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Changes AQP4-siRNA Treatment Varying Degrees of Non-Traumatic Brain Injury and Brain Tissue Edema ADC Values

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Abstract

Objective: To observe the different degrees of non-traumatic brain injury side (Contralateral brain tissue, CBT) changes in brain edema and the therapeutic effect of AQP4-siRNA application, while the feasibility of monitoring the CBT area ADC value edema change.

Methods: Healthy March age 198 rats, according randomly divided into sham group (n=18), fell injury group (n=90) and AQP4-siRNA treatment group (n=90), using controlled cortical impactor (controlled cortical impact, CCI) prepared mild and severe trauma rats with brain contusion and treatment groups after contusion press time 1 h, 24 h, 48 h, 72 h, 168 h each divided into five sub-group (n=18), each group were sacrificed at the above time and randomly selected three death row before MR imaging followed by pathological changes in electron microscopy CBT edema and BBB (blood-brain barrier, BBB) blood-brain barrier (blood-brain barrier, BBB) integrity (n=2), and Western blot AQP4 expression (n=6), brain water content measured (n=10), analysis of changes of CBT brain injury edema, AQP4-siRNA interference treatment and MRI features.

Results: AQP4 expression of mild group CBT, ADC value and pathological changes in 1 h, 24 h and 48 h normal, 72 h after AQP4 appears the slightly reduced after 7d appears significantly higher, ADC value 72 h appears significantly reduced, then increased 7d pathological 72 h CBT display area of the cells within the current mild edema, 7d appear mild hybrid edema; prophylactic treatment after AQP4-siRNA AQP4 in 48 h, 7d group showed significantly lower than the trauma ($P<0.05$), ADC display 48 h somewhat lower than the trauma group, 72 h significantly increased, 7d significantly lower ($P<0.05$), showed that the treatment group compared with the pathological trauma group at 72 h and 7d relieve cerebral edema. AQP4 expression of severe injury group CBT region 1 h is significantly higher than the control group, 24 h, 48 h, 72 h sustained decline, then to 7d recovery, AQP4-siRNA treatment after 24 h to 72 h CBT of AQP4 in significantly lower ($P<0.05$), pathology showed severe injury group CBT area in the early (1 h) that is out now

vasogenic edema, 24 h to 48 h in the main intracellular edema, 72 h to 7d was mixed edema; treatment group within 24 h to 48 h cell edema improve, 7d hybrid significantly reduced edema, severe trauma group CBT area ADC value in the early (1 h) that is significantly higher in 24 h to 48 h reduction, early treatment group ADC value (1 h) no significant difference between the trauma group, 24 h significantly higher than the bruise.

Conclusion: Severe contusion and mild contusion after CBT area was now different pathological changes in edema after severe brain contusion early CBT region appears vasogenic edema, which subsequently led to brain tissue edema and mixed edema, AQP4-siRNA cannot early mitigation vasogenic brain edema, but may then occur within AQP4 expression by reducing brain edema and mild reduction of cell hybrid edema. Mild group CBT area in the late (72 h) appears cytotoxic edema, AQP4-siRNA can alleviate cerebral edema. ADC value change of AQP4 expression and edema have a certain type of contact that can provide valuable information for the image after TBI diagnosis and treatment of cerebral edema judgment.

It can be used as a means to assess the body image depending on the degree of injury at different time periods to observe changes in brain edema and treatment CBT area.

Keywords: AQP4; siRNA; Brain injury

Introduction

With the rapid development of the transport industry and the construction industry, the incidence of brain trauma was significantly higher (about accidental injury 50%) [1], and traumatic brain trauma as one of the main pathological changes in brain tissue, not only important cause dysfunction of brain cells, often is a direct factor [2] leads to death of. Mechanisms of traumatic brain edema is very complex [3-6], common features include vasogenic edema and cytotoxic brain edema, including, most research focused on the observation and treatment of injury or damage to surrounding brain tissue area, and often CBT changes ignore the damage [4,5], this

group of researchers found that CBT area after TBI injury also occurs corresponding change in brain edema, to a certain extent, increased the degree of brain edema and neurologic injury, how to effectively control CBT area brain edema has important clinical significance to minimize the increased intracranial pressure after TBI, improve prognosis.

Studies have shown that brain tissue AQP4 is the most abundant water channel protein, and its distribution position, the amount of change in brain edema formation, development has an important role in the formation and improve prognosis by AQP4-siRNA able to reduce cerebral edema have been a number of [6-10] study confirmed, but preventive AQP4-siRNA treatment after TBI CBT region if we do not know enough to also play a role. In addition, the degree of progress of the evaluation and treatment of cerebral edema in the body is still the focus of our efforts to study and reflect ADC is based on magnetic resonance diffusion imaging obtained in the effective technical body brownian motion of water molecules, which can display the number of the unit cells and volume differences, to a certain extent reflects the quantitative indicators within the tissue extracellular space size and the number of water molecules in flux, intracellular edema ADC is reduced due to the extracellular space decreases when vasogenic edema due to ADC increased flux of water molecules rises [4,7,8].

Therefore, the main purpose of this study was to explore whether all the wounds are swollen with water CBT pathological change? Its edema formation mechanism consistent? Whether CBT can reduce cerebral edema progress through preventive AQP4-siRNA treatment? ADC is able to provide functional magnetic resonance imaging to monitor complex after TBI clinical changes and treatment of brain edema *in vivo* imaging means of observation?

Materials and Methods

Animals and grouping

198 adult male SD rats were purchased from experimental animal center of Sichuan university, (animal license number: SCXX (Chuan) 2008-2024), weighing 300 g to 350 g, separate feeding, holding a bright 12 h, 12 h dark, at 22~25 range, free water and food intake. Were randomly divided into sham group, contusion and interference agents group, sham group 18, the latter two groups divided according to time points after contusion 1 h, 24 h, 48 h, 72 h, 7d 5 sub-groups 18 rats. Each group were two samples for pathological observation, taking six proteins for semi-quantitative Western blotting of brain tissue AQP4, more than 10 used to measure the water content of the brain.

Animal model

By improved Feeney method [11] model of cerebral contusion, brain injury using PinPoint™ impactor (Hatteras

instruments, Cary, NC, USA). With 100 g/L chloral hydrate (4 ml/kg) in rats on intraperitoneal injection of anesthesia. The rats in the prone position the head fixed in a stereotactic head frame (ST-7Setagaya-Ku, Tokyo, Japan company), the head skin preparation, routine disinfection, cut along the midline of the scalp for about 2 cm. Desktop machines using dental drill (307-2Btype, Shanghai LZQ precision tool technology Co.), 1.5 mm drill (speed 4000 r/min) in between the front and rear halogen, middle and right next to open 2.5 mm drilled open skull, as a center opened with mosquito forceps 5 mm diameter round bone window, keep the dura intact, targeting bone window hit, establishing the right side of the brain to moderate brain contusion model (based on pre-test selection parameters: impact time 0.1 s, hit speed 2.5 m/s, respectively, to different types of striking head (resolution 4 A group, the 6th group C) and hit the strike depth (A group of 1.5 mm, group C 3.5 mm) prepared of mild and severe rats with brain trauma [12]. In addition to not hit the guise surgery group than the other groups operating with trauma treatment group using the Hamilton syringe immediately after injury model (the 30th) at the center of the vertical needle injury to damage the surface of the pitch 3 mm siRNA injected a total of 10 μ l, injection speed 1 μ l/min after injection needle stop one minute after suturing tissue, then place it in the 37° temperature heating pad until waking up the next day and then injected once a siRNA targeting AQP4 expression plasmid: According to NCBI (national center for biotechnology information) in the mRNA coding sequence of rat AQP4 gene, using gene silencing target sequence design software bio edit software design siRNA is: 5'-AGATCAGCATGCCAAGTC-3' (registration number: 012,825, located AQP-4 cDNA first 326-3456p), the target dsRNA justice chain 5'-AGAUCAGCAUCGCCAAGUCUU-3', antisense strand is 5'-UUUCUAGUCGUAGCGGUUCAG-3'.

MR examination

Applications respiratory monitoring pad (SA instruments, Inc., Stony Brook, NY) placed in the abdomen to monitor breathing in rats, blowing insulation in the 37°C thermostat MR airflow aperture. The image forming apparatus using Bruker 7T BioSpin MR spectrometer (Bruker, Germany), a pore diameter of 16 cm, the maximum gradient strength of 300 mT/m, 24 channel pulse coil surface. Imaging sequence T2WI: TR: 1200.0 ms, TE: 50.0 ms, matrix: 256 × 256, excitation: 4, thickness of 1.0 mm, the interlayer spacing 0.00 mm, FOV: 3.6 cm × 3.6 cm; DWI using echo planar imaging sequence, TR 9000 ms, TE 102 ms, b value of 0 s/mm² and 800 s/mm², FOV=3 cm, NEX=2. MR scan is complete after the image transfer to the workstation using imaging J software measurement within CBT ADC value. By the two experienced physicians to take a double-blind test, to select three CBT region ROI (**Figure 1**), the resulting data is x () ± s representation.

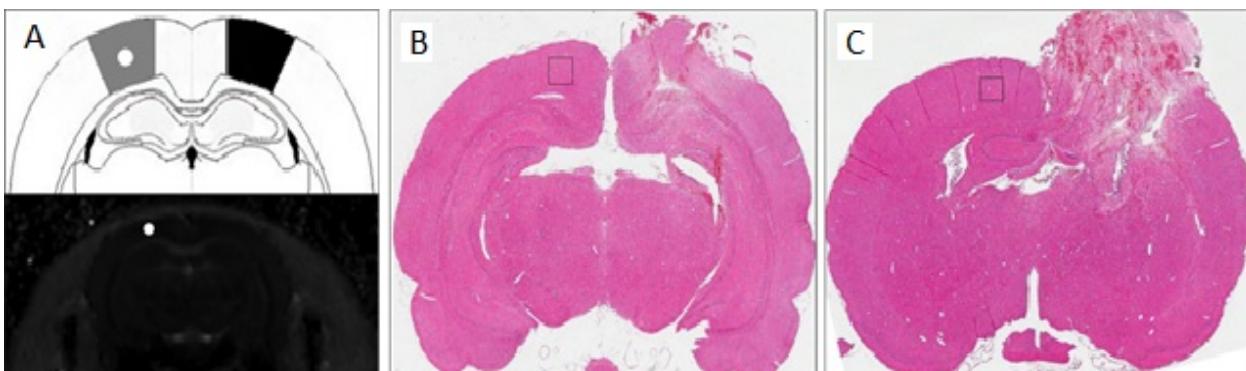


Figure 1 A: a schematic trauma, representative's trauma area, on behalf of CBT area, B: normal ADC maps, C: on behalf of ADC map ROI takes a value in the region and a corresponding range (A/b indicated on the diagram on the right in **Figure 2** should be marked mild and severe brain damage large slices).

Pathological morphological observation

HE staining

Each group takes a sample to 100 g/L chlral hydrate (4 ml/kg) in anesthetized rats, the left ventricle infusion 40 g/L paraformaldehyde until right atrial outflow colorless liquid, brains were removed, 40 g/L paraformaldehyde external fixation 24 h, coronal cut the damaged brain tissue. Embedded in paraffin, sectioned. HE staining sections were routine light microscope and photographed.

IgG immunohistochemical staining BBB permeability

Take HE staining adjacent levels of IgG using the two-step immune histochemical staining, apply EnVision color system (GLK500705, Denmark DAKO company) Paraffin sections were deparaffinized and water, washed 3 times with PBS, the 16.7 mol/L hydrogen peroxide incubated at room temperature 10 min, washed 3 times with PBS, dropping antibody (IgG, 1: 8000, Santa Cruz, USA), and incubated overnight at 4° washed with PBS three times, dropping two anti (Zhongshan Golden Bridge Biotechnology Inc.) incubated at room temperature 20 min, washed 3 times with PBS, plus DAB color liquid, immediately into the water stops, were mounted light microscope after coloration.

Western blot protein expression of AQP4

The injured parts of the brain tissue using liquid nitrogen and grinding with rapid tissue lysate (RIPA) extracting total protein lysate samples in each group, total protein concentration was measured by BCA method. The protein gel was transferred to a polyvinylidene fluoride (PVDF) film, plus antibody (rabbit anti-rat AQP4, 1: 200, Chemicon USA Inc.) was added after washing the membrane secondary antibody (goat anti-rabbit AQP4, 1:200, Beijing Zhongshan Golden Bridge Biotechnology Co., fir). Analysis of gray value target band of (D) with Bio-RAD gel imaging system (Beijing Zhongshan Golden Bridge Biotechnology Co.).

Determination of brain water content

Injury setback rats at time points corresponding sodium pentobarbital anesthesia (n=10), were killed off the head, removed brain damage from rat brain tissue contralateral hemisphere, with exhaust filter surface water and pools of blood after placed on quality has been dried and said foil, said the electronic balance (BSAI24S-CW, Beijing Sartorius Scientific Instrument Co., Ltd.) wet weight placed in an electric oven after drying 48 h 105°C to constant weight (two times the difference between the dry weight of <0.0002 g), brain water content calculated according to Blliot formula: Cerebral water content=(wet weight-dry weight)/wet weight × 100%.

Statistical analysis

Application of statistical software SPSS 13.0, the measurement data are $x \pm s$ representation. T-test and One-way-ANOVA analysis row. P<0.05 was considered statistically significant.

Results

CBT area AQP4 expression and pathological edema characteristics

Mild group CBT in 1 h, 24 h and 48 h pathology showed nerve cell morphology, space was normal, IgG show BBB structural integrity (**Figure 2**), semi-quantitative Western blot AQP4 expression compared with control group had no significant change (**Figure 3**), indicating mild early injury group CBT no trauma involved; 72 h after pathology showed CBT appears cytotoxic edema, 7d appears mixed edema (**Figure 2**), semi-quantitative Western blot show AQP4 rose rapidly (**Figure 3**) after a small decline, indicating CBT pathologic changes in edema and injury of late and AQP4 expression changes related to analysis of the reasons may be affected by the blood flow caused by compression of the ipsilateral cerebral perfusion reduce edema and other causes of cytotoxic edema [13-15], and then cause 7d mixed cerebral edema.

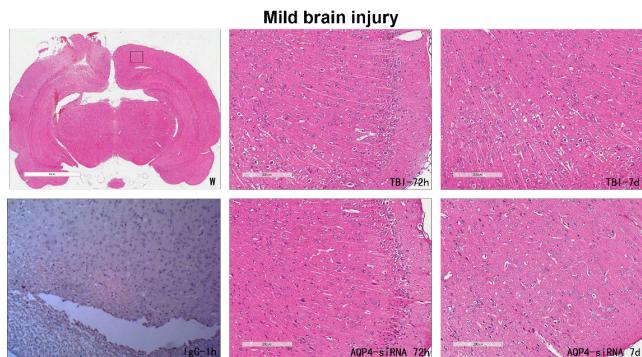


Figure 2 Mild trauma group. W: full brain specimens, on behalf of CBT on pathological collection area; IgG-1 h: BBB structural integrity (no significant positive cells); TBI-72 h: 72 h of trauma group within the current cell edema; AQP4-siRNA 72 h: treatment 72 h intracellular edema in the group significantly reduced; TBI-7d: 7d trauma group appears mixed edema; AQP4-siRNA 7d, the treatment group mixed edema.

early vasogenic edema formation process has a regulatory role; followed by pathological damage CBT display area 24 h to 48 h within the current cell edema, 72 h to 7d was significantly mixed edema (**Figure 4**), and semi-quantitative display AQP4 in 24 h, 48 h, 72 h sustained decline (**Figure 5**), which may be the role of the body's self-protection mechanism results [16-18], and then to 7d mildly elevated.

AQP4-siRNA interference preventive action to reduce damage to cerebral edema formation CBT

AQP4-siRNA treatment after the mild expression of AQP4 in 48 h, 7d appears significantly reduced ($P<0.05$) (**Figure 3**), pathology showed intracellular edema abated within 72 h, 7d mixed edema reduced, microglia improved (**Figure 2**); severe injury group CBT area AQP4 slightly decreased after 1 h, 24 h to 72 h significantly reduced, 7d return to normal levels (**Figure 5**), pathology showed that the treatment group 1 h vasogenic edema and no significant changes significant improvement within 24 h to 48 h intracellular edema, 7d mixed edema significantly reduced, microglia activity enhancement (**Figure 4**).

Functional magnetic resonance (ADC) to monitor changes in brain edema CBT area and AQP4-siRNA therapeutic effect

Mild contusion group CBT region 1 h to 48 h ADC value no significant changes, 72 h appears significantly lower, consistent with the time intracellular edema, followed 7d significantly increased, and the degree of external water swollen cells aggravated coincide (**Figure 6 and Table 1**); after AQP4-siRNA interference ADC display 48 h group decreased compared with trauma, but this time pathological damage CBT display area for normal, AQP4 table Damien was reduced, confirming AQP4 expression and there is a certain correlation between ADC values [7,8,19], and 72 h display AQP4 semi-quantitative rapid increase, ADC but lower (**Figure 6 and Table 1**), and within this tissue cells showed intracellular edema, explained mainly by the ADC water flux influence the extracellular space, while AQP4 permeability of water molecules in the cell membrane is relatively low. Treatment ADC value increased significantly at 72 h, in 7d significantly reduced (**Figure 6 and Table 1**), and after treatment within 72 h 7d hybrid cell edema and relieve edema same; therefore, ADC value through functional magnetic resonance imaging can be used as light monitor the dynamic changes early CBT area AQP4 protein amount of brain contusion after siRNA treatment, while providing monitoring tools for advanced (72 h to 7d) change edema treatment.

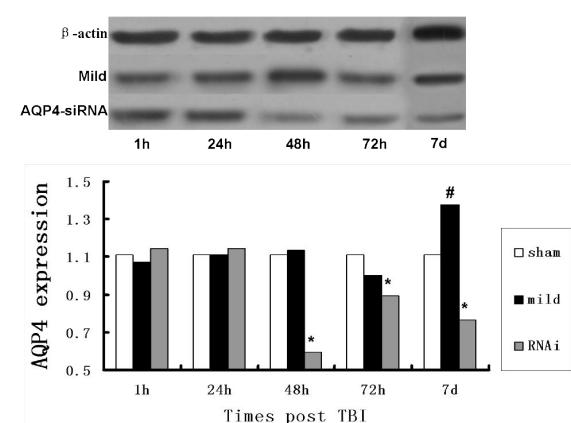


Figure 3 AQP4 expression CBT area and AQP4-siRNA treatment after the organization: the treatment group AQP4 in 48 h, 72 h and 7d significantly lower than the control group, (# represents compared with the control group, $P<0.05$; * on behalf of the more trauma group $p<0.05$).

Severe injury group: pathology revealed severe injuries CBT area in the early (1 h) that is out now vasogenic edema, IgG show BBB damage (**Figure 4**), indicating severe trauma group CBT area with a certain degree of hedging injury, AQP4 expression in damage After 1 h showed rapid significantly increased (**Figure 5**), which means AQP4 CBT area in injury

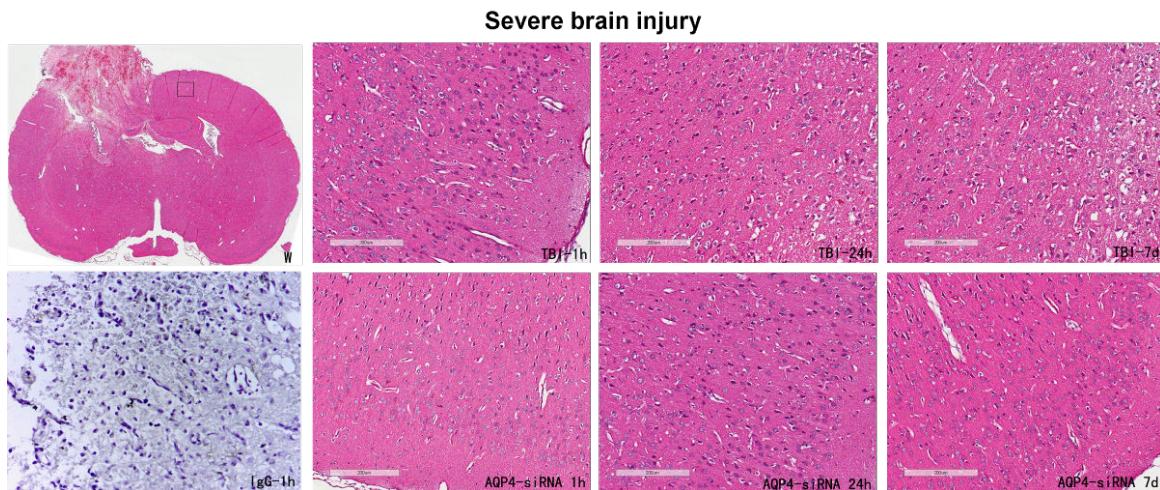


Figure 4 Severe trauma groups. W: Full brain Specimens, on behalf of CBT on pathological collection area; IgG-1 h: BBB structural damage (significantly more positive cells); TBI-1 h: 1h trauma group appears vasogenic edema; AQP4-siRNA-1 h: treatment Group 1 h vasogenic edema no significant relief; TBI-24 h: 24 h of trauma group within the current cell edema; AQP4-siRNA 24 h, in the treatment groups were edema. TBI-7d: trauma group 7d mixed edema aggravation; AQP4-siRNA 7d, the treatment group mixed edema.

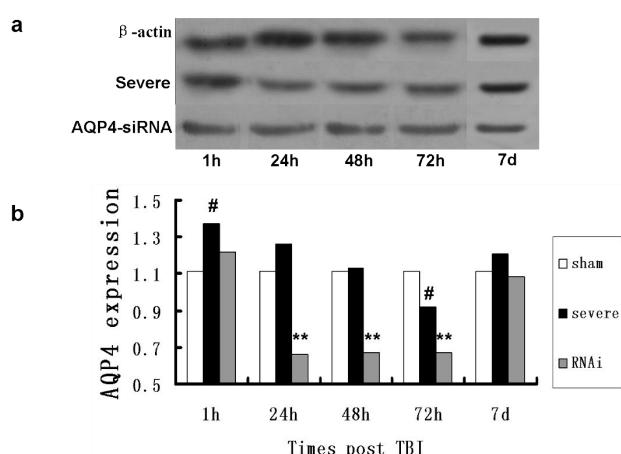


Figure 5 Severe trauma group CBT area and inside AQP4-siRNA tissue AQP4 expression after treatment: treatment group AQP4 in 24 h, 48 h and 72 h was significantly lower than the trauma (# represents compared with the control group, P<0.05; ** on behalf of the more trauma group p<0.01).

Severe setback injury group CBT area (**Figure 7 and Table 1**) early (1 h) ADC value was significantly higher, ADC value AQP4-siRNA interference did not change significantly, which CBT region appears vasogenic edema and no significant improvement after treatment consistency; at 24 h significantly decreased in the treatment group and reduce the extent of decline, and the pathology revealed the extent of inner tissue edema and, after treatment to reduce the consistency, 48 h to 7d CBT area ADC value is gradually increased slightly, but the treatment group showed volatility changes in the organization and extracellular edema and vasogenic edema related coexist.

Therefore, by functional magnetic resonance ADC value can be early (1 h to 24 h) severe injuries CBT brain tissue edema type, degree and treatment monitoring tools provided.

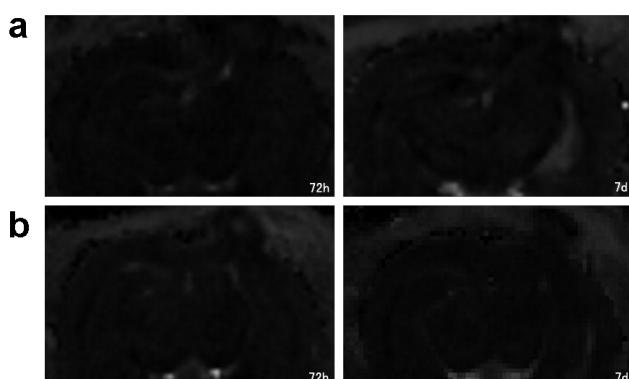


Figure 6 Mild ADC maps (Representative), a: trauma group, after TBI 72 h, ADC compared with the control group (**Figure 1b**) significantly reduced, 7d, ADC lower than the control group after TBI; b: AQP4-siRNA mild trauma group, TBI after 72 h, ADC was significantly higher than the trauma, after TBI 7d, ADC lower than the trauma.

Discussion

The study group found that varying degrees of cerebral contusion can cause damage CBT region have different pathological edema and prognosis, the reason lies in the different degrees of brain contusion area led CBT produced different pathological damage. Mild trauma group CBT area in the early (1 h to 48 h) Display BBB structure, cell morphology and cell gap were normal and no abnormal, corresponding

AQP4 expression level not see significant changes, indicating that CBT are early normal tissue structure, fluid within the

organization balance is not affected by the contralateral wound.

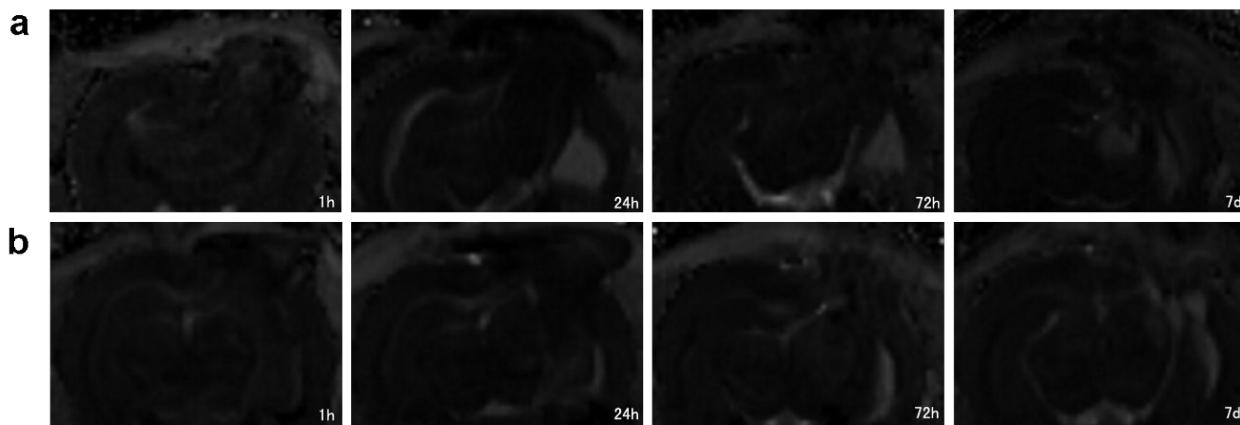


Figure 7 Severe trauma group ADC map (representative), a: severe trauma group, after TBI 1 h, ADC compared with the control group (**Figure 1b**) significantly increased, TBI after 7d, ADC was significantly higher than the control group; b: Severe trauma AQP4-siRNA treatment group, a: after TBI 1 h, ADC compared with trauma group (**Figure 1b**) significantly increased, no significant difference between the trauma group, 7d, ADC lower than control group after TBI; TBI after 24 h, treatment ADC group than in the control group increased; 72 h, ADC treatment group compared with the trauma group increased after TBI.

Table 1 CBT groups in different time zones ADC value: ADC value in mild trauma group compared with the control group after TBI 72 h (**Figure 1**) significantly reduced, while the treatment group 72 h, 7d group was significantly higher than the wound, indicating that AQP4-siRNA treatment of mild trauma CBT region within 72 h produced hybrid cell edema and edema have a preventive effect produced 7d; after severe trauma and treatment groups than in the control group TBI ADC 1 h significantly increased, indicating an early and severe trauma group that formed vasogenic edema and AQP4-siRNA treatment had no significant effect, 24 h, TBI group decreased ADC value after TBI, AQP4-siRNA significantly increased after treatment, indicating that after AQP4-siRNA treatment alleviated the trauma early cells within edema.

ADC values ($\times 10^4$ mm/s 2)					
	1 h	24 h	48 h	72 h	7d
Sham	8.58 ± 0.14	8.58 ± 0.14	8.58 ± 0.14	8.58 ± 0.14	8.58 ± 0.14
mild	8.76 ± 0.12	8.42 ± 0.09	8.26 ± 0.11	6.22 × 0.08 $^\Delta$	8.92 ± 0.18
Mild-siRNA	8.46 ± 0.21	8.21 ± 0.17	7.63 ± 0.22	7.48 ± 0.10*	7.74 ± 0.08
Severe	10.38 ± 0.26 $^\Delta$	8.04 ± 0.21	8.21 ± 0.29	9.01 ± 0.32	10.26 ± 0.24 $^\Delta$
Severe-siRNA	10.96 ± 0.37	9.82 ± 0.42*	8.54 ± 0.33	10.47 ± 0.46*	9.55 ± 0.18

* (p<0.05); $^\Delta$ (p<0.05)

Then late in injury (72 h), CBT region appears intracellular edema and mild, has increased to seven days to analyze the reason may be damaged cerebral edema caused by compression of the contralateral hemisphere reduced perfusion caused CBT region, and AQP4 expression? The damage zone 72 h apparent mixed edema peak also confirmed this inference [13-15]. After through AQP4-siRNA interference preventive CBT area 48 h AQP4 protein was significantly lower now and then showing up and down the small-scale fluctuations, the interference therapy CBT region 7d 72 h and brain edema has been significantly improved, so preventive AQP4-siRNA dry mild brain contusion scrambling may CBT area

of advanced cytotoxic brain edema play a good preventive effect.

Severe injury group CBT area in the early (1 h) that is out now obvious vasogenic edema, by pathology revealed BBB main incentive is to destroy the organization, followed by extravasation of blood vessels within the tissue fluid, extracellular osmotic pressure, which led to successive cell within edema (24 h to 48 h) and mixed edema (72 h to 7d) produced the damage zone with pathological changes similar to our previous report [20], and AQP4 expression CBT zone with the previous reported a small decline early damage zone was different, increased slightly for the fast, which may explain BBB indirect damage is insufficient to cause the body's self-

protection mechanism AQP4 degradation caused by rapid opening [17], but then AQP4 expression to 72h manifested as continued downward trend, indicating that kind of self-regulating mechanisms of the body showed relatively delayed. After severe injury group CBT region implement preventive interference AQP4, AQP4 seems to inhibit the expression of the Ming (in 24 h, 48 h obvious), pathology and treatment of brain water content observation showed no significant difference in brain edema and trauma 1 h group, illustrated by AQP4-siRNA does not improve due to the damage caused by BBB vasogenic edema, which is consistent with our previous reports, and in the subsequent 24 h to 7d brain edema has been significantly improved, indicating that AQP4 through preventive interference can significantly reduce the expression of AQP4 CBT area, improve brain edema.

DWI able to reflect the Brownian motion of water molecules and water molecules inside and outside the cell membrane of the transfer movement, according to ADC value detected water molecules can change the morphology of water swollen state intracellular and extracellular space is converted into image information visible [4,7,8]. This group of experiments showed that in the early ADC can discern whether caused varying degrees of damage CBT district BBB damage caused by vasogenic edema, and therefore can be more sensitive in the early to judge whether there are pathological changes CBT zone early in severe injury group CBT area (1 h) appears while vasogenic edema appears significantly increased ADC value at the subsequent (24 h) cell edema premise ADC value rapidly decreased, to a certain extent reflects the extracellular space narrowing, however, 48 h to 7d ADC value fluctuating trend changes, and the degree of synchronization of cerebral edema, which was mainly due to intracellular edema and vasogenic edema, etc. at the same time increase the comprehensive result, the former the ADC value decreased, which makes ADC value increased, two effects cancel each other out, resulting in the presence of a certain degree of volatility ADC value, ADC value and therefore difficult to evaluate by severe trauma CBT zone complexity edema late changes, but can detect early sensitive to vasogenic edema (1 h) intracellular edema (24 h) out now and increase the degree of progress. In the mild group CBT area 72 h appears modest reduction, consistent with the time intracellular edema, it is worth noting that in mild trauma group AQP4 preventive interference, ADC value occurs when a small decline in 48 h, which and semi-quantitative analysis of the treatment group significantly reduced the expression of AQP4 synchronized inherent links between AQP4 and the mean ADC of [7,8], but from the data we can see that AQP4 is significantly lower ADC values and small the magnitude of reduction was not an exact match, but at 72 h semi quantitative display AQP4 rapid increase, ADC but lower, and within this only occurs when the tissue cells in the mild edema, mean relative mobility of water molecules changes in extracellular organizations, ADC value of AQP4 be less sensitive to exchange water molecule (72 h appears intracellular edema) in the cell membrane. However, the organizational structure of the display area 48 h CBT normal, and AQP4 and ADC value has changed at this point, indicating that the earlier ADC capable of reproducing function reflects

the movement of water molecules in brain tissue microscopic changes, be able to monitor AQP4-siRNA dry by pathological observation provide technical means interference effect, but its sensitivity remains to be improved.

In short, the different levels of brain contusion CBT zone will produce different pathological changes, severe brain contusion after CBT in early vasogenic edema appears, and then in the secondary cell edema and mixed edema, and mild brain contusion to the late major secondary to cytotoxic edema. AQP4-siRNA can both reduce CBT region through AQP4 expression and relieve brain edema in progress, but does not alleviate the severe injuries CBT zone early vasogenic edema. Functional magnetic resonance ADC values can be in the early to discern whether there are pathological changes CBT region, and Mild injury CBT area late (72 h to 7d) and after severe injuries early (24 h within) the AQP4-siRNA dry CBT area pre-treatment. So ADC value changes as cerebral edema and interference assessment therapeutic effect observed after TBI in the means of the image depending on the degree of injury at different time periods.

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