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Basic Investigation

Effect of Batroxobin on Expression of C-Jun in Left Temporal Ischemic Rats with Spatial Learning and Memory Disorder

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The effect of Batroxobin on expression of c-Jun in left temporal ischemic rats with spatial memory disorder was investigated by means of Morri's water maze and immunohistochemistry methods. The results showed that the mean reaction time and distance of temporal ischemic rats for searching a goal were significantly longer than those of sham-operated rats, and at the same time c-Jun expression of left temporal ischemic region was significantly increased. However, the mean reaction time and distance of Batroxobin-treated rats were shorter and they used normal strategies more often and earlier than those of ischemic rats. The number of c-Jun immune reactive cells of Batroxobin-treated rats was also less than that of ischemic group. In conclusion, Batroxobin can improve spatial memory disorder in temporal ischemic rats, and the down-regulation of the expression of c-Jun is probably related to the neuroprotective mechanism.

Snake venom is a well-known traditional Chinese medicine used to treat some diseases including ischemic cerebrovascular diseases. Batroxobin is a thrombin-like enzyme isolated from snake venom, which enables to remove specifically fibrinogen. Our previous studies found that Batroxobin was an excellent drug for treatment of acute ischemic cerebrovascular diseases.[1] Batroxobin could attenuate cerebral edema in ischemic gerbils and inhibit c-fos gene expression of rat brains during ischemia and reperfusion.[2-4] We also found that Batroxobin could improve learning and memory

disorder of rats with left temporal ischemia[5]. But the mechanism remains unclear.

C-Jun is one of immediate early genes (IEGs) known to have rapid but brief response to stimulus. In the central nervous system, these IEGs are mostly rapidly induced by various stimuli including epilepsy and ischemia[6]; they encode for transcription factors, which in turn regulate the expression of a number of target genes or late genes, some of which may contribute to neuronal death or survival. However, the association of memory disorder induced by temporal ischemia and c-Jun expression as well as the effect of Batroxobin are unclear.

A recently developed spatial memory task is Morri's water maze (MWM)[7] which has been proven very useful for characterizing the neurochemical basis of spatial learning and memory in rodents[8]. In this study, we used Morri's water maze to investigate the effect of Batroxobin on the expression of c-Jun in temporal ischemic rats with spatial learning and memory disorders.

Materials and Methods

Animal and grouping: Thirty-six male Wistar rats weighing 180~220g were randomly divided into three groups, ischemia group (n=12), Batroxobin-treated group (n=12) and sham-operated group (n=12). The rats were in experimental environment for two days for habituation before behavioral training, and then a five-day behavioral training was carried out during 10:00am~5:00pm. The rats were free for food and water during the whole process of experiment. After the behavioral experiment had been finished, the rats were killed and c-Jun expression of brain tissue was studied by immunohistochemical method.

Animal model: Selective left temporal ischemia of rat model was induced by the photochemical method[5]. Each rat was anaesthetized with 10% chloral hydrate (0.3g/kg, i.p.) .The rats were placed in a stereotaxic frame and rectal temperature was kept at 36.5 ℃ ± 0.7 ℃ with a heating lamp. The left temporal scalp incision was performed to expose the skull surface. For skull illumination, standard equipment was used. A light guide fiber cool light was positioned on the left temporal skull surface. Rose bengal dye (Sigma Chemical Ltd; 20mg/ml in sterilized 0.9% NaCl) was injected slowly via a lateral tail vein, and the left temporal skull surface was illuminated for 20 minutes. The light was then interrupted, the wound was sutured, and the animal allowed to recover. Batroxobin-treated rats received an injection of Batroxobin (8BU/kg, i.p.) 30min before operation two days late. Sham-operated control animals were prepared with the same procedures except illumination.

Morri's water maze behavioral experiment:[7] The behavioral experiment equipment consists of Morri's water maze and Morri's Maze Experimental Assistant System (MMEAS). Morri's water maze is a large circular pool (d=94mm, h=55cm) filled with water rendered opaque with milk powder. Submerged just below the water surface somewhere within the pool is a platform (s=20cm^2) onto which the rat can climb to emerge from water and escape from the necessity of swimming. MMEAS consists of image collect card, video camera and automated computer tracking system. The experimental data were collected and analyzed automatically.[7]

The behavioral experiment was started on the fourth day after operation and continued for five days including 1 day of pretraining trial and 4-day training trial. Each rat accepted 6 trials per day with a 15 min

interval for each and the total number of training trial is 24. The experimental data included response time (s), distance (cm) and strategies. The mean value of three trials for response time and distance was considered as one unit respectively. F and X^2 test were analyzed with SPSS software package.

Immunohistochemistry: After the behavioral experiment was finished, six rats from each group were anaesthetized with 10% chloral hydrate (0.3g/kg, ip), brains were fixed by transcardiac perfusion with 4% paraformaldehyde 40 ml , then the brains were quickly removed and kept in the fixation of 4% paraformaldehyde at 4℃. They were subsequently sectioned (slice thickness, 30 μ) in cryostat and stained for c-Jun by using monoclone antibody (Santa Cruz Ltd) and SP kit[9]. Five slices of ischemic brain tissue from each rat were observed under microscope and c-Jun immune positive cells were counted (10× 10).

Results

Morri's water maze behavioral experiment: In brief, the mean reaction time and distance of temporal ischemic rats for searching a goal were significantly longer than those of sham-operated rats. However, the mean reaction time and distance of Batroxobin-treated rats were shorter and they used normal strategies more often and earlier than those of ischemic rats (details see elsewhere).[7]

Expression of c-Jun: The number of c-Jun immune reactive cells of left temporal ischemic rats was significantly increased as compared with that of sham-operated group. However, the number of c-Jun immune reactive cells of Batroxobin-treated rats was less than that of ischemic group (see the following Table and Fig.).

Table. Number of c-Jun immune reactive cells

Group	n	c-Jun ($\bar{x} \pm s$)
Ischemia	6	$52.3 \pm 9.2^{*}$
Batroxobin-treated	6	$33.8 \pm 3.2^{*\#}$
Sham-operated	6	12.8 ± 3.8

$^{*}P<0.01$, compared with the sham-operated group

$^{\#}P<0.01$, compared with the ischemic group

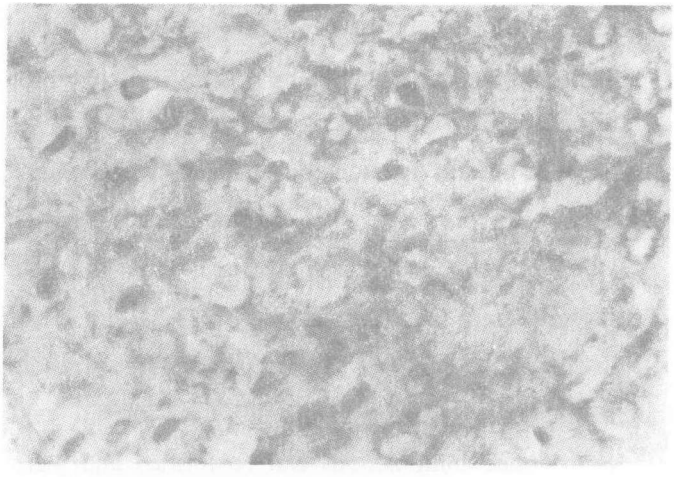

Fig 1. C-Jun positive cells of the cortex in cerebral ischemic rats

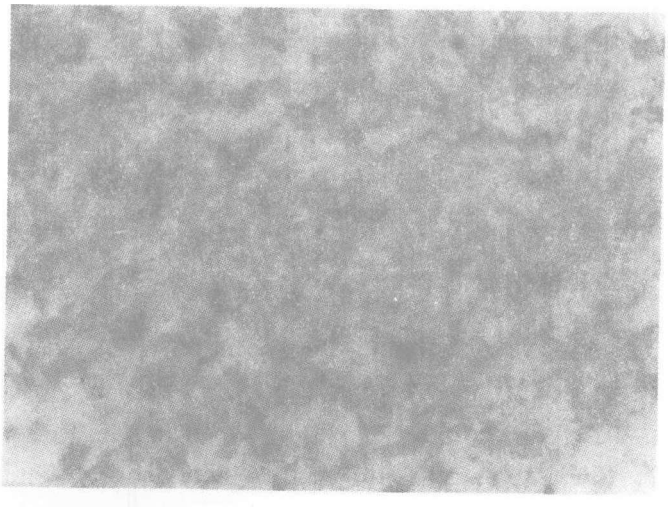

Fig 2. C-Jun positive cells of the cortex in Batroxobin-treated rats

Discussion

In the present Morri's water maze behavioral experiment and immunohistochemical study, we found that the spatial learning and memory ability was disordered in left temporal ischemic rats[7] and the c-Jun expression of ischemic temporal cortex was increased. This result was in accordance with our previous study that c-fos gene expression was increased during cerebral ischemia[4] and the present result further suggested that there may be a relation among left temporal ischemia, spatial memory disorder and c-Jun expression.

The protein products of c-fos and c-Jun gene are thought to form heterodimers that directly contact with DNA binding sites and function as transcriptional regulators at AP-1 binding sites. The binding of these heterodimers to the AP-1 sites is modulated by phosphorylation. Various homo- and heterodimer combinations can be formed and there are some evidences to suggest that the relative levels of these multiple homo- and heterodimers may have positive as well as negative transcriptional function. C-Jun phosphoprotein is required to form active heterodimers before binding to AP-1 sites and initiating transcription of target gene. It is conceivable that c-Jun sets a cascade of events in action that leads to neuronal destruction in some cell group. It is possible that a sudden surge in glutamate receptor production could initiate excitotoxic injury in the postischemic brain by leading to a vicious cycle of receptor activation, calcium influx, IEGs activation and consequent increased receptor synthesis[10]. There are some evidences to suggest that IEGs expression of ischemic brain tissue is linked to NMDA glutamate receptor activation, and NMDA receptor antagonists MK-801 may inhibit the induction of IEGs.[11]

Our MWM study have found that the mean reaction time and distance of Batroxobin-treated rats are shorter and they use normal strategies more often and earlier than those of ischemic rats[7]. The result is in accordance with our previous study that Batroxobin may ameliorate the dysfunction of learning and memory in temporal ischemic rats measured by step-down methods.[5] The MWM result further indicates that the Batroxobin may also ameliorate the dysfunction of spatial learning and memory in temporal ischemic rats measured by Morri`s water maze. It has been demonstrated that Batroxobin can reduce the morbidity and mortality of ischemic stroke in gerbils and rats, ameliorate cerebral edema, and attenuate the dysfunctions of arginine vasopression, excitatory amino acids and nitric oxide, and down-regulation of endothelin-1 gene expression.[1-4,12] Based on these mechanisms, it is reasonably proposed that Batroxobin may be of down-regulation of c-Jun expression in ischemic brain tissue, ameliorate ischemic brain injury, and may therefore improve the spatial learning and memory disorder of left temporal ischemic rats.

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