Insulin-Like Growth Factor I
A Potential Neuroprotective Compound for the Treatment of Acute Ischemic Stroke?

Ron Kooijman, PhD; Sophie Sarre, PhD; Yvette Michotte, PhD; Jacques De Keyser, MD, PhD

Background and Purpose—Insulin-like growth factor I (IGF-I) exerts neuroprotective effects in both white and gray matter under different detrimental conditions. The purpose of this review is to collect the evidence whether IGF-I is a candidate neuroprotective drug in patients with acute ischemic stroke.

Results—IGF-I was found to be neuroprotective in animal models of focal brain ischemia when given ≥2 hours after the insult. Different routes of administration (eg, cerebroventricular, intravenous, and intranasal) were found to be effective. In addition to inhibition of apoptosis and reduction of the infarct volume, IGF-I also improved neurological outcome. Furthermore, there are strong indications that IGF-I can also stimulate the regeneration of neural tissue.

Conclusions—Additional studies are required to reveal the neuroprotective mechanisms of IGF-I in detail and to elucidate the role of IGF-binding proteins. Preclinical studies in relevant animal models for studying stroke (ie, hypertensive, diabetic, or aged animals) should be done testing different doses and routes of IGF-I administration and different combinations of IGF-I and IGF-binding proteins. (Stroke. 2009;40:e83-e88.)

Key Words: cerebral stroke ■ insulin-like growth factor I ■ ischemia

The only approved acute treatment of ischemic stroke is reperfusion by tissue plasminogen activator. However, because of the limited time window for treatment and the rigorous inclusion criteria, a minority of the patients benefit from this treatment.1 Many clinical trials with neuroprotective drugs in patients with acute ischemic stroke have yielded disappointing results.2 The ischemic cascade has multiple pathways by which it can lead to damage and involves membrane depolarization, calcium overload, excitotoxicity, oxidative stress, and inflammation, many of which trigger necrosis or apoptosis. One of the reasons for the failure of many of the previously tested neuroprotectants is that they only target a single aspect of the ischemic cascade. One should expect more effective results by therapies that are able to interfere with multiple cellular targets in the brain and act further downstream in the ischemic cellular events leading to cell death.3,4

Insulin-like growth factor I (IGF-I) is a pleiotropic peptide that exerts prosurvival signals through the IGF-I receptor, which is present on virtually every cell type. As a consequence, IGF-I is able to stimulate cell survival in many different cell types. This can be explained by the ability of IGF-I to use at least 3 different signal transduction pathways to exert antiapoptotic effects through both posttranslational modification and transcriptional modification of pivotal regulators of cell survival.5 The purpose of this review is to briefly summarize the current knowledge on the role and therapeutic effects of IGF-I in animal models for focal cerebral ischemia and to evaluate the therapeutic potential of IGF-I for patients with ischemic stroke.

Physiology of the Insulin-Like Growth Factor System With Special Reference to the Central Nervous System
IGF-I plays a key role in embryonic and postnatal growth, exerts metabolic effects, and is involved in tissue homeostasis through regulation of cell proliferation and programmed cell death. During postnatal growth, IGF-I mediates the effects of growth hormone, which stimulates IGF-I production in the liver. In addition, IGF-I is also produced by many nonhepatic tissues and exerts autocrine or paracrine effects in addition to the endocrine effects of liver-derived IGF-I. Notably, serum contains an excess of IGF-I, and liver-specific ablation of IGF-I resulted in 75% reduction of serum IGF-I levels without effects on body growth.6 Because this reduction in IGF-I levels affected several brain functions, including neurogenesis in the hippocampus,7 it was speculated that high levels of serum IGF-I are required for certain processes in the brain.

Insulin-Like Growth Factor-Binding Proteins
The existence of at least 6 IGF-binding proteins (IGFBPs) renders the IGF system much more complex.8 IGFBPs are not
homologous to insulin or IGF receptors, but do bind IGF-I and IGF-II with a higher affinity than the IGF-I receptor. As a consequence, most of the circulating IGF-I (99%) is bound to IGFBPs. They may prolong the half-life of IGFs, regulate tissue distribution, and facilitate or block the binding to their cognate receptors in target tissues. IGFBPs may also prolong the actions of IGFs by ensuring slow release in the vicinity of target cells. Proteolytic cleavage and phosphorylation of IGFBPs are likely to be involved in the regulation of IGF release. IGFBPs have also been shown to exert direct cellular effects in an IGF-independent way, most likely through interaction with cell surface receptors such as integrins. IGFBP-2, -4, and -5 are the most predominant IGFBPs in the brain.9

**Role of the Insulin-Like Growth Factor System in the Central Nervous System With Special Reference to Stroke**

Overexpression and genetic ablation of components of the IGF system in animal models have shown that IGF-I plays a pivotal role in brain development.10 In addition to an endocrine role for IGF-I in the brain, also autocrine and paracrine effects have been postulated, because IGF-I is widely expressed in the central nervous system. IGF-I exerts several effects on cells of the central nervous system that may improve functional outcome after stroke. Because IGF-I not only affects neurons, but also oligodendrocytes and other glial cells, IGF-I may protect white matter as well as gray matter in the brain, which is of major importance for the clinical use of IGF-I, because the human brain consists for 50% of white matter compared with 10% in rodents.

IGF-I is a survival factor for both sensory and motor neurons, and it protects neurons against excitotoxicity and oxidative stress as indicated by in vitro experiments showing that IGF-I inhibits glutamate-, nitric oxide-, and hydrogen peroxide-induced apoptosis.11–13 IGF-I also protects oligodendrocyte precursors from cytotoxicity14 and mature oligodendrocytes from the death-inducing effects of tumor necrosis factor-α.15

In addition to these protective effects, IGF-I also has the potential to influence recovery from ischemic stroke through regeneration, because it stimulates in vitro proliferation and differentiation of neural and oligodendrocyte progenitors. Even more, IGF-I also enhances the proliferation of endogenous neural progenitors in rats.16 The effects on oligodendrocytes and the finding that IGF-I stimulates myelin expression17 are probably the underlying mechanisms for the stimulating effects of IGF-I on remyelination.18,19 Furthermore, IGF-I is able to modulate brain plasticity by influencing neurite outgrowth, synaptogenesis, neuronal excitability, and neurotransmitter release.20

A phenomenon that needs to be elucidated is the role of IGFBPs in the central nervous system. As outlined in the previous section, IGFBPs may either enhance or block the effects of IGF-I. An eloquent study, designed to increase the free concentration of IGF-I in the brain using compounds that interfere with the interaction between IGF-I and IGFBPs, indicated that IGFBPs can block the effects of endogenous IGF-I.

When given intracerebroventricularly (ICV) at 1 hour after middle cerebral artery occlusion (MCAO), the small compound NBI-31772 (1-[3,4-dihydroxybenzyl]-3-hydroxycarbonyl-6,7-dihydroxyisoxquinoline) and the peptide [Leu24, 59, 60, Ala31]hIGF-I were shown to increase the free level of IGF-I in the cerebrospinal fluid and to be neuroprotective in rats.21 However, another study showed that the in vitro effect of IGF-I on neurogenesis depended on its association with IGFBP-2.22 A role for IGFBPs in neuroprotection has also been implicated in a rat model for ischemia/hypoxia.23 It was shown that whereas ICV administration of IGF-I was neuroprotective, administration of the des(1-3)IGF-I analog that does not bind to IGFBPs was without effect. It is not clear whether binding to IGFBPs is required for transport from the ventricles to the parenchyma or whether IGFBPs serve to create a pool of IGF-I protected from degradation. Whether IGFBPs facilitate or inhibit the effects of IGF-I in the central nervous system may depend on different factors such as the target tissue or the way of administration.

**Serum Insulin-Like Growth Factor I Levels and Cerebral Ischemia**

Several epidemiological studies revealed an inverse relation between plasma IGF-I levels and the risk of ischemic stroke24 or the clinical outcome after stroke.25,26 However, it remains to be established whether this relation is causative. A good indication for a causative effect of IGF-I on survival after stroke has been obtained by van Rijn et al27 who studied the relation between the occurrence of a 192-bp allele in the IGF-I promoter region and survival after stroke. It was shown that noncarriers, exhibiting low plasma IGF-I levels, were more at risk of death after stroke than carriers. Several hormones, including leptin, insulin, and IGF-I,28 have been shown to cross the blood–brain barrier, and the current view is that the blood–brain barrier is not a simple barrier but a structure that orchestrates the transport of certain bloodborne factors to the brain parenchyma. Specific transport of IGF-I across the blood–cerebrospinal fluid barrier has also been reported.29 and this system has been implicated in neuroprotective effects of IGF-I. The finding that the blood–brain barrier is disrupted for several weeks after induction of cerebral ischemia30 also supports the notion that systemic IGF-I treatment could be beneficial. In addition, the observation that the free serum IGF-I concentrations are transiently increased by 70% after tissue plasminogen activator treatment in patients with stroke may be highly relevant.31

**Studies in Experimental Models of Ischemic Stroke**

IGF-I is neuroprotective in different models of hypoxic/ischemic brain injury.32 To explore the possibilities for the clinical use of IGF-I in ischemic stroke, we discussed the effects of IGF-I observed in animal models of focal brain ischemia. In all studies, focal ischemia was induced by transient and permanent MCAO.

To evaluate the therapeutic potential of IGF-I, it is of crucial importance to establish the effects of IGF-I when given after induction of focal ischemia and to investigate different modes of administration. Seven studies revealed that administration of IGF-I after induction of the injury reduces...
the infarct volume, increases cell survival, or improves functional outcome (Table). In one study, the effects of IGF-I treatment at 2, 4, or 6 hours after the insult were tested. Intranasal administration of IGF-I at 2 or 4 hours after induction of ischemia significantly reduced infarct size by 54% and 39%, respectively. A 29% reduction in infarct size obtained when IGF-I treatment was started 6 hours after the insult did not reach significance. A significant improvement of neurological function was obtained only when intranasal treatment with 150 μg IGF-I was delayed for no longer than 2 hours. Significant effects on motor sensory function were found on Days 5, 6, and 7, but not at earlier time points (Days 1 to 4). The effects on somatosensory function were only significant at Day 7. Remarkably, when the same treatment was applied at 10 minutes, 24 hours, and 48 hours, neurological functions were already improved at Days 1, 2, and 3. Infarct volume was also reduced by IVC injection of 33 μg IGF-I per day during 3 days starting 30 minutes after MCAO. Improved functional outcome in this study was observed at Days 1, 2, and 3. When IGF-I was given subcutaneously at a dose of 200 μg/day during 7 days starting from 30 minutes after the insult, the infarct volume was reduced and significant improvement of functional outcome was detected at Days 1 and 3 to 7. It remains to be established whether IGF-I treatment at later time points reduces or delays functional outcome. Taken together, these results indicate that further studies to the window of opportunity for different ways of administration at different doses are required. For instance, it would be interesting to find out whether higher doses of IGF-I can compensate for the delay of administration.

Other ways of IGF-I administration were also shown to be effective when applied after the insult. Topical administration in Gelfoam on the cerebral cortex 1 hour after MCAO reduced infarct size and increased cell survival. Moreover, intravenous administration of IGF-I 2 hours after the insult decreased the infarct volume and the number of apoptotic cells in diabetic rats. The proof of principle that IGF-I can be neuroprotective when given after the insult has also been demonstrated using models for global ischemia and hypoxia/ischemia.9 Also promising with respect to therapeutic applicability are the

### Table. IGF-I Treatment in Animal Models of Focal Cerebral Ischemia

<table>
<thead>
<tr>
<th>Species</th>
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<th>Stroke Model</th>
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<th>Window</th>
<th>Outcome Measurements</th>
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<tbody>
<tr>
<td>Male rats, n=9–10</td>
<td>1, 2, 9</td>
<td>60 minutes MCAO</td>
<td>20 μg topically at the cerebral cortex</td>
<td>1 hour</td>
<td>Reduced infarct volume increase in cell survival</td>
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<tr>
<td>Male rats, n=9</td>
<td>1, 2</td>
<td>60 minutes MCAO</td>
<td>20 μg topically at the cerebral cortex</td>
<td>1 hour</td>
<td>Reduced infarct area</td>
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<tr>
<td>Male rats, n=10–14</td>
<td>1, 2, 3, 5, 6</td>
<td>60 minutes MCAO</td>
<td>(1) 33 μg/day (3 days) intracerebroventricular; (2) 200 μg/day (7 days) subcutaneous</td>
<td>0.5 hour</td>
<td>Reduced infarct volume, improved functional outcome</td>
<td>35</td>
</tr>
<tr>
<td>Male rats, n=9–10</td>
<td>1, 2, 3, 5, 9</td>
<td>120 minutes MCAO</td>
<td>37.5 μg intranasal; 150 μg intranasal</td>
<td>10 minutes, 24 and 48 hours†</td>
<td>Reduced infarct volume; improved neurological function</td>
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<tr>
<td>Male rats, n=9</td>
<td>1, 2, 3, 5, 9</td>
<td>120 minutes MCAO</td>
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<td>Improved sensory, motor, and vestibulomotor function</td>
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<td>1, 2, 3, 5, 9</td>
<td>120 minutes MCAO</td>
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<td>1, 2, 7, 9</td>
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<tr>
<td>Mice, n=8</td>
<td>1, 2, 9</td>
<td>MCAO electric coagulation</td>
<td>AAV-IGF-I construct in the left caudate nucleus</td>
<td>−3 weeks</td>
<td>Improved functional outcome, enhanced neurogenesis and neovascularization</td>
<td>39</td>
</tr>
</tbody>
</table>

*Numbers indicate fulfillment of the following STAIR criteria for animal studies on stroke: (1) publication after peer review; (2) statement of control of temperature; (3) randomization of animals; (4) blinded induction of ischemia; (5) blinded assessment of outcome; (6) anesthetic without neuroprotective activity; (7) use of appropriate animal model for stroke (diabetic, hypertensive, or aged); (8) sample size calculation; (9) compliance with animal welfare regulations; (10) statement of potential conflict of interest.

†IGF-I was given at all time points indicated.

AAV indicates adenoassociated virus.
findings that IGF-I may be given subcutaneously or intravenously.\textsuperscript{35,38} One study has tested IGF-I gene transfer using adenoassociated virus–IGF-I constructs in mice.\textsuperscript{39} In this study, the construct was introduced in the caudate nucleus 3 weeks before MCAO and proved to stimulate neurogenesis, neovascularization, and improved functional outcome. That gene therapy can also be effective when started after an ischemic insult was shown in a gerbil model for global cerebral ischemia. Gene transfer using a Sendai virus proved to be neuroprotective and to increase survival when applied 30 minutes after bilateral artery occlusion.\textsuperscript{40}

Although these data indicate that IGF-I can be used as a neuroprotectant after ischemic stroke, more studies on the window of opportunity for different kinds of IGF-I administration are needed. In a rat model for hypoxia/ischemia, it was shown that application of hypothermia after the insult delays cell death and broadens the window for IGF-I treatment.\textsuperscript{41} Because hypothermia is also protective in different rat models for focal cerebral ischemia,\textsuperscript{42} early application of hypothermia after stroke could broaden the window of opportunity for IGF-I treatment after stroke.

The quality of animal studies on stroke has been discussed as a result of many unsuccessful clinical trials. The observation that many preclinical studies on stroke exhibit important deficits\textsuperscript{43} has led to the Stroke Therapy Academic Industry Round table (STAIR) criteria for preclinical research on stroke.\textsuperscript{44} These criteria have been taken into account in our evaluation of the literature and are given in the legend of the Table. The fulfillment of the STAIR criteria for each study is indicated and it appears that the quality score of these studies ranged from 2 to 5 on a scale of 0 to 10. A major drawback with respect to the STAIR criteria observed in these studies concerns the use of neuroprotective anesthetics such as halothane, isoflurane, or pentobarbital\textsuperscript{45,46} in most studies. The use of anesthetics may be circumvented by induction of vasoconstriction using endothelin-1 in awake animals.\textsuperscript{47} Another major caveat to the results in the Table is that most studies have been done with normal adult rats, whereas in humans, ischemic stroke prevalently occurs in the elderly and in patients with hypertension or diabetes. Moreover, aging, hypertension,\textsuperscript{48} and diabetes\textsuperscript{49} also have been shown to influence the short-term effects of MCAO and the recovery.

Therefore, the STAIR committee recommended that preclinical testing of therapies should be performed using aged, hypertensive, or diabetic animals to increase the predictive validity of animal studies for stroke. Only 2 studies to the effects of IGF-I on focal cerebral ischemia have been performed using one of these animal models. One study using diabetic rats showed that intravenous administration of IGF-I reduced infarct size and the number of apoptotic cells in the L5 and L6 layers in the cerebral cortex and the CA3 region of the hippocampus.\textsuperscript{50} Another study using hypertensive rats revealed that IGF-I when given ICV increased the proliferation of endogenous neural progenitors.\textsuperscript{16} Other problems with respect to the STAIR criteria concern the lack of power analysis, randomization of animals, and blind induction of treatment and assessment of outcome (Table). Additional caveats with respect to these in vivo experiments concern the following points: (1) the use of only male rats to test for the effects of IGF-I in MCAO. Indeed, IGF-I has been shown to exert synergistic neuroprotective effects with estrogens\textsuperscript{50}; (2) in most cases, IGF-I has been given centrally, whereas systemic administration is more appropriate in a clinical setting; and (3) the use of a model for transient ischemia instead of thromboembolic models with and without thrombolysis.

In addition to limiting brain damage within the first day after ischemic stroke, IGF-I has also been implicated in stimulating the regeneration of neurons. For example, ICV infusion of IGF-I in an MCAO rat model resulted in increased proliferation of progenitors in the ipsilateral dentate gyrus of the hippocampus.\textsuperscript{16} Furthermore, endogenous IGF-I has also been implicated in regeneration, because ICV infusion of an anti-IGF-I antibody decreased the proliferation of progenitors, which was peaking between Days 2 and 4 after MCAO. Indeed, IGF-I was upregulated after MCAO and expressed in activated astrocytes and progenitors.\textsuperscript{51} Proliferation of neural progenitors was also enhanced by adenoassociated virus vector-mediated transfer of the IGF-I gene to the caudate nucleus in mice 3 weeks before the insult.\textsuperscript{39} This treatment also resulted in amelioration of the functional outcome and an increased vascular density in the treated hemisphere 3 weeks after MCAO.

**Conclusions and Perspectives**

Although there is ample evidence that IGF-I when given shortly after induction of ischemic stroke is neuroprotective in rats, additional preclinical testing is needed. Further preclinical studies using aged, diabetic, or hypertensive rats are required to establish the time windows for different ways of IGF-I administration. Basic research in relevant animal models should also reveal whether IGF-I treatment during the regeneration phase (between 1 and 3 weeks) would ameliorate functional outcome.

IGF-I is approved in the United States for treatment of growth disorders in individuals with growth hormone resistance. Although side effects occur in children treated with recombinant human IGF-I (rhIGF-I), the treatment is rarely adapted or interrupted due to side effects and the long-term safety is considered to be good.\textsuperscript{52,53} However, the dose-dependent side effects observed in clinical studies with adults were considered to be unacceptable for treatment of patients with diabetes.\textsuperscript{54} When patients were treated with a combination of IGF-I and IGFBP-3, the side effects were markedly reduced even at higher doses.\textsuperscript{55} However, serious side effects, including hypoglycemia, paresthesias, and episodes of Bell’s palsy, were still observed in some patients with Type II diabetes.\textsuperscript{55} Also, the finding that IGF-I treatment can lead to intracranial hypertension\textsuperscript{56} deserves special attention with respect to the use of IGF-I in patients with ischemic stroke. This side effect and its consequences may not have occurred after ICV administration of IGF-I due to the induction of a hole in the skull and meninges. We therefore believe the effects of IGF-I administration in combination with IGFBPs should be tested in relevant experimental models and the minimal effective dose should be determined. Special attention should be addressed to the efficacy of more preferable ways of administration, ie, intravenously or subcutaneously.
Although it has been suggested that IGFBPs influence the passage of IGF-I over the blood–brain barrier, there is no direct evidence for this and so this area needs further investigation, especially with respect to changes in permeability induced by cerebral ischemia. Ongoing clinical studies testing the effects of IGF-I in combination with IGFBP-3 should provide more insight into the safety of short-term IGF-I/IGFBP-3 treatment in nondiabetic subjects. Absence of side effects in ongoing clinical trials and positive results in the required animal studies could lead to Phase II clinical studies to assess the effects of short-term (5 to 7 days) subcutaneous administration of IGF-I/IGFBP-3 on penumbra-saving, clinical outcome, and to determine possible adverse effects in patients with acute ischemic stroke.

Because plasma levels of IGF-I and IGFBP-3 in patients with ischemic stroke are markedly reduced as a function of the infarct size and treatment with tissue plasminogen activator transiently increases free IGF-I levels in these patients, monitoring of IGF-I and IGFBP-3 levels during treatment is essential. This will allow to correlate beneficial and adverse effects to serum levels of IGF-I and IGFBP-3 and to adjust the treatment to individual plasma levels in patients.

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Disclosures
None.

References


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