2006; 54:826-39.

[21] Rama Rao KV, Chen M, Simard JM, Norenberg MD.Increased aquaporin-4 expression in ammonia-treated cultured astrocytes. *Neuroreport* 2003;14:2379-82.