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Original Research

Alteration of basic fibroblast growth factor expression in rat during cerebral ischemia[1]

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KEY WORDS basic fibroblast growth factor; cerebral ischemia; fluoroimmunoassay; confocal microscopy

ABSTRACT

AIM: To investigate the distribution and expression changes of basic fibroblast growth factor (bFGF)-like immunoreactivity (IR) in focal cerebral ischemic rat brain. **METHODS**: The expression of bFGF was observed by fluoro-immunohistochemistry. The focal cerebral ischemic injury was carried out using middle cerebral artery occlusion (MCAO) model. **RESULTS**: Confocal images demonstrated that both the striatum and the frontoparietal cortex showed increases of bFGF-like IR after 2-h MCAO and 24-h reperfusion. In striatum, the increase patterns of bFGF-like IR were different according to the ischemic extent. In the core of infarct and its surrounding region (with more IR induction), bFGF-like IR was mainly located in astrocytes. In region adjacent to infarct (with most IR induction, the grey ratio of bFGF-like IR increased from sham-98 % ± 10 % to ischemia-125 % ± 6 %), some neurons also showed an upregulation of bFGF-like IR. In frontoparietal cortex, strong induction of bFGF-like IR was mostly seen in neurons (The grey ratio of bFGF-like IR increased from sham-104 % ± 11 % to ischemia-132 % ± 28 %), although it was observed in astrocytes as well. **CONCLUSION**: The expression of bFGF increased after focal cerebral ischemia in rats, suggesting that: in striatum, astrocytes may play an important role in the protection of neurons via the overexpression of bFGF; whereas in cortex, neurons probably exert an autoprotection through expressing bFGF themselves.

INTRODUCTION

Basic fibroblast growth factor (bFGF) is a polypeptide with potent trophic effects on brain neurons, glia, and endothelial cells. Several papers[1,2] reported that bFGF mRNA and bFGF-like immunoreactivity (IR) in the rat brain increased following cerebral ischemia. bFGF could protect neurons against boosting of $[Ca^{2+}]_i$, nitric oxide toxicity, excitatory amino acids, *in vitro*[3-5], as well as against ischemic, neurotoxic and chemical hypoxic brain damages *in vivo*[6-8]. The functional recovery was enhanced by bFGF following focal cerebral infarction in the rat[9]. The exact protective mechanism of bFGF remains poorly understood due to its considerable complexity. Some researchers indicated that there was also enhancement of bFGF receptor (bFGFR) mRNA expression, parallel with the expression of bFGF mRNA in ischemic rat brains[10-12]. In the present study, triple fluoro-immunohistochemic staining combined with confocal laser scanning microscopy (CLSM) was used to investigate the expression and distribution changes of bFGF-like IR in several regions of rat brain following MCAO. In addition, the sum of grey for the bFGF-like IR in peri-infarct striatum and pyramidal cell layer in frontoparietal cortex were measured, and the subcellular location of the IR was investigated.

MATERIALS AND METHODS

Animal preparation Adult male Sprague-Dawley rats (260 - 300 g, Shanghai Experimental Animal Center, Chinese Academy of Sciences, Grade Ⅱ, Certificate № 005) were randomized into two groups: sham-operated ($n = 6$), ischemic ($n = 8$). Anesthesia was

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induced by 10 % chloral hydrate 320 mg·kg^{-1} ip. Rectal temperature was continuously monitored with a rectal probe inserted to a 4-cm depth from the anal ring and maintained at (36.8 – 37.1) ℃ with a heating lamp during the experiment. Focal brain ischemia was induced by the intraluminal suture MCAO method[13]. After 2-h MCA occlusion, the suture was withdrawn out of internal carotid artery. Sham-operation was performed using the same protocol as in the ischemia group, but without MCAO.

Triple fluoro-immunohistochemic labeling After 24-h reperfusion, rats were sacrificed by transcardial perfusion with 4 % paraformaldehyde fixative. The brains were removed and cryoprotected in PBS 0.1 mol·L^{-1} (pH 7.4) containing 30 % sucrose at 4 ℃ overnight. Coronal sections of 50 μm were continuously cut with a cryostat from bregma 1.60 mm to – 4.16 mm, according to the Atlas of Paxinos & Watson (3rd edition). By using fluoro-immunohistochemical assay (Bio-Rad Fluorescence Labeling Kit), one of the 50-μm sections (bregma: – 0.40 mm, containing caudate putamen and frontoparietal cortex) was prepared for observing the bFGF-like IR expression and distribution. The fluorescence labeling procedure was carried out as described in the instruction manual of the kit. The sections were treated with primary antibody solution containing guinea pig anti-GFAP diluted at 1∶250 and affinity purified polyclonal rabbit anti-bFGF IgG (3 mg·L^{-1}, Calbiochem, reacting with a 18 kDa protein) which does not exhibit cross-reactivity with aFGF, then added secondary antibodies (goat anti-guinea pig Cy5, goat anti-rabbit FITC) and Radiant Red stain, each diluted at 1∶100. The specificities of each primary antibody were verified by replacing the antibodies with PBS, or preadsorbing the anti-bFGF primary antibody with excess bFGF. These control-staining experiments consistently showed negative results.

Confocal laser scanning microscopy observation The labeled tissue slices were scanned and photographed by Leica confocal laser scanning microscope. The observing sites included the core of infarct, peri-infarct, region surrounding the infarct in striatum, as well as the pyramidal layer, multiform layer in frontoparietal cortex (as described by the triangles and circles in Fig 1). The Band Pass 530/32, 600/30, and the Long Pass 665 filters were selected for FITC (excitation maxima 494 nm, emission maxima 520 nm), Radiant Red (excitation maxima 500 nm, emission maxima 610 nm), and GFAP (excitation maxima 650 nm, emission maxima 670 nm), respectively. The CLSM system was operated by Leica TCSNT software. The sample lens used for image capture was Leica 50 × water lens/NA 0.75. The zoom was 1.0, while the thickness of each optical scan slice was 1.25 μm. The scan images were saved with 512 × 512 pixel type.

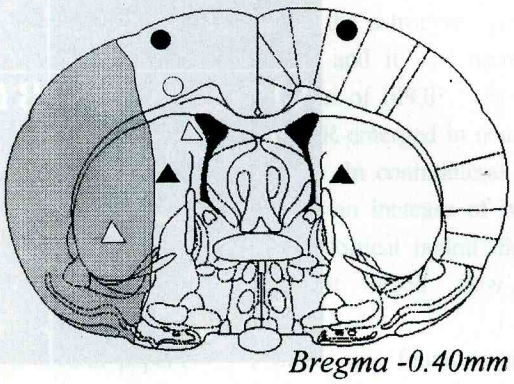

Fig 1. The typical extent of cerebral infarct (shaded area) after MCAO, and the locations observed using CLSM. Triangles indicate the observed sites (core of infarct, adjacent region to the infarct, surrounding region to the infarct) in striatum and circles indicate the observed sites (multiform layer, pyramidal layer) in frontoparietal cortex. Filled triangles and circles also show the measure sites of grey for bFGF-like IR.

Measurement of bFGF-like IR expression For semi-quantitative assessment of bFGF-like IR expression, the sum of grey for the IR in peri-infarct areas (filled triangles delineated fields in both sides of striatum) and pyramidal layer of frontoparietal cortex (filled circles delineated fields in both sides of frontoparietal cortex) were measured with Leica Q500IW image processing system. The grey value was averaged from that of five different fields in the same region. The grey ratio of bFGF-like IR in the lesion side versus the contralateral side was calculated.

Statistical analyses Data were presented as $\bar{x} \pm s$, and analyzed by t-test.

RESULTS

By using CLSM, the bFGF-like IR expression could be seen in striatum and frontoparietal cortex, and the triple labeling of brain sections with Cy5, FITC, Radiant Red indicated the cellular location of bFGF-like IR (Fig 2, 3). In sham-operated group, the bFGF-like IR was rarely found in astrocytes of caudate putamen (Fig 2D)

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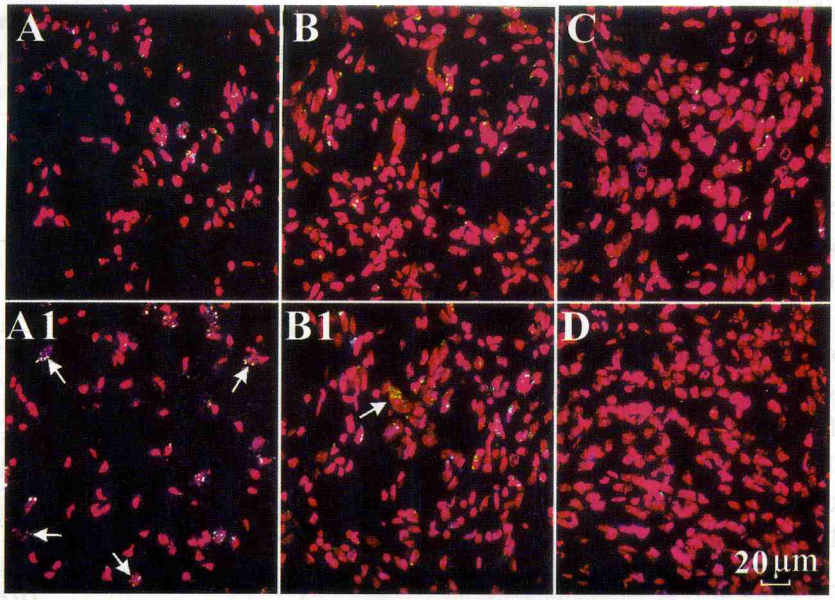

Fig 2. Confocal images showing the expression of bFGF-like IR in striatum after a 2-h MCAO and a 24-h reperfusion. Neurons were exhibited by red colour, astrocytes by pink, and bFGF-like IR by yellow. (A) In the core of infarction, mild increase of bFGF-like IR was observed mainly in astrocytes. (A1) A few badly damaged neurons of infarct core also showed the IR. (B) In region adjacent to the infarction, intense IR was detected. (B1) some neurons in the adjacent region showed as well upregulation of bFGF-like IR. (C) In the surrounding region from the infarction, more IR appeared primarily in astrocytes. (D) Sham control.

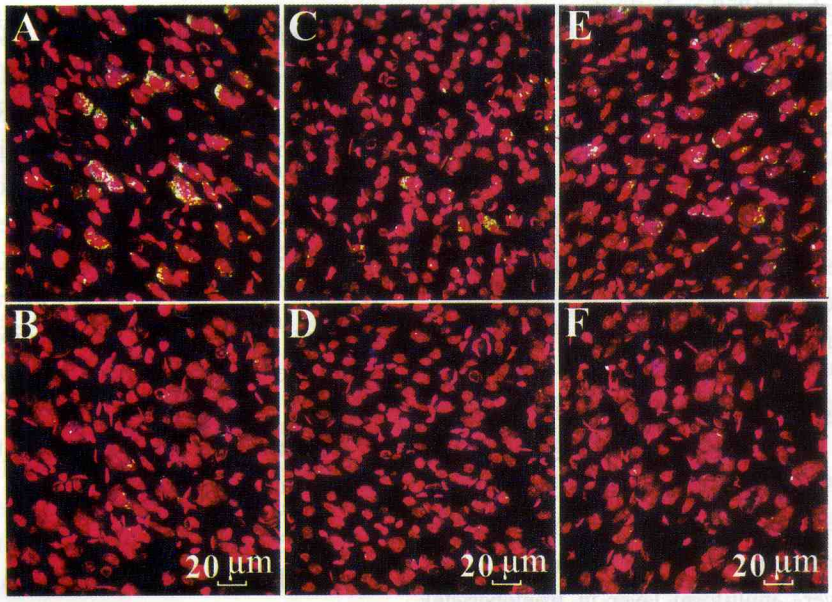

Fig 3. Confocal images showing the expression of bFGF-like IR in frontoparietal cortex after a 2-h MCAO and a 24-h reperfusion. (A,E) In pyramidal layer (both sides), the strongest increase of bFGF-like IR was mostly seen in neuronal cytoplasm. (C) In multiform layer, strong increase of the IR was evidently seen in neurons. (B,D,F) Sham control.

and neurons of frontoparietal cortex (Fig 3B, 3D, 3F).

In rats exposed to 2-h MCAO and 24-h reperfusion, distinct areas of striatum revealed different increase mode of bFGF-like IR expression (Fig 2). In the core and surrounding region of infarct (2A), notable neuronal damage was observed, more bFGF-like IR appeared primarily in astrocytes, but a few badly damaged neurons in the core also showed the bFGF-like IR (2A1); in region adjacent to the infarct (2B), intense bFGF-like IR was detected (Fig 2), the grey ratio of bFGF-like IR increased from 98 % ± 10 % ($n = 6$, sham) to 125 % ± 6 % ($n = 8$, ischemia, $P < 0.01$). In addition, some neurons showed upregulation of bFGF-like IR as well (2B1). However, different layers of frontoparietal cortex displayed similar increase mode of bFGF-like IR expression (Fig 3). In either pyramidal layer (3A) or multiform layer (3C), strong increase in the bFGF-like IR was evidently seen in neurons, although it was observed in astrocytes as well. Moreover, the pyramidal layer exhibited the strongest increase in the bFGF-like IR (Fig 3), in which the grey ratio of bFGF-like IR increased from 104 % ± 11 % ($n = 6$, sham) to 132 % ± 28 % ($n = 8$, ischemia, $P < 0.05$). In the frontoparietal cortex of contralateral side (3E), there was also upregulation of bFGF-like IR, particularly in the neurons of pyramidal layer.

We also observed the subcelluar location of bFGF-like IR. In neurons, the majority of the bFGF-like IR was located in neuronal cytoplasm, and a little bFGF-like IR was observed in the nuclei. The model example for this are the pyramidal neurons of frontoparietal cortex. For astrocytes, the bFGF-like IR was observed in the nuclei but because no typical branches except the nuclei of astrocytes were shown, the exact reasons are unclear. Speliotes *et al*[1] provided a clue that bFGF mRNA increased to a peak at d 1 while GFAP mRNA peaked at d 3 after permanent MCAO. He also found that the increase on the number and size of GFAP-like IR astrocytes, as well as increase in GFAP staining intensity in peri-infarct cortex began at d 3, and persisted for 7 – 14 d after permanent MCAO.

DISCUSSION

In the present study, we found that increase of bFGF-like IR was present in several regions of striatum and frontoparietal cortex following focal cerebral ischemia. In striatum, the increasing mode of bFGF-like IR varied according to the infarct locations. bFGF-like IR mainly lay in the astrocytes in the care of the infarct and its surrounding areas. Speliotes *et al*[1] and Teng *et al*[2] reported that the majority of bFGF-like IR was in nuclei of astrocytes, which coincided with the result we found in striatum. Further more, in striatum, we found a few badly damaged neurons in the infarct and more neurons adjacent to the infarct showed enhancement of bFGF-like IR. The results suggest that the astrocytes in striatum responded to ischemia firstly and it was more endurable partly due to the expression of bFGF. In frontoparietal cortex, most bFGF-like IR emerged in neurons, in pyramidal and multiform layer. In contralateral frontoparietal cortex, we also detected an increase of bFGF-like IR, which may lie in corticocortical neural disconnection and/or the changes of blood flow and metabolism.

In several papers[14,15], bFGF was found to stimulate the expression of GFAP as well as other functional proteins in astrocytes. On the other hand, cerebral ischemia could also induce an upregulation of bFGF mRNA and bFGF receptor mRNA (bFGFR mRNA) expression in astrocytes[10,11], furthermore, bFGFR mRNA was also found in both neurons and nonneuronal cells in the peri-infarcted area after MCAO[12]. Our present study also revealed the increase and distribution of bFGF-like IR parallel with that of bFGF receptor as mentioned above by other groups.

In summary, MCAO-induced increases of bFGF-like IR in striatum and bilateral frontoparietal cortex were evidenced in our study. The bFGF-like IR was mainly located in astrocytes in striatum, while it mostly appeared in neurons of frontoparietal cortex. Our observations in confluence with others may result in a possible mechanism of neuroprotection against ischemia. In striatum, astrocytes may play an important role in the protection of neuron via the overexpression of bFGF; whereas in cortex, neurons probably exert an autoprotection through secreting bFGF themselves.

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大鼠脑缺血时碱性成纤维细胞生长因子表达的变化[1]

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关键词 碱性成纤维细胞生长因子；脑缺血；荧光免疫测定；共聚焦显微镜检查

目的：观察局灶性脑缺血时，大鼠脑内碱性成纤维细胞生长因子(bFGF)表达分布的改变. **方法**：用荧光免疫组化方法观察 bFGF 的表达. 用 MCAO 模型造成大鼠局灶性脑缺血损伤. **结果**：缺血后，bFGF 在纹状体及额顶叶皮质均显著升高. 在纹状体梗塞核心及周边区，bFGF 主要在星状胶质细胞内表达；在邻近梗塞的区域，bFGF 灰度比率从98 % ± 10 % 增至125 % ± 6 %，神经元内也少量表达. 在额顶叶皮质，bFGF 灰度比率从104 % ± 11 % 增至132 % ± 28 %. bFGF 主要分布于神经元内. **结论**：局灶性缺血大鼠脑内不同区域 bFGF 表达的增加有所差异，提示纹状体内，可能主要由星状胶质细胞起神经保护作用；而在皮质，可能由神经元起自我保护作用.

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