Effect of Xingnaojing injection on cerebral edema and blood-brain barrier in rats following traumatic brain injury

XU Miao 徐妙*, SU Wei 苏伟, XU Qiu-ping 徐秋萍 and HUANG Wei-dong 黄卫东

【Abstract】Objective: To explore the effects of Xingnaojing injection on cerebral edema and blood-brain barrier (BBB) in rats following traumatic brain injury (TBI).

Methods: A total of 108 adult male Sprague-Dawley rats were used as subjects and randomly assigned to three groups: sham-operation, TBI and Xingnaojing injection groups (10 ml·kg⁻¹·d⁻¹, intraperitoneal injection). TBI in rats was set up by the improved device of Feeney’s weight-dropping model with the impact of 600 g. Brain water content and BBB permeability expressed as Evans blue content were measured at 1, 3, 5 and 7 days after surgery.

Results: In sham-operation group, brain water content and Evans blue content in brain tissue were 78.97±1.22% and 5.13 μg±0.71 μg. Following TBI, water content in brain tissue was increased significantly at 1, 3, 5 and 7 days (83.49%±0.54%, 82.74%±0.72%, 80.22%±0.68%, 79.21%±0.60%), being significantly higher than that in sham operation group (P<0.05). Evans blue content was increased in TBI group (16.54 μg±0.60 μg, 14.92 μg±0.71 μg, 12.44 μg±0.92 μg, 10.14 μg±0.52 μg) as compared with sham-operation group (P<0.05). After treatment with Xingnaojing injection, brain water content decreased as compared with TBI group (81.91%±1.04%, 80.38%±0.72%, 79.54%±0.58%, 78.60±0.77%, P<0.05). Xingnaojing injection also reduced the leakage of BBB as compared with TBI group (15.11 μg±0.63 μg, 13.62 μg±0.85 μg, 10.06 μg±0.67 μg, 9.54 μg±0.41 μg, P<0.05).

Conclusion: Xingnaojing injection could alleviate cerebral edema following TBI via reducing permeability of BBB.

Key words: Medicine, Chinese traditional; Brain injuries; Brain edema; Blood-brain barrier

Brain edema, one of the most frequent secondary injuries of traumatic brain injury (TBI), often leads to a rise in intracranial pressure and is a key contributor to the morbidity and mortality associated with TBI. The cellular and molecular mechanisms contributing to the development or resolution of TBI-associated brain edema are poorly understood. Current treatments for brain edema such as hyperosmolar agents and surgical decompression have changed little since they were used more than 80 years ago and their efficacy is limited. One goal in TBI research as well as in clinical practice, is to develop a protocol for an immediate and sustained reduction of cerebral edema, and recovery of cerebral function.

Xingnaojing injection (XNJ), consisting of Chinese herbs such as Moschus, Borneol, Radix Curcumae, Fructus Gardeniae, was extracted by modern biotechnology according to Chinese Traditional Medicine named An Gong Niu Huang Wan. Modern pharmacological studies confirmed that XNJ can directly act on the central nervous system through blood brain barrier (BBB). It is now well documented that XNJ can reduce brain injury and enhance functional recovery after TBI and stroke in different clinical trials and animal models of injury. Despite the demonstrated benefits of XNJ in treating TBI, little is known about how this medicine specifically produces its salutary effects at the cellular level after TBI. To elucidate further the mechanisms can help to prevent edema formation and inflammation. Therefore we studied the brain water content and permeability of BBB in response to TBI and then the effects of treatment with XNJ using a rodent brain injury model.
METHODS

Instruments
HP8453 spectrophotometer (HP, USA), AB104 electronic analytical balance (Mettler-Totado, Switzerland), ZBY149-83 electric baking oven (Shanghai, China), DHP-9082 calorstat (Shanghai, China), and dentistry drill (Ningbo, China) were used.

Reagents and medicines
XNJ (Wuxi Jimingkexing Shanhe Pharmaceutic Co, Ltd. China, batch number: 061233), Evans blue (Sigma, USA), formamide (Merck, USA), 10% chloral hydrate (Guoyao Chemicals Co, Ltd. China) were used.

Animals and models
A total of 108 male Sprague-Dawley rats weighing 300-340 g provided by Zhejiang Medical Institute, were used as subjects and randomly assigned to three groups: sham-operation, TBI and XNJ groups. The later two groups were divided into 4 subgroups, i.e. postinjury 1, 3, 5 and 7 day groups. In each subgroup, the number of rats was 12, of which 4 were for brain water content test, 4 for BBB test and 4 for histological test.

Rats were anesthetized with 10% chloral hydrate (2 ml·kg⁻¹, intraperitoneal injection). Under aseptic conditions, a sagittal incision was made in the right scalp and the fascia was retracted to expose the cranium. Then, a dentistry drill was used to open the skull immediately at the point of 1.5 cm posterior to the bregma and a 5-mm diameter bone window was made with intact cerebral dura mater. TBI in rats was set up by a free fall of 20 g weight from a height of 30 cm with the impact of 600 g·cm according to the improved device of Feeney's weight-dropping model. After TBI, bone window was closed by bone wax and the fascia and scalp were sutured. In the XNJ group, XNJ was injected intraperitoneally (10 ml·kg⁻¹·d⁻¹) within 10 minutes after the models were set up and then the same dose was given every day. TBI group received only normal saline. Sham-operated rats were anesthetized and received craniectomy, but were not submitted to brain injury or treatment.

Determination of brain water content
Brain edema was measured at 1, 3, 5 and 7 days after surgery. Briefly, samples from the peri-contusion area were taken and weighed. Then they were placed in an oven and dried at 110°C for 24 hours. After drying, all weighings were done on the same balance. The water content was calculated by the following equation:

\[ \text{water content (\%)} = \frac{(\text{wet sample weight} - \text{dry sample weight})}{\text{wet sample weight}} \times 100\% \]

Determination of BBB permeability
2% Evans blue (EB) dye (3ml·kg⁻¹) was injected into the femoral vein at 1 hour before decapitation. The chest was opened 60 minutes later and the heart was perfused with 500 ml saline at 37°C through the left ventricle until the colorless perfusion fluid was obtained from the right atrium. After decapitation, tissue samples about 0.2 g in weight from the peri-contusion area were harvested and weighed. Then, the samples were placed in 5 ml formamide solution and incubated for 24 hours at 45°C then centrifuged at 1 500 r·min⁻¹ for 30 minutes. The optical density (OD) of the EB formamide solution was determined by spectrophotometry at 635 nm, then the content of EB (µg) was obtained according to the standard curve. BBB permeability was expressed as EB per gram of tissue (µg·g⁻¹).

Histological examination
Brain samples from the peri-contusion area for histological examination were taken at 1, 3, 5 and 7 days after surgery. Then they were immediately immersed in 10% buffered formalin and embedded in the paraffin. Paraffin sections were cut off, mounted on glass slides, and stained with hematoxylin and eosin (HE).

Statistical analysis
All data were expressed as mean ± SD. Statistical analysis was carried out with One-Way ANOVA using SPSS 11.5 statistical package. \( P < 0.05 \) was considered statistically significant.

RESULTS

Gross appearance of the brain tissues
Blood vessel dilated gently along the bone window, but no capillary hemorrhage or other abnormal findings were seen in brain tissues with negative EB-stain in sham-operation group. Conspicuous focal contusion and laceration of the brain concurrent bleeding spots and positive EB-stain were found in TBI group. Milder focal brain injury and EB-stain were shown in XNJ group than in TBI group.
Pathologic changes of brain tissue by HE staining

In sham-operation group, no abnormality could be found. Neurons in the brain tissues were cone-shaped, star-shaped or fusiform-shaped with clear outline and a narrow bright band surrounded neurons. Large round nuclei with light staining were located in the center of cells. Weak eosinophilic cytoplasm was shown by HE staining (Figure 1). In TBI group, brain tissue loosening, increased intercellular and intervascular spaces as well as bleeding in the brain parenchyma and subarachnoid space could be seen. Neurons were swelling with light staining, the nuclei were small and deviated, and the cytoplasm was cancellated with unclear boundaries (Figure 2). In XNJ group, the pathologic changes were milder than those in TBI group (Figure 3).

Effect of XNJ on brain water content

In TBI group, cerebral edema developed and peaked from day 1 to day 3, which was more severe than that of sham operation group \( (P<0.05) \). XNJ reduced the level of cerebral edema at 1, 3, 5 days as compared with TBI group \( (P<0.05) \). At the 7th day, it was very close to sham operation group \( (P>0.05, \text{Table 1}) \).

Effect of XNJ on BBB permeability

BBB permeability in TBI group increased and peaked at 1 day, and then declined gradually at every measure time point, but was much higher than that in sham operation group \( (P<0.05) \). XNJ reduced the level of BBB as compared with TBI group \( (P<0.05) \). But it was still higher than that of sham operation group \( (P<0.05, \text{Table 2}) \).

Table 1. Changes of brain water content in brain tissues \( (\%, \overline{x} \pm s, n=6) \)

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 d</th>
<th>3 d</th>
<th>5 d</th>
<th>7 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBI</td>
<td>83.49±0.54*</td>
<td>82.74±0.72*</td>
<td>80.22±0.68*</td>
<td>79.21±0.60*</td>
</tr>
<tr>
<td>XNJ</td>
<td>81.91±1.04*δ</td>
<td>80.38±0.72*δ</td>
<td>79.54±0.58*δ</td>
<td>78.60±0.77</td>
</tr>
<tr>
<td>Sham</td>
<td>78.97±1.22</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

\* \( P<0.05 \) vs sham-operation group; \δ \( P<0.05 \) vs TBI group; _: unmeasured

Table 2. Changes of EB content in brain tissues \( (\mu g \cdot g^{-1}, \overline{x} \pm s, n=6) \)

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 d</th>
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<td>10.06±0.67*δ</td>
<td>9.54±0.41*δ</td>
</tr>
<tr>
<td>Sham</td>
<td>5.13±0.71</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

\* \( P<0.05 \) vs sham-operation group; \δ \( P<0.05 \) vs TBI group; _: unmeasured
DISCUSSION

Traumatic brain edema is thought to be a mixed form of brain edema consisting of vasogenic and cytotoxic (cellular) edema. For vasogenic edema, the primary mechanism is the disruption of BBB due to endothelial damage, widening of tight junctions or induction of abnormal vesicular transport, all of which subsequently allow components of blood plasma to enter the brain. So the disruption of BBB is one of the early harmful events of TBI, allowing infiltration of peripheral macrophages and leukocytes into cerebral tissue and aggravation of the inflammatory response. Central nervous system inflammation is an intrinsic part of many neurodegenerative disorders including TBI. Inflammation mediated by proinflammatory cytokines (TNF-α, IL-1β, IL-6), reactive oxygen species (ROS) and prostaglandins might promote brain damage or augment cytotoxicity. Cytotoxic edema is caused by translocation of interstitial water into the intracellular compartment, in association with ionic and metabolic dysregulation. The primary cause of cytotoxic edema is due to the fact that the transmission of kinetic energy after TBI causes massive tissue damage from which to produce toxic metabolites. Restoring the integrity of BBB and reducing inflammatory cytokine activity are thereafter critical for the treatment of traumatic brain edema.

Since the damaged areas are dominated by necrotic tissue, we observed the tissue in the peri-contusion area known as penumbra area but not the dead tissue in the lesion core. Our findings, being consistent with other studies, showed that brain edema formation peaked at 24 hours after TBI and was significantly higher than sham levels (P<0.05). Brain edema continued during the initial 2-3 days following TBI, and decreased gradually. EB as a classical labeling compound cannot penetrate the normal BBB because it exists as EB-albumin compound in serum. When BBB is disrupted after TBI, EB could enter the brain. So EB extravasation has been used widely for simple quantitative evaluation of BBB disruption in vasogenic brain edema. A peak level of EB was observed in the peri-contusion area 24 hours after injury. But significant EB extravasation was still observed on the 7th day. BBB permeability was coincident with brain edema development with good correlation (n=0.898, P<0.01), suggesting that traumatic brain edema in the acute stage is directly associated with alterations of BBB permeability, with increasing permeability to macromolecules allowing fluid movement from intravascular to extravascular spaces. Neal et al found that the number of rat endothelial cell antibody 1 (RECA1), a marker of endothelial cells, decreased significantly in the penumbra of injury following TBI. Previous studies found transient and moderate BBB opening in the perifocal zone of lesion, and demonstrated that the secondary inflammatory cascade will exacerbate BBB dysfunction following TBI.

XNJ is the intravenous agent of An Gong Niu Huang Wan, which is the representative prescription for dissolving the turbid with aromatics and eliminating the phlegm for resuscitation. XNJ injection is mainly made up of such traditional Chinese medicines as Moschus, Borneol, Radix Curcumae and Fructus Gardeniae. Firstly, it can induce resuscitation and refreshment, and relieve brain edema. Thirdly, it can purge pathogenic fire and detoxify, antagonize inflammation and spasm, and then plays a protective role in the neurons after ischemia. Pharmacological studies confirm that Moschus and Borneol can enhance the tolerance of central nervous system to hypoxia and ischemia. Fructus Gardeniae can obviously ameliorate endothelial function through the pathway of nitric oxide. Radix Curcumae can remove phlegm and clear away toxic substances. In the clinical practice, it is mainly used for treating the disturbance of consciousness, anoxia neonatorum, viral encephalitis, meningitis and cerebrovascular accidents including cerebral hemorrhage, cerebral infarction and TBI.

In the present study, injury-induced water content increase in brain tissue, indicating edema formation in the contusion area after TBI, was substantially reduced by XNJ treatment. Administration of XNJ significantly inhibited EB extravasation, indicating a reduced BBB opening in response to XNJ treatment. The mechanisms by which XNJ attenuates BBB permeability and edema after TBI are currently unknown; however, a number of possibilities have been proposed. Lipid peroxidation and free radical generation are well known to increase BBB permeability and edema formation. XNJ has been shown to inhibit membrane lipid peroxidation and described as antioxidants. XNJ also has effects on cerebral blood flow via modulation of nitric oxide formation, and the ensuing vasodilation is thought to protect against BBB damage. In addition, XNJ has been shown to increase
the expression of antagonist excitatory amino acid receptors and restrain neuronal excitation and protect cultured cortical neurons from excitotoxicity of glutamic acid.\textsuperscript{9} XNJ is also well known to have anti-inflammatory effects after TBI, including effects on proinflammatory cytokines and prostaglandins.\textsuperscript{10} Finally, recent evidence suggests that XNJ, by regulating the differential expression of aquaporin water channels, may be implicated in the development and resolution of brain edema after TBI. Thus, XNJ may attenuate BBB damage and resultant edema formation by a number of different mechanisms. Irrespective of the mechanisms involved, it is clear that XNJ contributes to the edema and BBB permeability attenuation observed after TBI.

REFERENCES


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