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Delayed Treatment with Intravenous Basic Fibroblast Growth Factor Reduces Infarct Size Following Permanent Focal Cerebral Ischemia in Rats

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Summary: Basic fibroblast growth factor (bFGF) is a polypeptide that supports the survival of brain cells (including neurons, glia, and endothelia) and protects neurons against a number of toxins and insults in vitro. This factor is also a potent dilator of cerebral pial arterioles in vivo. In previous studies, we found that intraventricularly administered bFGF reduced infarct volume in a model of focal cerebral ischemia in rats. In the current study, bFGF (45 μg/kg/h) in vehicle, or vehicle alone, was infused intravenously for 3 h, beginning at 30 min *after* permanent middle cerebral artery occlusion by intraluminal suture in mature Sprague–Dawley rats. After 24 h, neurological deficit (as assessed by a 0- to 5-point scale, with 5 = most severe) was 2.6 ± 1.0 in vehicle-treated and 1.5 ± 1.3 in bFGF-treated rats (mean ± SD; $N = 12$ vs. 11; $p = 0.009$). Infarct volume was 297 ± 65 mm^3 in vehicle- and 143 ± 135 mm^3 in bFGF-treated animals ($p = 0.002$). During infusion, there was a modest decrease in mean arterial blood pressure but no changes in arterial blood gases or core or brain temperature in bFGF-treated rats. Autoradiography following intravenous administration of ^{111}In-labeled bFGF showed that labeled bFGF crossed the damaged blood–brain barrier to enter the ischemic (but not the nonischemic) hemisphere. Whether the infarct-reducing effects of bFGF depend on intraparenchymal or intravascular mechanisms requires further study. **Key Words:** Basic fibroblast growth factor—Cerebral infarction—Focal cerebral ischemia.

"Neurotrophic" growth factors are polypeptides that, acting through specific receptors, initiate cascades of signal transduction resulting in increased neuronal survival. Recently, it has been appreciated that these factors also protect neurons against various insults and toxins. In particular, basic fibroblast growth factor (bFGF) is a 154-amino acid, 18-kD polypeptide that supports the survival of a wide variety of neurons from the embryonic rat brain in vitro and protects cultured neurons against excitatory amino acid (EAA) toxicity, anoxia, hypoglycemia, free radicals, and nitric oxide (Walicke, 1988; Mattson et al., 1989; Freese et al., 1992; Finklestein et al., 1993; Maiese et al., 1993; Mattson and Scheff, 1994). In vivo bFGF protects brain neurons against mechanical and excitotoxic injury (Anderson et al., 1988; Nozaki et al., 1993). Moreover, this multipotential factor has trophic effects on brain glial and endothelial cells and is a potent systemic and cerebral vasodilator (Pettman et al., 1985; Gospodarowicz et al., 1986; Cuevas et al., 1991; Rosenblatt et al., 1994).

In previous studies (Koketsu et al., 1994), we found that intraventricular administration of bFGF, starting at 3 days before ischemia, reduced infarct volume in a model of focal cerebral ischemia in mature rats. In the current study, we extended these

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Abbreviations used: bFGF, basic fibroblast growth factor; BSA, bovine serum albumin; EAA, excitatory amino acid; MCA, middle cerebral artery; NO, nitric oxide; TTC, 2,3,5-triphenyltetrazolium hydrochloride.

findings to show that the *intravenous* administration of bFGF, beginning *after* ischemia, also reduced infarct volume and improved behavioral outcome in a model of permanent middle cerebral artery (MCA) occlusion.

METHODS

Male Sprague–Dawley rats (300–360 g) were allowed food and water ad libitum. Rats were then weighed and anesthetized with chloral hydrate (400 mg/kg, i.p.), and polyethylene catheters were inserted into the tail artery to monitor blood pressure and blood gases and into the inferior vena cava through the left femoral vein for intravenous infusions. Core temperature was maintained between 36.8 and 37.8°C during surgery and infusions by a heating lamp connected to a rectal temperature probe (Model 73A; Yellow Springs Instruments).

Permanent occlusion of the MCA was produced by intraarterial suture, as described previously (Minematsu et al., 1992, 1993; Meadows et al., 1994). Briefly, a 4–0 monofilament nylon suture with a rounded tip was inserted into the right common carotid artery and then advanced through the internal carotid artery into the MCA for a distance of approximately 17 mm, until mild resistance was felt. Using this technique, the tip of the suture is advanced into the origin of the anterior cerebral artery, occluding the MCA. A large infarct in the territory of the MCA is typically produced (Minematsu et al., 1992, 1993; Meadows et al., 1994).

Recombinant human bFGF was obtained as concentrated stock (1.15 mg/ml in 20 m*M* sodium citrate, 0.6 *M* NaCl, pH 5.0) as a generous gift from Creative Biomolecules (Hopkinton, MA, U.S.A.) and stored at −80°C before use. Stock solution was then diluted into vehicle containing 0.9% NaCl and 100 μg/ml bovine serum albumin (BSA, Boeringer-Mannheim, Mannheim, Germany), pH 7.4 ~1:38 dilution), to give a final bFGF concentration of 30 μg/ml. Animals were infused intravenously with this solution at a rate of 0.5 ml/h for 3 h, beginning at 30 min after MCA occlusion, to deliver a bFGF dose of 45 μg/kg/h, or 135 μg/kg for the entire infusion period. Control animals received intravenous infusions of 0.9% saline (N = 8) or 0.9% saline with 100 μg/ml BSA (N = 4). We found no differences in infarct volume or physiological parameters among these control animals, so the data were pooled in the analysis. In addition, in other control experiments, we found that the "extra" trace amounts of sodium citrate (0.513 m*M*) and NaCl (16 m*M*) present in bFGF solutions (due to dilution of the stock solution) had no effect on infarct size or physiological parameters.

Anesthesia was maintained during infusions with supplemental doses of chloral hydrate (100 mg/kg, i.p.). Mean arterial blood pressure (MABP) and arterial blood gases were recorded just before, 30 min after, and 3 h after MCA occlusion (the last two time points were just after and 2.5 h after the initiation of bFGF infusions). After infusions, catheters were removed, and animals were allowed to awaken from anesthesia, returned to home cages, and allowed food and water ad libitum. Intraarterial sutures were left in place until sacrifice.

At 24 h after ischemia, animals were assessed by behavioral rating scale (0 = no deficit to 5 = dead), as described previously (ZeaLonga et al., 1989). Animals were then reanesthetized with chloral hydrate and decapitated. Brains were removed and the appropriate location of the intraarterial suture was confirmed. Brains were then sectioned into 2-mm coronal slices, which were incubated in 2% 2,3,5-triphenyltetrazolium hydrochloride (TTC) for 30 min, and fixed in 10% buffered formalin. This procedure stains viable tissue red; infarcted tissue remains unstained (Koketsu et al., 1994). After 48 h, sections were photographed (Zeiss microscope, 0.8× magnification), and infarct size in each of five slices was determined using a computer-interfaced digitizer (Signa Scan V 3.10; Jandel Scientific, Costa Madera, CA, U.S.A.; and Numonics 2200, Numonics, Montgomeryville, PA, U.S.A.; slices 1–5—bregma coordinates +2.4, +0.4, −1.6, −3.6, and −5.4, respectively). Infarct area was determined separately for cortex and caudoputamen for slices 1, 2, and 3. Infarct areas on each slice were summed and multiplied by slice thickness to give infarct volumes. All infusions, behavioral ratings, and infarct analysis were done in a randomized, blinded manner.

To examine the possible effects of bFGF on brain temperature, a separate group of intact animals (N = 3) received an intravenous infusion of bFGF (45 μg/kg/h) starting at 30 min after induction of chloral hydrate anesthesia and lasting for 3 h. Body temperature was controlled as above, and temperature of the lateral cerebral cortex, measured by a temperature probe (Omega Group, Stamford, CT, U.S.A.) inserted through a small burr hole, was recorded before and at half-hour intervals during bFGF infusion.

All data are expressed as mean ± SD. Continuous data were analyzed by analysis of variance or unpaired two-tailed t tests with Bonferroni correction for multiple comparisons. Noncontinuous behavioral data were analyzed by Mann–Whitney U test.

To determine whether intravenously administered bFGF might cross the damaged blood–brain barrier to enter ischemic brain tissue, an additional three animals received radiolabeled bFGF and the distribution of radioactivity in the brain was determined by autoradiography. To label bFGF, the protein was dissolved in 0.2 *M* sodium bicarbonate buffer, pH 8.1, and modified with diethylenetetraminepentaacetic acid anhydride (anhydride:bFGF, 200:1 molar ratio; Sigma). The reaction was allowed to proceed for 20 min, and the solution was dialyzed overnight. $^{111}InCl_3$ (DuPont) in 0.1 *M* sodium citrate buffer, pH 5.3, was then added for 1 h, and nonbound ^{111}In was removed by gel chromatography using a Sephadex G-100 column. The specific activity of labeled protein was 50 μCi/μg.

Three hours after MCA occlusion, rats received an intravenous infusion of ^{111}In-labeled bFGF (20 μg) lasting 30 min. (This time period coincides with the last 30 min of 3-h infusions given to treated animals; see above.) Four hours later, animals were anesthetized with sodium pentobarbital (100 mg/kg i.p.) and perfusion-fixed with 0.9% saline, followed by 2% paraformaldehyde, 0.01 *M* sodium-*m*-periodate, and 0.075 *M* L-lysine monohydrochloride, in 0.1 *M* sodium phosphate buffer, pH 7.4. Brains were removed and the total radioactivity determined by gamma counter. Brains were then postfixed for 20 h and cut on a vibratome (150-μm sections). Sections were then mounted on polylysine-coated slides, allowed to dry, dehydrated in alcohol, and put on film (β-Hypermax, Amersham) for 7 days at −80°C. Autoradiograms were then

developed with Kodak D-19 developer and nonhardening fixer.

To determine that the label found in brain remained associated with bFGF, one additional animal received ischemia surgery and intravenous ^{111}In-bFGF as above, and bFGF in brain homogenate was immunoprecipitated with specific anti-bFGF serum. Tissue was homogenized (1:4, wt/vol) in 2 *M* NaCl, 0.1 *M* Tris–HCl, pH 7.56 with aproteinin, pepstatin A, leupeptin, and trypsin inhibitor (all at 2.5 μg/ml). The homogenate was then centrifuged, passed through a low-protein-binding filter, and concentrated by centrifugal ultrafiltration. Concentrated homogenate was then incubated with an excess of rabbit polyclonal antiserum to bFGF (R16; kindly obtained from Dr. Joachim Sasse, Tampa, FL, U.S.A.), and the antigen–antibody complex was precipitated using an immobilized protein G kit according to the protocol of the manufacturer (Pierce). Radioactivity in the precipitate was determined by γ counter. In preliminary studies, we determined that at least 50% of authentic ^{111}In-bFGF was precipitated under these conditions, whereas <1.0% of ^{111}In-BSA could be precipitated.

RESULTS

As described previously (Minematsu et al., 1992, 1993; Meadows et al., 1994), infarcts produced by permanent intraarterial suture occlusion of the MCA involved large regions of the lateral cerebral cortex and underlying caudoputamen in the ipsilateral hemisphere. Overall, infarct volume was reduced (ca. 50%) in bFGF-treated versus control animals (t = 3.5, df = 21, p = 0.002; Table 1). This effect was seen to a similar degree in both cortex and caudoputamen and among all brain slices examined (Table 1).

Similarly, neurological deficit at 24 h after ischemia was reduced in bFGF-treated versus control animals (z = 2.6, p = 0.009; Table 2). There were no changes in physiological parameters between these groups, with the exception of a moderate (20–25%) decrease in blood pressure during bFGF infusion (Table 2).

Core temperature was monitored and kept constant in both bFGF-treated and control animals. To examine possible independent effects of bFGF on brain temperature, we directly monitored cortical temperature in a separate group of intact rats (N = 3) both before and at 30-min intervals for 3 h during intravenous bFGF infusion. We found no differences in brain temperature over time in these animals [$F(2,14)$ = 0.3, n.s.].

To determine whether systemically administered bFGF might cross the damaged blood–brain barrier to enter ischemic tissue, ^{111}In-bFGF was infused intravenously in three animals for 30 min, starting at 3 h after the onset of ischemia (corresponding to the last 30 min of the infusion period in treated animals; see above). Four hours later, brains were removed, sectioned, and subjected to autoradiography. Gamma counting showed that only a small fraction

TABLE 1. *Infarct volume and size in bFGF-treated versus control animals*

	Control (N = 12)	bFGF (N = 11)	Percentage change	p value	
				Uncorrected	Corrected
Infarct volume (mm^3)					
Total	297 ± 65	143 ± 135	−52	0.002	0.006
Cortex	232 ± 60	113 ± 112	−51	0.004	0.012
Caudoputamen	65 ± 14	30 ± 25	−54	0.007	0.02
Infarct area (mm^2)					
Slice 1					
Total	34 ± 8	17 ± 17	−50	0.004	0.046
Cortex	23 ± 4	12 ± 12	−48	0.004	0.048
Caudoputamen	12 ± 5	5 ± 6	−58	0.009	n.s.
Slice 2					
Total	46 ± 9	23 ± 22	−50	0.003	0.034
Cortex	30 ± 7	15 ± 16	−50	0.008	n.s.
Caudoputamen	16 ± 5	8 ± 7	−50	0.003	0.027
Slice 3					
Total	38 ± 8	18 ± 16	−53	0.001	0.008
Cortex	33 ± 6	15 ± 16	−55	0.001	0.015
Caudoputamen	4 ± 3	2 ± 2	−50	n.s.	n.s.
Slice 4					
Total	20 ± 10	11 ± 13	−45	n.s.	n.s.
Cortex	20 ± 10	11 ± 13	−45	n.s.	n.s.
Caudoputamen	—	—	—	—	—
Slice 5					
Total	11 ± 12	3 ± 7	−73	n.s.	n.s.
Cortex	11 ± 12	3 ± 7	−73	n.s.	n.s.
Caudoputamen	—	—	—	—	—

Data are mean ± SD. Uncorrected p value reflects results of unpaired two-tailed t tests. Corrected p value reflects the Bonferroni correction. n.s. (nonsignificant), $p > 0.05$.

TABLE 2. *Neurological deficit at 24 h after ischemia and physiological parameters in bFGF-treated versus control animals*

	Control (N = 12)	bFGF (N = 11)	Percentage change	p value	
				Uncorrected	Corrected
Body weight (g)	333 ± 20	328 ± 15	−2	n.s.	n.s.
Baseline					
pH	7.30 ± 0.03	7.30 ± 0.03	0	n.s.	n.s.
P_{CO_2}	34 ± 10	38 ± 5	12	n.s.	n.s.
P_{O_2}	95 ± 20	90 ± 18	−5	n.s.	n.s.
MABP	80 ± 17	75 ± 12	−6	n.s.	n.s.
30 min					
pH	7.30 ± 0.05	7.40 ± 0.04	1	n.s.	n.s.
P_{CO_2}	34 ± 8	34 ± 6	0	n.s.	n.s.
P_{O_2}	95 ± 13	90 ± 12	−5	n.s.	n.s.
MABP	73 ± 14	55 ± 16	−25	0.008	n.s.
3 h					
pH	7.30 ± 0.03	7.30 ± 0.04	0	n.s.	n.s.
P_{CO_2}	32 ± 4	30 ± 5	−6	n.s.	n.s.
P_{O_2}	102 ± 21	100 ± 10	−2	n.s.	n.s.
MABP	79 ± 18	62 ± 16	−22	0.029	n.s.
Behavior	2.6 ± 1.0	1.5 ± 1.3	−42	0.009	—

Data are mean ± SD. Uncorrected p value reflects results of unpaired two-tailed t tests. Corrected p value reflects the Bonferroni correction. Behavior data were analyzed by Mann–Whitney U test as a lone comparison. n.s. (nonsignificant), $p > 0.05$.

of the total administered dose of radioactivity (0.01 ± 0.004%) was present in brain at the time of sacrifice. Autoradiography showed that this radioactivity was localized at the site of suture occlusion of the MCA and, in addition, was visualized faintly in the cortex and striatum and more intensely in the hippocampus of the ischemic (right) but not the nonischemic (left) hemisphere (Fig. 1). To verify that the radioactivity found in brain remained associated with bFGF, one additional animal underwent ischemia surgery and received intravenous ^{111}In-bFGF, and labeled bFGF in brain homogenate was immunoprecipitated using specific anti-bFGF serum. Under the conditions used, 30% of the total radioactivity in the homogenate was precipitated (equivalent to 60% of the amount of labeled bFGF standard that could be immunoprecipitated under the same conditions). These data indicate that at least some of the radioactivity found in brain remained associated with bFGF.

DISCUSSION

Our results show that the intravenous infusion of bFGF, starting 30 min after the onset of ischemia, reduced infarct volume and improved neurological outcome in a model of permanent MCA occlusion in the rat. Infarct volume was reduced in both cortex and caudoputamen. No changes in physiological parameters, except for a moderate decrease in blood pressure, were seen during bFGF infusion.

These results are consistent with several previous reports showing that exogenously administered bFGF limits the extent of cell death in models of focal and global cerebral ischemia. Nozaki et al. (1993) found that the intraperitoneal administration of bFGF (100 μg/kg), given 30 min before hypoxia/ischemia, reduced the size of focal cerebral infarcts in neonatal rats. Nakata et al. (1993) found that the intraventricular injection of bFGF (96–960 ng) 2 h after global forebrain ischemia in gerbils reduced CA-1 neuronal loss. Consistent with these findings, MacMillan et al. (1993) found less CA-1 neuronal loss in transgenic mice overexpressing bFGF than in nontransgenic controls following global hypoxia/ischemia. Recently, Koketsu et al. (1994) found that the intraventricular infusion of bFGF (4.8- and 25-μg total dose, respectively), beginning before ischemia, reduced infarct volume in models of focal cerebral ischemia in mature rats. The current report is the first to show reduction in size of focal ischemic infarcts following *systemic* (*intravenous*) bFGF administration in mature animals. Moreover, infusion of bFGF was begun *after* the onset of ischemia in our study. More recently, we obtained similar results using a reperfusion model of focal cerebral ischemia (Jiang et al., 1995).

The dose of bFGF chosen for the current studies of intravenous administration (45 μg/kg/h for 3 h) was considerably higher than that used in previous studies of direct intracerebral administration (see above) and was chosen empirically as a dose that

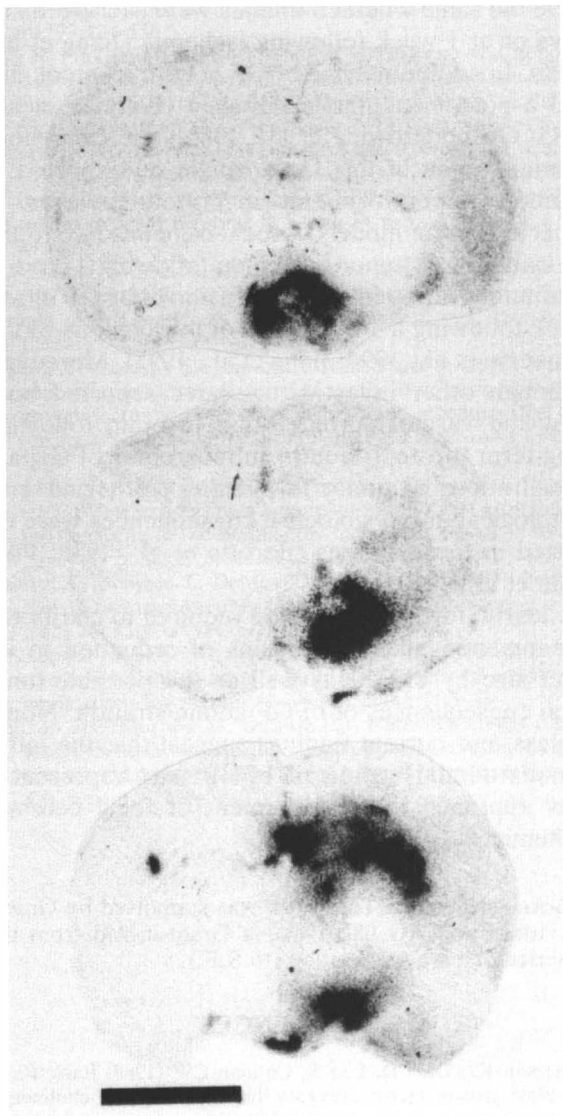

FIG. 1. Brain autoradiograms following intravenous ^{111}In-bFGF administration. Intravenous ^{111}In-bFGF was administered continuously during 3–3.5 h after MCA occlusion. Four hours later, animals were sacrificed, and brains sectioned and subjected to autoradiography. Dense labeling was seen at the base of the brain at the site of arterial occlusion. In addition, faint labeling was seen in the cerebral cortex **(top)** and striatum **(middle),** and denser labeling was seen in the hippocampus **(bottom)** of the ischemic (right) but not the nonischemic (left) hemisphere. Scale bar = 30 mm.

might be high enough to maximize potential infarct-reducing effects while avoiding significant side effects. In particular, Cuevas et al. (1991) found that 300 ng of bFGF administered as a rapid intravenous bolus to mature rats (equivalent to about 1 μg/kg/min) resulted in a significant drop in systemic blood pressure. Since lowered systemic blood pressure might be expected to increase infarct size after focal cerebral ischemia, we chose a dose (45 μg/kg/h, or 0.75 μg/kg/min) that was below the reported hypotensive dose. On the other hand, it was anticipated that the administered dose of intravenous bFGF might be high enough to cross the damaged blood–brain barrier and enter ischemic brain tissue at a sufficient concentration to exert potential infarct-reducing effects. Indeed, our results using radiolabeled bFGF showed that while intravenous bFGF did not enter the intact hemisphere, it did enter regions of the ischemic hemisphere, including the cortex, striatum, and especially, hippocampus. The relatively high labeling of the hippocampus may reflect the high density of bFGF receptors in this structure (Wanaka et al., 1990). Although the MCA remained occluded by intraluminal suture during the time of bFGF infusion, bFGF may have entered ischemic tissue via leaky vessels at the borders of ischemic zones and subsequent diffusion. Moreover, although only a small percentage of the total administered dose of radioactivity (0.01%) was present in the ischemic hemisphere at the time of sacrifice, this does not preclude the possibility that sufficient concentrations of bFGF were attained to exert trophic effects or that bFGF was being delivered and "turned over" continuously during the period of infusion. Indeed, assuming, based on our radiolabeling data, that only 0.01% of the total administered bFGF dose (45 μg for a 330-g animal) reached ischemic brain tissue, this means that up to 4.5 ng of bFGF might be available. Assuming an extracellular hemispheric volume of 1 ml, the bFGF concentration would then be 4.5 ng/ml. In vitro, bFGF has potent neurotrophic and neuroprotective effects at concentrations of 1–10 ng/ml (Walicke, 1988; Mattson et al., 1989; Freese et al., 1992; Finklestein et al., 1993).

Potential mechanisms of bFGF-induced reduction in infarct size include (a) direct protective effects on brain cells, including neurons, and (b) effects on cerebrovascular tone and cerebral blood flow (CBF). At least one of the currently identified high-affinity bFGF receptors (*flg*) is widely distributed on neurons and on endothelial cells in the intact rodent brain and, also, becomes localized on glia after brain injury or ischemia (Wanaka et al., 1990; Logan et al., 1992; Endoh et al., 1994). As noted above, bFGF is a potent neurotrophic factor that also protects neurons against a number of toxins and insults in vitro, including anoxia, hypoglycemia, EAAs, Ca^{2+} ionophore, free radicals, and nitric oxide (NO) (Mattson et al., 1989; Freese et al., 1992; Finklestein et al., 1993; Maiese et al., 1993; Mattson and Scheff, 1994). In particular, EAAs, NO, and free radicals are thought to play important roles in the pathogenesis of neuronal death after ischemia (Choi and Hartley, 1993). Al-

though the molecular mechanism of neuroprotection by bFGF is incompletely understood, it may depend on new neuronal gene transcription and protein synthesis (Mattson et al., 1989). Neurons at the margins ("penumbra") of focal infarcts appear to retain the capacity for synthesis of at least some cellular proteins [e.g., heat shock proteins (Li et al., 1992)], and it is possible that bFGF reduces infarct size, in part, through direct effects on these cells. Basic FGF also has trophic effects on brain glial and endothelial cells (Pettman et al., 1985; Gospodarowicz et al., 1986), and it is possible that bFGF protects these cells by similar mechanisms. Indeed, the focal infarcts observed in the current study involved all brain cellular elements, including neurons, glia, and endothelia ("pannecrosis"), so that the infarct-reducing effects of bFGF may have been due to direct protective effects on all of these cell types. As noted above, our radiolabeling data indicate that intravenously administered bFGF may have entered ischemic brain tissue at concentrations sufficiently high enough to be compatible with the trophic effects of bFGF observed in in vitro studies.

Another possible mechanism of bFGF-induced reduction in infarct size is through regulation of cerebrovascular tone and CBF. As noted above intravenous bFGF (at doses of 300–1,000 ng as a single bolus) lowers blood pressure by systemic vasodilation in mature rats, and recent studies show that topically applied bFGF (at doses of 5–200 ng/ml) is a potent dilator of cerebral pial arterioles through NO-dependent mechanisms (Cuevas et al., 1991; Rosenblatt et al., 1994). Although the dose of intravenous bFGF administered in the current study was chosen to be below that previously reported to cause hypotension in rats (Cuevas et al., 1991), we nonetheless saw a moderate but significant drop in MABP in bFGF-treated animals. However, in spite of this drop in MABP (and presumably also in cerebral perfusion pressure), we also saw smaller infarcts in bFGF-treated animals. These data suggest that, at the dose used, bFGF may have a greater dilatory effect on cerebral than systemic vessels, thus resulting in a net increase in CBF.

Other potential questions concerning the effects of bFGF administration following focal ischemia include a possible delay in the evolution of infarct size and the possible long-term effects of bFGF. Since infarct volume was assessed only 24 h after ischemia in the current study, it might be argued that the effect of bFGF is simply to delay the eventual evolution of cerebral infarcts. However, in recent studies using a reperfusion model of focal ischemia, we found that the infarct-reducing effects of bFGF were the same whether animals were sacrificed at 2 days or at 1 week following ischemia (Jiang et al., 1995). In addition, since bFGF is both a potent glial and a potent endothelial mitogen (Pettman et al., 1985; Gospodarowicz et al., 1986), the exogenous administration of this factor might be expected to promote tumor formation in brain. However, in other studies in models of focal ischemia, we found no evidence of tumor formation following 4 days of continuous intraventricular infusion of bFGF or at 1 week following a 3-h infusion of intravenous bFGF (Koketsu et al., 1994; Jiang et al., 1995). Moreover, although other investigators have reported both glial and vascular proliferation in brain following long-term (up to 1-month) intracerebral bFGF administration, no tumor formation or other adverse histological or physiological consequences were reported in these studies (Barotte et al., 1989; Puumala et al., 1990).

Clearly, further studies are required to clarify the phenomenon and mechanisms of reduction in infarct size by bFGF, as well as the possible long-term consequences of bFGF administration. Nonetheless, our current findings suggest that the intravenous administration of bFGF may represent a new approach to the treatment of focal cerebral ischemia.

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