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Effect of basic fibroblast growth factor on focal ischemic injury and antioxidant enzyme activities

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KEY WORDS basic fibroblast growth factor; cerebral ischemia; catalase; superoxide dismutase; glutathione peroxidase; glutathione transferases

ABSTRACT

AIM: To explore the effect of basic fibroblast growth factor (bFGF) on focal cerebral ischemic injury and antioxidant enzyme activities. **METHODS**: Rats underwent 24-h middle cerebral artery occlusion by intraluminal suture. Infarction volume was shown with staining and quantitated by image analysis system. Neurologic deficit scores were determined with a 0 - 5 grade scale. Antioxidant enzyme activities of forebrains were detected. **RESULTS**: bFGF (45 $\mu g \cdot kg^{-1} \cdot h^{-1}$ iv for 3 h, started 5 min after the onset of ischemia) showed potent neuroprotective effects. Infarction volumes were decreased from 272 mm^3 ± 22 mm^3 (saline-treated) to 201 mm^3 ± 30 mm^3 (bFGF-treated). Neurologic deficit scores were decreased from 3.6 ± 1.5 (saline-treated) to 2.3 ± 1.6 (bFGF-treated). Focal cerebral ischemia induced an increase in the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), but a decrease in the level of glutathione *s*-transferase (GSH-ST). Treatment of bFGF further increased the CAT activity but had no effect on the activities of SOD, GSH-PX, and GSH-ST. **CONCLUSION**: bFGF has a neuroprotective effect against focal cerebral ischemic injury. The elevation of CAT activity by bFGF may be involved in this effect.

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INTRODUCTION

Basic fibroblast growth factor (bFGF) is a 18-kDa polypeptide with potent survival-promoting and protective effects on various cells, including neurons, glia, and endothelial cells. Intraventricular or intravenous injection of bFGF reduced the volume of cerebral infarction of focal ischemia in rats[1-3]. In the current study, the neuroprotective effect of bFGF was studied and the effect of bFGF on levels of antioxidant enzymes activities was also explored.

MATERIALS AND METHODS

Chemicals Human recombinant bFGF was produced by Torita Bio-Pharm Co. (Zhuhai, China). 2,3,5-Triphenyltetrazolium chloride (TTC) was the product of the Third Reagent Factory of Shanghai (Shanghai, China). Kits to determine the activities of antioxidant enzymes (CAT, SOD, GSH-PX, GSH-ST) were purchased from the Nanjing Jianchen Institute of Biotechnology.

Focal cerebral ischemia model[4] Adult ♂ SD rats (obtained from Shanghai Experiment Animal Center, Chinese Academy of Sciences, Grade Ⅱ, Certificate № 005), weighing 375 g ± 25 g were anesthetized with 8 % chloral hydrate 400 $mg \cdot kg^{-1}$ ip. Rat body temperature was maintained at 36.5 - 37.5 ℃ with a heating lamp during anesthesia. A nylon suture with

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0.26 mm of diameter was introduced into the left external carotid artery (ECA) of rat after temporarily clipping the left common carotid artery (CCA) and the internal carotid artery (ICA). The nylon suture was then advanced from ECA into ICA until slight resistance was felt. The distance from the bifurcation of CCA to the tip of suture was about 22 mm. These methods placed the tip of the suture at the proximal segment of the anterior cerebral artery (ACA), thus blocked the origin of middle cerebral artery (MCA). The suture was left in place until death.

Experiment protocol Rats were randomly divided into three groups: 1) sham-operated; 2) ischemia control treated with saline; 3) treated with bFGF 45 $\mu g \cdot kg^{-1} \cdot h^{-1}$ iv for 3 h, starting at 5 min after the onset of ischemia from the femoral vein via a microinjection pump (CMA/100) at 0.33 $mL \cdot h^{-1}$.

Neurologic deficit evaluation The neurologic deficit status of rats were evaluated at 24 h after surgery by observers blinded to the treatments. Grade 0, no observable neurologic deficit; Grade 1, failure to extend the right forepaw fully; Grade 2, intermittent circling; Grade 3, sustained circling without moving forward; Grade 4, unable to walk spontaneously with a depressed level of consciousness; Grade 5, death.

Measurement of infarction volume Twenty-four hours after ischemia, rats were decapitated, and their brains were removed and washed with saline. Coronal sections of 2 mm thick were dissected with a brain slicer. Then, the slices were immersed in saline containing 2 % TTC at 37 ℃ in the dark for 30 min. The stained slices were fixed with 10 % formalin and photographed. The infarction area of each slice was traced and calculated using an image analysis system (T-90). Total infarction volume for each brain was calculated by summation of infarction areas of the subsequent slices (total area × thickness).

Measurement of antioxidant enzyme activities Twenty-four hours after ischemia, rats were decapitated, and their brains were removed and stored at −20 ℃. On the day to assay, each brain hemisphere was defrosted and homogenized with a buffer 4 mL consisting of sucrose 250 $mmol \cdot L^{-1}$, Tris-HCl 5 $mmol \cdot L^{-1}$, and edetic acid-2Na 0.1 $mmol \cdot L^{-1}$ (pH 7.5). The homogenate was centrifuged at 4000 × g at 4 ℃ for 15 min and the supernatant was used for enzyme assays. The protein of the supernatant was determined[5].

The activities of antioxidant enzymes were determined with kits. The assay for CAT activity was based on its ability to decompose H_2O_2. The absorbance of supernatant at 254 nm changed when the H_2O_2 solution was injected into the cuvette. The change of the absorbance reflected the CAT activity.

The assay for SOD activity was based on its ability to inhibit the oxidation of oxymine by O_2^- produced from xanthine-xanthineoxidase system. The red product (nitrite) produced by the oxidation of oxymine had absorbance at 550 nm. One unit of SOD activity was defined as the amount that reduced the absorbance at 550 nm by 50 %.

GSH-PX activity was determined by quantifying the rate of oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) by the H_2O_2 as catalyzed by GSH-PX. A! yellow product which had absorbance at 412 nm could be formed as GSH react with dithiobisnitrobenzoic acid. One unit of GSH-PX was defined as the amount that reduced the level of GSH by 1 $\mu mol \cdot L^{-1}$ in one minute per mg protein.

GSH-ST activity was determined by quantifying the rate of GSH to react with 1-chloro-2,4-dinitrobenzene as catalyzed by GSH-

ST. One unit of GSH-ST was defined as the amount that reduced the level of GSH by 1 μmol·L^{-1} in one minute per mg protein.

Statistics Data were expressed as $\bar{x} \pm s$ and assessed by two-tailed t test.

RESULTS

Effect of bFGF on focal ischemia Large infarct in the ipsilaterial dorsolaterial cerebral cortex and underlying striatum was produced by permanent unilaterial occlusion of the proximal MCA by intraluminal suture in rats. In saline-treated group, 6 of 16 rats died and others developed visible infarction volumes under TTC staining. After treatment with bFGF, the infarction volumes and the neurologic deficit scores were both drastically decreased (Tab 1). Because both the rats underwent infarction volume measurement and those underwent enzyme assay were used for neurologic deficit evaluation, the sample number of neurologic deficit evaluation was higher than that of others.

Effect of bFGF on CAT, SOD, GSH-PX, and GSH-ST activities In saline-treated group, the CAT activity elevated in the left (ipsilateral) but not in the right hemisphere by 24-h left side focal ischemia. In the bFGF-treated rats, the CAT activity levels in both left and right hemispheres increased. The CAT level in the left hemisphere of bFGF – treated rats was higher than that of saline-treated rats.

In saline-treated group, the SOD and GSH-PX activities elevated in both left and right hemispheres of rats by focal ischemia. But, SOD activity of bFGF-treated rats was lower than that of saline-treated rats. As to GSH-PX activity, there was no difference between bFGF-treated group and saline-treated group. The GSH-ST activities were decreased in the left but not right side hemispheres of rats by left side focal ischemia in both the saline-treated and the bFGF-treated group (Tab 2).

DISCUSSION

Our results indicated that intravenous infusion of human recombinant bFGF exhibited

Tab 1. Neurologic deficit scores and infarction volumes in rats after 24-h MCA occlusion in sham-operated group, saline-treated group, and bFGF (45 μg·kg^{-1}·h^{-1} for 3 h)-treated group. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* saline treated group.

Group	Grade of neurologic deficit 0	1	2	3	4	5	Neurologic deficit score ($n = 16$)	Infarction volume/mm^3 ($n = 7$)
Sham-operated	16	0	0	0	0	0	0	0
Saline-treated	0	2	2	2	4	6	3.6 ± 1.5	272 ± 22
bFGF-treated	0	8	3	1	1	3	2.3 ± 1.6^b	201 ± 30^c

Tab 2. Effect of bFGF (45 μg·kg^{-1}·h^{-1} for 3 h) on level of antioxidant enzyme activities. $n = 8 - 10$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* sham-operated group. $^fP < 0.01$ *vs* saline-treated group (left side).

Groups	CAT/ U·g^{-1} (protein)	SOD/ kNU·g^{-1} (protein)	GSH-PX/ kU·g^{-1} (protein)	GSH-ST/ kU·g^{-1} (protein)
Sham-operated	174 ± 27	23.1 ± 0.7	13.6 ± 1.1	31.8 ± 1.2
Saline-treated (right side)	185 ± 21	27.8 ± 1.6^c	16.1 ± 0.4^c	30.8 ± 1.8
Saline-treated (left side)	212 ± 18^c	32.4 ± 1.5^c	17.5 ± 0.5^c	25.7 ± 4.2^c
bFGF-treated (right side)	206 ± 23^b	22.0 ± 1.5^f	15.8 ± 0.9^c	30.9 ± 0.9
bFGF-treated (left side)	304 ± 57^{cf}	24.6 ± 1.2^f	17.9 ± 1.2^c	25.7 ± 1.9^f

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neuroprotective effects on focal ischemic injury in rats. These data are consistent with several previous reports showing that intraventricular or systemic administration of bFGF limited infarct size when given within the first few hours after focal ischemia[1-3].

The effect of bFGF against ischemic injury may be based on its direct cytoprotective potential. *In vitro*, bFGF protects cultured neurons against a number of toxins and insults thought to be important in the pathogenesis of cell death after ischemia and these effects appear to be mediated through signal transduction cascades initiated by gene expression and protein synthesis[6-8].

Since reactive oxygen species were believed to play a central role in excitotoxic/ischemic injury to neurons, we tested the effects of bFGF on the activities of antioxidant enzymes under ischemic situation in the current study. The dose of bFGF used in testing its influence on antioxidant enzymes is the dose at which bFGF could exhibit remarkable neuroprotective effects. CAT is an important scavenging enzyme against reactive oxygen species, as it removes H_2O_2 produced during metabolic processes. The enzyme is present in virtually all neural cells, albeit at different concentration[9,10]. The elevated CAT level of saline-treated group indicated that oxygen radicals were involved in postlesional reactions of ischemia and suggested that the impaired regions protected themselves by increasing their CAT activity. The ability of bFGF to further induce the expression of CAT may contribute to its protective effect against ischemic injury.

Our finding that SOD, GSH-PX activity increased in both the left (ipsilaterial) and right (contralaterial) hemisphere of rats was consistent with previous reports[11-13]. SOD is the enzyme that dismutases superoxide. It is interesting that SOD activity of bFGF-treated rats was lower than that of saline-treated rats. The ameliorated ischemic injury of bFGF-treated rats may contribute to this fact. GSH-PX is the enzyme involved in the reduction of cytosolic H_2O_2. The fact that there was no difference between bFGF-treated group and saline-treated group indicated that bFGF did not exert its neuroprotective effect through elevating the activity of GSH-PX. GSH-ST is also a kind of enzyme involved in the antioxidant system of brain to decompose lipid peroxides. It is known as a family of enzymes which play an important role in the detoxification and excretion of xenobiotics[14]. In this study, we found that ischemia caused a decrease of GSH-ST activity level in the hemisphere ipsilaterial to the ischemic injury. The decrease of GSH-ST level may reflect the death of neural cells. Our results also indicated that bFGF had no influence on GSH-ST activity.

bFGF could induce the expression of SOD and GSH-PX *in vitro*[8,15]. In our opinion, at least at the time point we tested (24-h after the onset of ischemia), bFGF-treated rats did not exhibit elevation of SOD, GSH-PX or GSH-ST activities. However, the effect of bFGF on these enzymes may be remarkable at other time points.

In summary, activities of CAT, SOD, GSH-PX, and GSH-ST were differently affected by ischemic injury. CAT, SOD, and GSH-PX activities were increased while GSH-ST activity was decreased after ischemic injury. Extraneous bFGF had a remarkable neuroprotective effect and the effect may be partly due to its potential to increase CAT activity under ischemic condition.

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碱性成纤维细胞生长因子对局部脑缺血损伤及抗氧化酶活性的影响

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关键词 碱性成纤维细胞生长因子；脑缺血；过氧化氢酶；超氧化物歧化酶；谷胱甘肽过氧化物酶；谷胱甘肽转移酶类

目的：考察碱性成纤维细胞生长因子(bFGF)对局部脑缺血损伤和抗氧酶活性的影响. **方法**：大鼠用插丝法阻塞大脑中动脉 24 h，梗塞灶用 TTC 染色显示，图象分析测量；神经功能缺损采用 0 – 5 级评分. 分别分析每只大鼠左右两侧大脑的 4 种抗氧化酶活性水平. **结果**：bFGF(缺血 5 min 后以 45 $\mu g \cdot kg^{-1} \cdot h^{-1}$给予 3 h)可降低梗塞灶体积和神经功能缺损评分. 局部脑缺血升高过氧化氢酶(CAT)，超氧化物歧化酶(SOD)和谷胱甘肽过氧化物酶(GSH-PX)的活性，但使谷胱甘肽 S-转移酶(GSH-ST)的活性水平降低. bFGF 可在缺血状态下进一步升高 CAT 的活性，但对 SOD、GSH-PX 和 GSH-ST 的水平没有影响. **结论**：bFGF 对局部脑缺血损伤具有神经保护作用，此作用可能与 CAT 活性水平的升高有关.

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