Growth Factors and Stroke

David A. Greenberg and Kunlin Jin

Buck Institute for Age Research, Novato, California 94945

Summary: Current options for the treatment of stroke are extremely limited, partly because of the rapidity with which brain cells die when deprived of their blood supply. Several recent studies suggest that growth factors can produce improvement in animal models of stroke, even when administered at postischemic intervals of many hours to days, when conventional neuroprotective approaches are typically futile. Several growth factors can access the brain after systemic administration, making them more attractive as therapeutic agents. Finally, growth factors are key mediators of neurogenesis in the adult brain, which could have a role in brain repair and functional recovery following stroke. **Key Words:** Stroke, ischemia, growth factor, neuroprotection, neurogenesis.

INTRODUCTION

Growth factors are broadly defined as substances required for the growth of cells or organisms, but this definition could also fit a wide range of molecules that we do not consider growth factors.[1] Cytokines, for example, also regulate cell growth, but tend to show inducible rather than constitutive expression and act at least partly on hematopoietic cells. Hormones are characteristically produced by a single, specialized cell type and, in contrast to both growth factors and cytokines, usually act at a distance on target cells. To add to the confusion, receptors for growth factors and cytokines are sometimes structurally related.

It is not intuitively obvious that growth factors, as defined above, should have any effect in the acute stage of stroke, and it is still unclear if they will turn out to be clinically useful. But several growth factors and transgenics with altered growth factor expression have been tested in animal models of stroke, and this is probably a reasonable place to begin. Aspects of this subject have been reviewed recently,[2] including in this journal,[3] and the reader is referred to these papers for other emphases and viewpoints.

In principle, one can imagine at least 3 settings in which the effect of growth factors on stroke might be tested. First, growth factors could have a short-term preventive effect. For example, a growth factor that stimulates angiogenesis, such as vascular endothelial growth factor (VEGF), might be administered prior to stroke, in an effort to increase the vascularity of a marginally perfused brain territory. Second, growth factors might have an acute protective effect when administered in the immediate aftermath of vascular occlusion. Several growth factors activate signaling mechanisms, such as the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway or antiapoptotic (e.g., Bcl2-family) proteins, which promote cell survival in a variety of settings. Because at least part of ischemic neuronal death is thought to involve delayed, programmed cell death, this approach could be salutary even if it were too slow to block necrosis in the most severely ischemic brain tissue. In addition to VEGF, growth factors that employ these pathways include insulin-like growth factor-1 (IGF-1) and brain-derived neurotrophic factor (BDNF). Third, growth factors might enhance brain recovery after ischemia has resolved, by augmenting brain plasticity, which could include increased neurogenesis. A growing list of growth factors has been implicated in this process, including fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), heparin-binding epidermal growth factor-like growth factor (HB-EGF), nerve growth factor (NGF), IGF-1, BDNF, erythropoietin (EPO), stem cell factor (SCF), granulocyte colony-stimulating factor (G-CSF), and VEGF.

Growth factors can also have adverse clinical effects, which may limit their use in the treatment of stroke. These include hyperalgesia (NGF), bone pain (G-CSF),

Address correspondence and reprint requests to: David A. Greenberg, M.D., Ph.D., Buck Institute for Age Research, 8001 Redwood Boulevard, Novato, CA 94945. E-mail: dgreenberg@buckinstitute.org.

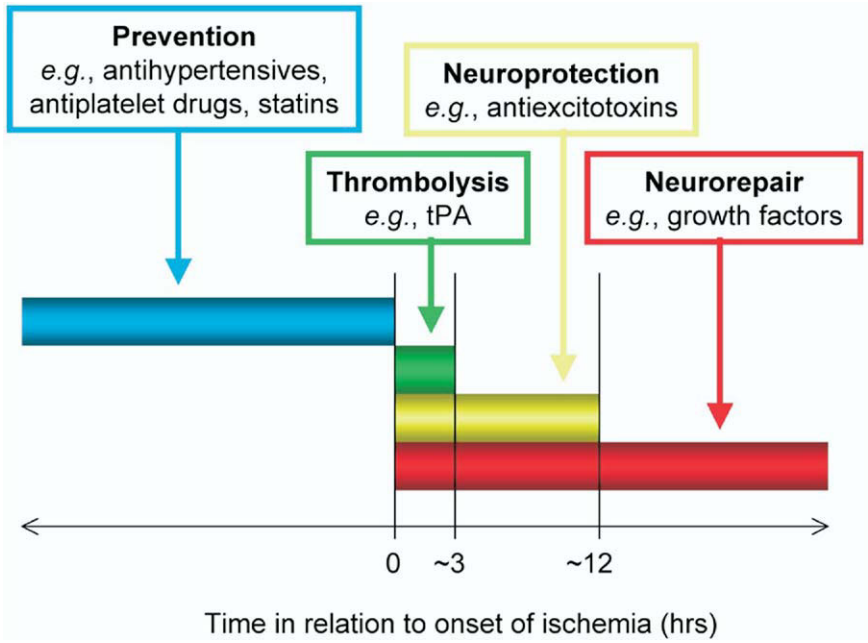

FIG. 1. Therapeutic strategies in relation to the time course of stroke.

increased vascular permeability and tumor angiogenesis (VEGF), and weight loss (ciliary neurotrophic factor).

This review will focus on 3 aspects of growth factors in stroke. First, we will consider studies involving the delayed, postischemic administration of growth factors in animal models of stroke. The greatest unmet need in stroke treatment is probably the absence of drugs that are effective in reducing the severity of deficits even when given many hours or more after stroke onset (FIG. 1). As is well known, for example, thrombolytic agents are effective only within about the first 3 hours poststroke. It is notable therefore that some animal studies have shown that delayed administration of growth factors can reduce stroke size or functional deficits.

Second, we will consider nasal delivery of growth factors as a possible route of administration in stroke. A perceived problem in the clinical application of growth factors is that they gain access to the brain with difficulty, and are often given by the intracerebroventricular (i.c.v.) route in animal studies. In addition, growth factors may have adverse systemic effects involving, for example, potentiating the growth of tumors or tumor vasculature. However, some studies suggest that growth factors and other proteins of similar size may enter the brain directly when instilled intranasally (i.n.).

Third, we will address the role of growth factors in stimulating postischemic neurogenesis. Neurogenesis is now known to continue throughout life in the adult brain, and is enhanced by a variety of neuropathological processes, including ischemia. Several growth factors also stimulate neurogenesis, suggesting that administration of growth factors might augment injury-induced neurogenesis to levels capable of effecting brain repair. With the caveat that the functional potential of stroke-induced neurogenesis remains uncertain, it is nevertheless of interest to consider that growth factors might help to promote long-term brain recovery after stroke by amplifying endogenous injury-induced neurogenesis.

DELAYED NEUROPROTECTION

A major limitation of many neuroprotective strategies is the brief time window during which intervention can be helpful. In fact, experimental approaches often depend on beginning treatment before the onset of ischemia, which is inapplicable in most clinical settings. Considerable value has, therefore, been placed on therapies that could be started after the onset of symptoms, preferably several hours after onset, when patients can reasonably be expected to have reached the hospital and received a diagnosis.

One way to devise effective delayed treatment in stroke might be to target downstream steps in signaling pathways that mediate ischemic neuronal death. Growth factors are among the molecules that interact with such steps, including the activation of protein kinases and antiapoptotic proteins. For this and other reasons, they have received considerable attention in the search for therapies directed against neurodegenerative diseases but also against stroke. The effect of preischemic treatment with growth factors, or transgenic overexpression or knockout of growth factor genes, has been assessed in many studies. For example, the severity of stroke is increased in mice lacking FGF-2[4] or VEGF-B,[5] and reduced in VEGF-A-overexpressing mice.[6] However, the therapeutic implications of these studies are uncertain,

because such genetic manipulations may be accompanied by adaptations that obscure the primary genetic effect.

Brain-derived neurotrophic factor

Brain-derived neurotrophic factor was given at 1 μg/h by the intracisternal (i.c.) route, beginning approximately 15 minutes after permanent middle cerebral artery occlusion (MCAO) in the rat and continuing for 24 hours.[7] At 24 hours, infarct volume was reduced by 33% and cortical infarct volume by 37% in BDNF-treated animals, although functional outcome was not assessed. In another study, BDNF (20 μg intravenous [i.v.] bolus, given one hour, three days, and five days after photothrombotic occlusion in rat parietal cortex) had no effect on infarct size, but improved functional motor recovery at 1 and 6 weeks.[8]

Erythropoietin

Erythropoietin is a hypoxia-inducible hematopoietic factor that promotes the differentiation of megakaryocyte/erythroid progenitors to lineage-committed erythroid progenitors, and maturation of the latter to erythrocytes, and which is used to treat anemia related to renal failure, cancer, and other chronic disorders.[9] Like VEGF (see below), EPO is transcriptionally activated by hypoxia-inducible factor-1α (HIF-1α). Brines et al.[10] first reported that in rats with MCA-distribution strokes, induced by permanent MCA and ipsilateral carotid artery occlusion followed by reversible (60 minutes) occlusion of the contralateral carotid artery, recombinant human EPO (5,000 units/kg intraperitoneal [i.p.]) reduced infarct volume measured at 24 hours. This was the case when EPO was given prior to ischemia, at the onset of ischemia, or 3 to 6 hours postischemia, although the extent of reduction at the 6 hour time point was less (~30% compared with ~60%). AsialoEPO (44 μg/kg i.v.), which lacks hematopoietic activity, was similarly protective against MCAO in rats.[11] Another modification of EPO, carbamylation, abolishes hematopoietic activity, as well as affinity for the EPOR2 receptor.[12] Carbamylated EPO (CEPO) reduced infarct volume at 24 hours in rats by approximately 50% (when given at 5 to 50 μg/kg i.v. one hour post-MCAO) or by approximately 33% (when given at 50 μg/kg i.v. 4 hours post-MCAO).[12]

Wang et al.[13] reported a study in which EPO (5,000 or 10,000 U/kg/d i.p.) was given to rats beginning 24 hours after occlusion of the MCA with an embolus, and continued for 7 days. Infarct volume at 7 days was unaffected by EPO, but performance on the foot-fault and corner tests was improved.

The use of intranasal EPO in experimental stroke is considered later.

FGF-2, or FGF-2 plus EGF

Fisher et al.[14] conducted extensive studies of FGF-2 in focal cerebral ischemia in rats. In an early study, FGF-2 (45 μg/kg/h i.v.) was given for 3 hours, beginning 30 minutes after permanent MCAO. Infarct volume, measured at 24 hours, was reduced by 52% and neurological deficits were improved, compared with vehicle-treated rats. When infarct volume was measured as late as 3 months postischemia, the effect of FGF-2 persisted, although the extent of improvement was reduced to 27%.[15] A more delayed treatment regimen, in which 1 μg of FGF-2 was given by intracisternal injection biweekly for 4 weeks beginning one day after permanent MCAO, produced no reduction in infarct volume, but increased the rate and extent of recovery of sensorimotor and reflex function.[16] Recovery may have involved axonal sprouting in the contralateral sensorimotor cortex, where increased expression of growth-associated protein-43 was observed.[17] In 2-hour MCAO with reperfusion, FGF-2 (45 g/kg/h i.v.), infused i.v. for 3 hours beginning at the onset of reflow, reduced infarct volume by 40%, and improved neurological outcome at 1 week.[18] A related study[19] showed that the therapeutic window for beginning intravenous FGF-2 (50 μg/kg/h for 3 hours) was between 3 to 4 hours postischemia. Dimeric FGF-2, given intracisternally 1 day and 3 days after MCAO, was also tested, and improved sensorimotor function over 3 weeks, although it failed to reduce infarct volume.[20] Bethel et al.[21] also tested FGF-2 (5 to 250 μg/kg/h i.v. beginning 1 hour after the onset of ischemia) in cats undergoing 4-hour MCAO. They found that infarct volume was reduced by 35% to 45%, but the amplitude of somatosensory evoked potentials was unchanged by FGF-2 treatment.

Other groups have also investigated the effects of FGF-2 in rats with MCAO. Given at a dose of 150 μg/kg i.v., 2 hours after permanent MCAO, FGF-2 reduced infarct volume by 32% and improved rotarod performance.[22] An adenoviral vector expressing FGF-2 was given intracerebroventricularly, 2 hours after MCAO, and reduced infarct volume at 2 days by 44%.[23]

The EGF and FGF-2 (0.48 μg i.c.v. each per day for 2 weeks, beginning 10 minutes after the onset of ischemia) were administered together to rats in whom the MCA was occluded by intracerebral injection of the vasoconstrictor peptide, endothelin-1.[24] Surprisingly, growth factor treatment increased infarct volume approximately 2-fold.

G-CSF, or G-CSF plus SCF

G-CSF is a hematopoietic growth factor involved in neutrophil production, whereas SCF participates in several phases of hematopoiesis, including the generation of common myeloid progenitors from hematopoietic stem cells and the differentiation of granulocyte progenitors to

cells of basophil/mast cell lineage.[9] Intravenous G-CSF (60 μg/kg), given over 90 minutes beginning 30 minutes after the onset of transient (90 minutes) MCAO in rats, reduced infarct volume at 24 hours by 53%.[25] Delaying the onset of treatment until 2 hours after MCAO still led to a 37% decrease in infarct volume.[26] Similar findings were observed when G-CSF (50 μg/kg i.v.) was given beginning one hour after combined common carotid artery/distal MCA occlusion, where infarct volume was reduced by 42%.[26] In addition, this latter regimen produced an improvement in recovery of sensorimotor function, measured at 72 hours.[26]

Another study[27] combined administration of G-CSF and SCF to mice that underwent permanent, proximal MCAO. The G-CSF (300 μg/kg) and SCF (100 μg/kg) were given as a single daily subcutaneous dose, beginning 24 hours after MCAO and continuing through day 10, or beginning on day 11 and continuing through day 20. Infarct volume at 4 weeks was reduced to a similar extent (by 70% to 80%) in both treatment groups, compared with controls. In addition, results of rotarod testing were markedly improved in the more delayed (11 to 20 days) treatment group, and performance in the Morris water maze was greatly improved in both treatment groups.

HB-EGF

HB-EGF acts through the EGF receptor, EGFR/ErbB1, as well as through the related tyrosine kinase ErbB4 and the metalloendopeptidase, N-arginine dibasic convertase. When HB-EGF (1 ng/h) was infused intracerebroventricularly in rats for 72 hours, beginning 24 hours after MCAO for 90 minutes, infarct volume was reduced by approximately 45% at 1 week and by approximately 35% at 2 weeks and 4 weeks,[28] Sensorimotor function was also improved in HB-EGF-treated rats at each of these 3 time points. In another study, rats received a single intracerebroventricular (i.c.v.) injection of an HB-EGF-expressing adenoviral vector, 3 days after transient (80-minutes) MCAO.[29] However, vector-treated rats had no reduction in infarct volume at eight days or 28 days post-MCAO. In contrast, vector administration improved rotarod performance at 14 days, 21 days, and 28 days.

IGF-1

IGF-1 (0.5 to 50 μg i.c.v., given 2 hours after ischemia) was tested in a model of focal cerebral ischemia consisting of carotid ligation and transient hypoxia (exposure to 6% O^2 for 10 minutes) in adult rats.[30] The rate of infarction at 5 days was reduced by treatment with 5 to 50 μg of IGF-1; at the higher dose, this rate was decreased from 87% to 26%. The same group used this model to examine outcome at longer postischemic intervals.[31] IGF-1 (50 μg i.c.v., given 2 hours after ischemia) failed to reduce infarct volume measured at 20 days, but improved sensorimotor function.

In a more standard MCAO model, rats were given IGF-1 (33.33 μg/d i.c.v. for 3 days, or 200 μg/d s.c. for 7 days) beginning 30 minutes after the onset of one hour of ischemia.[32] Infarct volume was reduced by 64% in intracerebroventricularly-treated and by 42% in subcutaneously-treated rats. Another group studied the N-terminal tripeptide of IGF-1, Gly-Pro-Glu (GPE).[33] GPE (3 mg/kg/h), started 75 minutes after MCAO and continued for three days, improved the neurological disability score and reduced cortical infarct size by 45%. Studies on intranasally administered IGF-1 in stroke are described later in this review.

VEGF

Initial interest in VEGF as a possible treatment for stroke was based on its angiogenic properties, but more recent studies have documented a direct neuroprotective effect, which may be more important in the early phases postischemia.[34] Zhang et al.[35] administered VEGF (1 mg/kg i.v. over 4 hours) to rats, beginning 48 hours after ischemia achieved by occluding the MCA at its origin with a fibrin-rich 24-hours old homologous clot. When evaluated by rotarod and adhesive-removal testing, VEGF-treated rats showed improved function at 1 week, 2 weeks, and 4 weeks post-MCAO. In a subsequent study,[36] rats underwent 90 minutes of transient MCAO, and VEGF (10 ng/h i.c.v) was given beginning 24 hours later and continuing for 3 days. Infarct volume was reduced by 36% at 3 days, 45% at 1 week, 34% at 2 weeks, and 48% at 4 weeks, in VEGF-treated compared with control rats. Neurological grade, which reflects motor, sensory, and reflex function, was also improved in VEGF-treated rats at these same time points.

Summary

Several growth factors can reduce infarct volume, improve functional recovery, or both, after experimental stroke. In some cases, functional improvement is observed even when the treatment is not begun until many hours to days after the onset of ischemia (TABLE 1). Such functional benefit can be observed even in the absence of effects on infarct volume.

INTRANASAL DELIVERY

Thorne and Frey[37] have been leading proponents of using an intranasal route to deliver large-molecule therapeutics, especially growth factors, directly to the brain. Although the precise route that such transnasal delivery involves is uncertain, both intracellular and extracellular routes have been proposed.[38,39] In addition, some have argued that molecules given intranasally actually reach the brain via the bloodstream. Advantages of intranasal administration, where feasible, include its ability to by-

TABLE 1. *Beneficial Effects of Delayed Growth-Factor Administration in Focal Cerebral Ischemia*

Growth Factor	Route of Delivery	Onset of Treatment‡	Infarct Volume	Functional Outcome	Reference
EPO	i.p.	6 h	↓	ND§	10
EPO	i.p.	24 h	↔	↑	13
FGF-2*	Intracisternal	24 h	↔	↑	20
G-CSF + SCF	s.c.	24 h	↓	↑	27
HB-EGF	i.c.v.	24 h	↓	↑	28
HB-EGF†	i.c.v.	72 h	↔	↑	29
VEGF	i.v.	48 h	ND	↑	35
VEGF	i.c.v.	24 h	↓	↑	36

EPO = erythropoietin; FGF-2 = fibroblast growth factor-2; G-CSF = granulocyte colony-stimulating factor; HB-EGF = heparin-binding epidermal growth factor-like growth factor; h = hour; i.c.v. = intracerebroventricular; i.p. = intraperitoneal; i.v. = intravenous; s.c. = subcutaneous; SCF = stem cell factor; VEGF = vascular endothelial growth factor. * Dimeric. †Adenoviral vector-expressed. ‡After onset of ischemia. §Not determined.

pass the blood–brain barrier, reduce adverse systemic drug effects, and achieve rapid delivery of drugs to the brain. In rats, volumes of approximately 50 μL are commonly used, [38] whereas smaller volumes (~20 μL) are preferable in mice.[40] In either case, the animal is placed in the supine position and anesthesia is sometimes used.

EPO

EPO (4.8 to 24 U i.n.) was given to rats 10 minutes after the onset of transient (1-hour) MCAO and again after one hour of reperfusion.[41] After reperfusion for 24 hours, infarct volume was reduced by up to 70% (at 4.8 to 12 U doses), while higher and lower doses were less effective (~40% reduction). Neurological score, reflecting primarily motor function, was also improved by EPO, with a similar dose–response relationship. The maximal improvement in both infarct volume and neurological score achieved with intranasal EPO was comparable with results obtained when EPO was administered intraperitoneally (i.p.).

IGF-1

Liu et al.[42,43] gave IGF-1 (37.5, 75, or 150 μg i.n.) to rats undergoing transient (2-hours) MCAO, at 10 minutes, 24 hours, and 48 hours after the onset of ischemia. At 72 hours, infarct volume was reduced by approximately 60% at the higher doses of IGF-1 compared with control, but not at the lowest dose. Tests of sensorimotor, vestibulomotor, and somatosensory function all showed improvement at 72 hours in rats given IGF-1 at the higher doses.

NGF

NGF (5.7 μg i.n.) was administered to rats 30 minutes and 24 hours after the onset of MCAO.[44] Infarct volumes in NGF-treated rats were reduced by 39% at 48 hours compared with controls, and the results of behavioral testing at 24 hours and 48 hours were also improved in the treated group.

Summary

Controversy as to its anatomical basis notwithstanding, intranasal administration of growth factors seems capable of improving histological and functional outcome after MCAO. However, the time window for achieving therapeutic effects has not been established. In particular, the studies cited above all involve early onset of treatment (within 30 minutes of MCAO), and how long initial administration of growth factor can be delayed is uncertain.

POSTISCHEMIC NEUROGENESIS

Innumerable growth factors have been shown to stimulate neurogenesis in neuroproliferative zones of the adult rodent brain, namely the subgranular zone of the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) in the walls of the lateral ventricles. Cerebral ischemia also enhances adult neurogenesis, which is accompanied by the directed migration of newborn neurons to sites of injury.[45–52] This has been proposed as an endogenous mechanism for brain repair, although only limited evidence for a beneficial effect of neurogenesis on outcome from ischemia exists.[53] In most studies, newborn cells are identified by the prior administration of bromodeoxyuridine (BrdU), which labels cells in S-phase of the cell cycle, and serves as a marker of recent cell division. The neuronal lineage of BrdU-labeled cells is then ascertained by staining for protein markers associated with an immature or mature neuronal phenotype. Here we focus specifically on studies in which growth factors have been shown to modify neurogenesis in the setting of focal cerebral ischemia.

EGF

Teramoto et al.[54] reported that administration of EGF (40 or 400 ng/d i.c.v.) to mice, beginning two days or 21 days after transient (20-minutes) MCAO in mice and

continuing for seven days, increased cell proliferation in the SVZ, migration of newborn cells into the adjacent ischemic medial striatum, and their differentiation to express mature neuronal marker proteins. The BrdU-labeled cells in the medial striatum were increased 11-fold at the lower and 17-fold at the higher dose of EGF, when treatment was started 2 days post-MCAO and cells were counted on day 9. A 10-fold increase was still observed at the higher dose when begun 21 days post-MCAO and measured on day 28. Thus, EGF increased cell proliferation in the SVZ and migration to the striatum, beyond the effects of ischemia alone, and its capacity to do so persisted for at least 4 weeks. In addition, EGF stimulated neuronal differentiation of recently proliferated cells, as evidenced by increased numbers of BrdU-labeled, doublecortin (Dcx)-immunoreactive cells (i.e., immature neurons) in the striatum three to five weeks post-MCAO, and of BrdU-labeled, neuronal nuclear antigen (NeuN)-immunoreactive cells (i.e., mature neurons) in the striatum 13 weeks post-MCAO. At this latter time point, BrdU/NeuN-immunopositive cells also expressed the neuronal subtype-specific marker parvalbumin, but not somatostatin or DARPP-32, consistent with their maturation to a phenotype associated with a discrete subpopulation of striatal interneurons.

EPO

Wang et al.[13] administered EPO (5,000 U/kg/d i.p.) to rats for 7 days, beginning 24 hours after MCAO. At the end of this period, EPO-treated rats showed increases of approximately 70% in the number of BrdU-labeled cells and approximately 80% in Dcx-immunoreactive cells in the SVZ ipsilateral to the infarct, as well as increased numbers of Dcx-positive cells in the ischemic striatum and cerebral cortex. Tsai et al.[55] produced brain-specific EPO receptor knockout mice, and found decreased numbers of Dcx-immunopositive cells in cerebral cortex 7 days after stroke induced by distal MCA branch cauterization plus transient (15-minutes) bilateral occlusion of the common carotid arteries. This appeared to be the combined result of decreased basal SVZ neurogenesis, a blunted neurogenesis response to ischemia, and defective neuromigration.

FGF-2, or FGF-2 plus EGF

Rats were given FGF-2 (0.5 μg i.c.) 24 hours and 48 hours after MCAO and were killed at 7, 14, or 21 days.[56] FGF-2 increased the number of BrdU-positive cells in the DG, of which 85% to 90% were also Dcx-positive, by 30% to 40% at 7 days; by 21 days, 75% to 85% of surviving BrdU-labeled cells were immunoreactive for NeuN, suggesting that they had become mature neurons. In contrast, SVZ neurogenesis was unaffected by FGF-2 in this study.

Two other studies employed combined administration of FGF-2 and EGF. Baldauf and Reymann[24] used endothelin-1 to occlude the MCA in rats and, 10 minutes later, began intracerebroventricular administration of FGF-2 plus EGF (0.48 μg/day each for 14 days). FGF-2 increased the number of BrdU/Dcx-immunopositive cells about 2-fold in the ipsilateral striatum, but reduced BrdU/Dcx labeling in the DG bilaterally at 14 days postischemia. Others[57] infused a combination of FGF-2 plus EGF (1.44 ng/d i.c.v. each for 3 days) in rats subjected to transient MCAO. At 21 days postischemia, the number of BrdU/NeuN-positive cells in the DG was increased by 46% in the growth factor-treated animals.

G-CSF, or G-CSF plus SCF

Rats underwent photothrombotic occlusion in the parietal cortex, followed 1 hour later by the first of 5 daily intravenous injections of G-CSF (15 μg/kg).[26] Six weeks postischemia, Dcx immunoreactivity in the ischemic neocortex was increased by 225% to 300%, and the number of BrdU/NeuN-positive cells was increased by approximately 70%, in FGF-2-treated compared with control rats. Another study combined administration of G-CSF (300 μg/kg/d s.c.) and SCF (100 μg/kg/d s.c.) to mice on days 11 to 15 after permanent, proximal MCAO.[27] Treatment increased the number of cells immunoreactive for both BrdU and the immature neuronal marker, Musashi-1, approximately 6-fold in the SVZ.

HB-EGF

HB-EGF (1 ng/h i.c.v. for 72 hours), given starting 24 hours after transient (90-minutes) MCAO in rats, increased BrdU labeling in DG (by ~20% at 4 days) and SVZ (by ~40% at 4 weeks), compared with rats not given HB-EGF.[28] Thus, the added effects of HB-EGF were small in relation to the neurogenesis-promoting effect of ischemia itself. In another study, an HB-EGF-expressing adenoviral vector was administered to rats intracerebroventricularly, 3 days after transient (80-minutes) MCAO.[29] In HB-EGF compared with LacZ-vector-treated rats, the number of Dcx/BrdU-positive cells was increased about 2-fold in the ipsilateral and contralateral SVZ at 8 days post-MCAO, and about 12-fold in the ipsilateral striatum at 28 days post-MCAO.

VEGF

VEGF, identified originally as an angiogenic and vessel-permeability factor, is also directly neuroprotective and stimulates neurogenesis.[34] When given to rats at 10 ng/h intracerebroventricularly, beginning 24 hours after 90 minutes of transient MCAO and continuing for three days, VEGF increased BrdU labeling in both ipsilateral and contralateral DG and SVZ at 28 days postischemia.[36] The extent of increase, compared with MCAO without VEGF treatment, averaged about 4-fold. The number of cells reactive for both BrdU and neuronal marker proteins was not determined, but BrdU colocalized exten-

sively with Dcx and the neuronal differentiation factor, NeuroD.

Summary

Focal cerebral ischemia stimulates neurogenesis in the adult brain and promotes the migration of nascent neurons from the SVZ into the ischemic striatum and cerebral cortex. The mechanisms involved are poorly understood, but may include the release of chemical mediators, including growth factors, from ischemic tissue.[58] If this is the case, then exogenous growth factors might have no additional effect in the ischemic brain, despite their ability to stimulate neurogenesis in the normal brain. However, the studies described above suggest this is not true. Several growth factors increase neurogenesis in the ischemic brain, beyond the effect of ischemia per se. Nevertheless, the functional implications of this phenomenon are unclear. First, it remains unclear in most cases if adult neurogenesis, including that induced by growth factors or by ischemia, yields functional neurons. Second, increased neurogenesis at some sites (e.g., DG) may be irrelevant to focal ischemia, because new neurons that arise in the DG do not appear to migrate to ischemic brain regions.

These reservations aside, the results described above are encouraging because they suggest that quantitatively significant increases in neurogenesis can be achieved by postischemic administration of growth factors. Factors like EPO and G-CSF are of particular interest because they can be administered systemically, and are already used clinically for other, hematological indications.

CONCLUSION

Every student of neuroscience has at least some familiarity with the story of the discovery of NGF, the prototypical neural growth factor. However, growth factors have been slow to enter clinical practice for neurological disorders, which contrasts with their important role in hematology and oncology. The studies reviewed above illustrate that growth factors may have a unique ability to modify outcome from stroke even when given many hours to days after the event. If so, this would remove a major limitation associated with most neuroprotective measures. The work described here also indicates that systemic administration permits access of several growth factors to the brain, which would also facilitate their therapeutic use. Finally, although research to date does not resolve whether growth factors will prove useful in the treatment of stroke, it does provide reason for optimism. From the standpoint of neurorehabilitation, an important unanswered question is for how long after stroke might growth factors be helpful. The studies cited above typically involve starting treatment within hours to a few days after the onset of ischemia, and determining the result after days to a few weeks. In future investigations, it will be invaluable to assess whether more delayed administration of growth factors, targeting the chronic phase of stroke, is also beneficial, and what mechanisms account for this.

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