Injury, Int. J. Care Injured 40 (2009) 963–967

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

# Protective effects of mutant of acidic fibroblast growth factor against cerebral ischaemia-reperfusion injury in rats

Hua Xu [a,b,*], Xiao-kun Li [c], Qing Zheng [a], Ya-dong Huang [c], Cheng-can Yao [a], Zhi-jian Su [c], Wen Zhao [a], Zhuan-you Zhao [d]

[a] Pharmacy College of Jinan University, Guangzhou 510632, PR China
[b] Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University, Guangzhou 510632, PR China
[c] Biopharmaceutical R&D Center of Jinan University, Guangzhou 510632, PR China
[d] Department of Pharmacology, Tianjing Institute of Medicine, Tianjing, 300193, PR China

ARTICLE INFO

*Article history:*
Accepted 14 January 2009

*Keywords:*
Mutant of human acidic fibroblast growth factor (MaFGF)
Middle cerebral artery occlusion (MCAO)
Cerebral ischaemia-reperfusion
Behaviour deficit
Infarct areas
Brain oedema
Anti-oxidation
Rats

ABSTRACT

*Objective:* To investigate the protective effect of a mutant of acidic fibroblast growth factor (MaFGF) against cerebral ischaemia-reperfusion injury in rats.
*Methods:* Sixty male Sprague–Dawley rats were randomly divided into six groups as follows: sham-operated group, untreated group, 20 μg/kg, 40 μg/kg and 80 μg/kg MaFGF-treated groups and also the positive control group. Cerebral ischaemia-reperfusion injury was induced by middle cerebral artery occlusion (MCAO) for 2 h followed by reperfusion for 24 h. Different dose of MaFGF were infused intravenously at 1 h after middle cerebral artery (MCA) occlusion. Nimodipine was used as positive control. The behaviour deficit score, brain-infarcted area, brain oedema degree, malondialdehyde (MDA) content and superoxide dismutase (SOD) activity were detected at 24 h after reperfusion.
*Results:* The results showed that MaFGF at the dose of 20 μg/kg, 40 μg/kg and 80 μg/kg significantly alleviated brain injury. Compared to untreated group, the behaviour deficits were much less severe, the brain oedema alleviated obviously, the MDA contents decreased and SOD activity increased dramatically in MaFGF-treated groups respectively. The efficacy of MaFGF was similar to that of nimodipine.
*Conclusion:* The results demonstrate that MaFGF has neuroprotective effect against brain injury resulting from focal ischaemia-reperfusion in Sprague–Dawley rats.


## Introduction

Acidic fibroblast growth factor (aFGF) is one of the important members of peptide growth factor families which was originally identified as peptides with mitogenic activity for fibroblasts and exist at high levels in brain extracts. It is reported that aFGF has neuroprotective and regenerative capabilities administered after focal cerebral ischaemia in different species,[18,20] so it may become a promising medicine for the treatment of acute focal cerebral ischaemia.

However, the therapeutic uses of aFGF are limited due to its mitogenic activity; it exhibits pleiotropic effects on cell proliferation, survival and differentiation, which may be related to tissue fibrosis and neoplasia.[12,19] Following the study results from Lozano et al., we modified the N-terminus of the aFGF gene by eliminating the N-terminal residues 1–27 and also substituting Met for Leu27 to obtain human acidic fibroblast growth factor mutant (haFGF27-154), namely, non-mitogenic haFGF (MaFGF), which weakened mitogenic property and preserved non-mitogenic properties which include vasodilatory, neuromodulatory, and cardio- and neuroprotective activities.[17,22,24] The aim of this study was to investigate whether MaFGF could protect against brain injury induced by cerebral ischaemia-reperfusion in rats.

## Materials and methods

### Chemicals

MaFGF, 0.329 mg/ml, was provided by Biopharmaceutical Research and Development Center of Jinan University. Its preparative method is as follows[22]: The MaFGF cDNA was amplified from the plasmid pUC-haFGF by a standard polymerase chain reaction, followed by subcloning the products into pET-3c vectors. MaFGF protein was expressed in BL21 (DE3) cells and purified on a CM-Sepharose column equilibrated with PBS buffer (Sigma). The purity of MaFGF was assessed using SDS-PAGE and the immunogenic activity was checked by Western blotting.

* Corresponding author at: Pharmacy College of Jinan University, Guangzhou 510632, PR China. Tel.: +86 20 85220850; fax: +86 20 85220850.
*E-mail address:* huax-mail@163.com (H. Xu).

0020–1383/$ – see front matter. Crown Copyright © 2009 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.injury.2009.01.110

Nimodipine, 0.2 mg/ml, was made in Tianjin people's pharmaceutical factory of amino acid produces (Tianjin, China). They were diluted in saline to necessary concentration before being used. The test kits of superoxide dismutase (SOD), malondialdehyde (MDA), blood urea nitrogen (BUN) and creatinine (Cr) were purchased from Nanjing Jiancheng bioengineering research institution (Nanjing, China).

*Experimental methods*

*Animals and groups*

Sixty male Sprague–Dawley rats, weight between 300 g and 350 g, were purchased from Medical Animal Center of Guangdong Province. The certification number was 2005A010 (Guangdong, China). They were maintained in a controlled environment at $24 \pm 2$ °C with a 12 h light/dark cycle and received food and water ad libitum, and acclimatised for at least 3 days prior to use. The animals were randomly divided into six groups according to administration of different treatment, including: sham-operated group (no occlusion of middle cerebral artery (MCA), $n = 10$), untreated group (performed MCAO, treated with saline, $n = 10$), MaFGF groups (performed MCAO, treated with MaFGF at dose of 20 μg/kg, 40 μg/kg and 80 μg/kg, respectively, 10 each group), and positive control group (performed MCAO, treated with nimodipine, 40 μg/kg, $n = 10$).

*Experimental procedure*

Rats were anaesthetised intraperitoneally with 350 mg/kg of chloral hydrate. The models of cerebral ischaemia-reperfusion injury preparation were performed by middle cerebral artery occlusion (MCAO) for 2 h and reperfusion for 24 h. MCAO was performed by intraluminal nylon suture occlusion method as described by Longa[16] and Belayev.[3] Briefly, the left common carotid artery was exposed and was carefully dissected free from surrounding nerves and fascia. The internal carotid artery was isolated and carefully separated from the adjacent vagus nerve, and the pterygopalatine artery was ligated close to its origin with a 5-0 nylon suture. Next, a 3-0 silk suture was tied loosely around the mobilised external carotid artery stump, and a 4-cm length of 3-0-monofilament nylon suture was inserted through the proximal external carotid artery into the internal carotid artery and thence into the circle of Willis, effectively occluding the middle cerebral artery. The suture was inserted 18–20 mm from the bifurcation of the common carotid artery. After the intraluminal suture was placed, the neck incision was closed and penicillin was dribbled in the incision to prevent infection. After 2 h of MCAO, the intraluminal suture was carefully removed and the internal carotid artery was reperfused. The internal carotid artery was isolated only in the sham-operated group but the middle cerebral artery was not occluded. The animals awakened from anaesthesia were returned to their cages to be allowed free access to food and water.

*Drug administration*

One hour after MCAO, the rats that did not demonstrate a right upper extremity paresis or died were excluded from further study. At the same time, the intervening drug (MaFGF and nimodipine) or untreated (saline) were intravenously administered to carry on selected valid rats in the volume of 1 ml/300 g body weight through a constant speed pump at a speed of 1 ml/h.

*Assays on brain ischaemia-reperfusion injury*

*Neurological function tests*

After MCAO had been performed for 24 h, neurological function tests were performed in all 60 rats. Neurological function was evaluated by an investigator who was blinded to the experimental groups, according to the method that were used previously[2,4]: (1) the postural reflex test: to examine upper body posture while the animal is suspended by the tail1, (2) the stretch reflex and pain reflex: examine the muscle strength by pulling forelimb with hand, and the painful feeling by clamping the forelimb, and (3) the resistance test: examine the resistance to lateral push toward contralateral side. Behavioural deficits were graded on a scale of 0–11 (normal score = 0, maximal deficit score = 11), which is given in Table 1.

*Estimation of infarct areas*

After neurological function test, all 60 rats were sacrificed by decapitation and the brains were then removed and placed in saline and the remaining water on brain was dried with filter paper. The brain was then rapidly frozen and sectioned into 3-mm-thick coronal slices. Half of brain slices obtained above were stained with 2,3,5-triphenyltetrazolium chloride technique to estimate the injury, which stains only viable tissue brick red, as previously described.[1,2] Briefly, the interval brain slice was placed in 2% 2,3,5-triphenyltetrazolium chloride for 30 min at 37 °C, away from light, then they were placed in 10% buffered formalin. One week later, every section of the brain was photographed with a COOLPIX955 digital camera and the pictures of infarcted zones were then video-digitised and saved as digital images to computer. The areas of infarction in percentages were determined with a computer-assisted image analytical system software v 4.0 designed by figure centre of Beijing aerospace university.

**Table 1**
Behavioural deficit score.

| Tests item | Description | Score |
|---|---|---|
| The postural reflex test: observing upper body posture while the animal is suspended by the tail | Normal | 0 |
| | Adduct or extend forelimb slightly | 1 |
| | Extend forelimb obviously or adduct forepaw | 2 |
| | Forelimb adduction manifested as obvious debility | 3 |
| | Failure to adduct forelimb fully | 4 |
| Stretch reflex and pain reflex: Observing the muscle strength by pulling forelimb with hand and the painful feeling by clamping the forelimb | Normal | 0 |
| | Muscle strength or painful feeling degrade | 1 |
| | Muscle strength and painful feeling degrade | 2 |
| | Muscle strength degrade obviously and deficit on painful feeling basically | 3 |
| Resistance test: observing the resistance to lateral push toward contralateral side | Normal | 0 |
| | Decreased resistance to lateral push | 1 |
| | Increased resistance to lateral push | 2 |
| | Turn to contralateral side obviously | 3 |
| | Same behaviour as grade 3, with circling | 4 |
| Maximum points | | 11 |