Insulin-Like Growth Factor I Serum Levels Influence Ischemic Stroke Outcome

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Background and Purpose—Insulin-like growth factor I (IGF-I) is neuroprotective in animal models of stroke. We investigated whether serum IGF-I levels in patients with acute ischemic stroke influence stroke severity and outcome.

Methods—Concentrations of IGF-I and IGF binding protein 3 were measured in serum samples obtained within 6 hours after stroke onset from 255 patients who took part in the placebo arm of the United States and Canadian Lubeluzole in Acute Ischemic Stroke Study. Stroke severity was assessed with the National Institutes of Health Stroke Scale. Multivariate analysis was performed to assess the overall shift in modified Rankin Scale score and changes in the National Institutes of Health Stroke Scale score at 3 months. Survival curves were plotted using the Kaplan-Meier method, and the Cox proportional hazard model was used for multivariate analysis to investigate factors influencing survival.

Results—After controlling for statistically relevant risk factors, subjects with high IGF-I levels or IGF-I/IGF binding protein 3 ratios had a better neurological and functional outcome at 3 months. Baseline stroke severity was not different between high and low IGF-I groups. In contrast to the low IGF-I group, neurological symptoms gradually improved from Day 3 in the high IGF-I group.

Conclusions—Our results suggest that high serum IGF-I levels just after ischemic stroke onset are associated with neurological recovery and a better functional outcome. (Stroke. 2011;42:2180-2185.)

Key Words: insulin-like growth factor I ■ ischemic stroke ■ outcome

Insulin-like growth factor I (IGF-I) is an endogenous survival factor for neurons, glial cells, and endothelial cells and may enhance recovery after injury by stimulating the differentiation of neural and oligodendrocyte progenitors. IGF-I has been shown to be a potent neuroprotective compound in rodent models of ischemic stroke. Most of the circulating IGF-I is found in a 150 kDa complex consisting of insulin-like binding protein 3 (IGFBP3), IGF-I, and an acid-labile subunit, which transports IGF-I in the circulation and prolongs its half-life. IGF-I is available for clinical use but has not been studied in a clinical trial in patients with stroke. A small study in elderly patients with stroke found an inverse relation between circulating IGF-I levels, determined within 24 hours of admission, and outcome, mainly death. Another study found a relationship between higher levels of IGF-I and improvement in functional and cognitive scores in revalidating patients with stroke. The aim of this study was to evaluate whether higher serum levels of IGF-I in the acute phase of ischemic stroke are associated with less severe strokes and better functional outcome.

Patients and Methods

Patients and Study Design
This study is a post hoc analysis of data from the placebo arm of the United States and Canadian Lubeluzole in Acute Ischemic Stroke Study (LUB-INT-9), which was a multicenter, randomized placebo-controlled, double-blind, parallel-group trial. The study group is well documented and not influenced by reperfusion therapies. The target population was patients at least 18 years of age who presented with substantial neurological deficit within 6 hours of the onset of an acute cerebral hemispheric ischemic stroke. They had to be alert or arousable to minor stimulation, exhibit a significant motor deficit of the arm or leg, and have a total National Institutes of Health Stroke Scale (NIHSS) score of >7 at baseline. Further classification of ischemic stroke subtypes was based on the Trial of Org 10172 in Acute Stroke Treatment criteria. Stroke severity was quantified using the NIHSS within 6 hours after stroke onset. Severe stroke was defined as a NIHSS score >15. The NIHSS was further assessed daily between Days 2 and 5 and at Weeks 4 and 12. Patients who died were given a maximum NIHSS score at their next specified assessment time point. Disability at 3 months after stroke onset was assessed using the modified Rankin scale (mRS). The mRS was assessed at baseline, at the end of study treatment, and at Weeks 4 and 12.

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The placebo group consisted of 346 patients with ischemic stroke. Baseline serum samples were available from 255 patients, which was the population used for the present post hoc analysis.

Biochemical Analyses
Venous blood samples were taken within 6 hours after stroke onset. Blood samples were immediately centrifuged and serum used for additional analyses was stored at −80°C until assay time. IGF-I and IGFBP3 levels were measured by radioimmunoassay kits according to the manufacturer’s instructions (Nichols Institute Diagnostics). The IGF-I kit has a sensitivity of 6 ng/mL and the IGFBP3 kit of 20 ng/mL. Samples from each individual were measured in duplicate in the same assay. To analyze the effects of IGF-I serum levels on stroke severity and outcome, patients were divided into a high and a low IGF-I serum level groups and high and low IGF-I/IGFBP3 ratio group according to whether the level or ratio was above or below the mean of the whole group. The IGF-I/IGFBP3 ratio is used as a measure for the bioavailable IGF-I.

Statistical Analyses
IGF-I levels and IGF-I/IGFBP3 ratios were normally distributed. Pearson \( \chi^2 \) test was used for categorical variables and the \( t \) test and Spearman rank correlation coefficient were applied for continuous variables. Statistical significance was defined as \( P<0.05 \).

Univariate testing was performed to identify associations between possible confounding factors (age, gender, center, admission NIHSS score, admission diastolic blood pressure, admission glucose level, stroke subtype, arterial hypertension, diabetes mellitus, atrial fibrillation, coronary artery disease, heart failure, smoking, previous stroke) and the outcome parameters (NIHSS scores, mRS score at 3 months, mortality at 3 months). To stabilize the variance of the NIHSS score, a log transformation was obtained.

Multivariate regression analysis by a backward stepwise method was performed with entry and removal criteria of 0.05 and 0.10, respectively, including all variables with probability values <0.05 in univariate analysis. Shift analysis of the mRS score at 3 months was assessed by the van Elteren Cochran-Mantel-Haenszel test.\(^a\) As reported by Lees and coworkers,\(^a\) the full range of the mRS as a 6-category polychotomous outcome (merging mRS scores of 5 with mortality) was used with adjustment for variables with significant association in univariate analysis. Covariates are entered as categorical parameters in the van Elteren Cochran-Mantel-Haenszel test. Continuous parameters therefore were divided into strata (baseline NIHSS score: 0 to 5, 6 to 10, 11 to 15, 16 to 20, and >20; serum glucose levels: percentile 0% to 25%, 26% to 50%, 51% to 75%, and 76% to 100%). Survival data were analyzed by both the Kaplan-Meier method and Cox proportional hazards multivariate analysis after verifying the proportional hazard assumption for each factor using Schoenfeld residuals. Results were expressed as adjusted hazard ratios and the log-rank test was used to assess differences between groups with high and low IGF-I serum levels.

Statistical computations were performed with the SPSS software package Version 17.0 (SPSS Inc, Chicago, IL), except for evaluation of the mRS shift, which was carried out in SAS Version 9 using the PROC FREQ function.

Data are presented as means±SD unless otherwise specified.

Results
IGF-I and IGFBP3 levels measured within 6 hours after stroke onset were 76.56 (±38.92) ng/mL and 2066.28 (±769.33) ng/mL, respectively. The mean IGF-I/IGFBP3 ratio was 0.039 (±0.024). Baseline characteristics of the high and low IGF-I serum level groups and high and low IGF-I/IGFBP3 ratio groups are presented in Table 1.

Stroke Severity
At admission, 118 patients (47%) had a severe stroke and 136 patients (53%) displayed mild to moderate strokes. When comparing low with high IGF-I levels and IGF-I/IGFBP3

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Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IGF-I Level</th>
<th>IGF-I/IGFBP3 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n = 142)</td>
<td>High (n = 113)</td>
</tr>
<tr>
<td>Mean age, y±SD</td>
<td>74±11</td>
<td>68±13</td>
</tr>
<tr>
<td>Male gender, %</td>
<td>63 (44)</td>
<td>76 (67)</td>
</tr>
<tr>
<td>Severe stroke (NIHSS &gt;15), %</td>
<td>60 (42)</td>
<td>58 (51)</td>
</tr>
<tr>
<td>Baseline mean NIHSS score±SD</td>
<td>15±5</td>
<td>16±6</td>
</tr>
<tr>
<td>Stroke subtype (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small vessel</td>
<td>20 (14)</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Large vessel</td>
<td>34 (24)</td>
<td>32 (28)</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>45 (32)</td>
<td>39 (35)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>43 (30)</td>
<td>27 (24)</td>
</tr>
<tr>
<td>Mean admission diastolic blood pressure, mm Hg±SD</td>
<td>82±16</td>
<td>86±16</td>
</tr>
<tr>
<td>Mean admission glucose level, mmol/L±SD</td>
<td>8.3±3.9</td>
<td>7.9±2.9</td>
</tr>
<tr>
<td>Risk factors, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>89 (63)</td>
<td>70 (62)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>33 (23)</td>
<td>21 (19)</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>42 (30)</td>
<td>27 (24)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>48 (34)</td>
<td>38 (34)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>19 (13)</td>
<td>16 (14)</td>
</tr>
</tbody>
</table>

IGF-I indicates insulin-like growth factor I; IGFBP3, insulin-like growth factor binding protein 3; NIHSS, National Institutes of Health Stroke Scale; SD, standard deviation.

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\( H11002 \)
ratios, mean NIHSS scores were not significantly different ($P=0.150$ and $P=0.394$, respectively; Table 1).

**Neurological Recovery**

Baseline high IGF-I levels and IGF-I/IGFBP3 ratio were associated with improvement in neurological recovery as assessed by the NIHSS score. Significant neurological recovery began on Days 3 and 4 and continued through the end of the study at 12 weeks (Figure 1).

Variables showing a statistically significant relation with the NIHSS score at 12 weeks were used for linear regression analysis (baseline NIHSS score, $P<0.001$; admission glucose level, $P=0.010$; atrial fibrillation, $P=0.033$; previous stroke, $P=0.003$; IGF-I, $P=0.028$; and the IGF-I/IGFBP3 ratio, $P=0.003$), which confirmed an association between both baseline IGF-I levels and IGF-I/IGFBP3 ratio and the NIHSS score at 3 months (Table 2).

**Disability and Mortality**

Univariate analysis identified significant associations between the mRS score at 3 months and the admission NIHSS score ($P<0.001$), admission glucose level ($P=0.010$), and
heart failure ($P<0.041$). Controlling for these variables, the distribution of the mRS score was significantly more favorable in the group with high IGF-I levels as compared with the low IGF-I level group ($P<0.031$; Figure 2). Similar results were obtained using the IGF-I/IGFBP3 ratio ($P<0.045$; Figure 2).

Three months after stroke onset, 186 patients (73%) had survived and 69 patients (27%) had died. Forty-six (32%) of the 142 patients in the low IGF-I level group died during the 3-month observation period compared with 23 (20%) of the 113 patients in the high IGF-I level group ($P<0.032$). Mortality was associated with age ($P<0.011$), admission NIHSS score ($P<0.001$), heart failure ($P=0.007$), stroke subtype ($P=0.008$), and atrial fibrillation ($P=0.045$) in univariate analysis. Cox proportional hazards multivariate analysis was used to adjust for these covariates, confirming a relation between both IGF-I concentration and the IGF-I/IGFBP3 ratio and mortality (IGF-I $\geq 76$ ng/mL: hazard ratio, 1.7 [1.01 to 2.86]; $P=0.045$ and IGF-I/IGFBP3 ratio $\geq 0.039$: hazard ratio, 2.09 [1.23 to 3.56]; $P=0.007$). Patients with high IGF-I serum levels and a high IGF-I/IGFBP3 ratio at onset displayed a better survival at 3 months ($P=0.043$ and $P=0.012$, respectively), as demonstrated with Kaplan-Meier survival curves (Figure 3).

### Discussion

In this post hoc analysis of the placebo arm of the previously reported LUB-INT-9 trial, we found that higher serum IGF-I levels and IGF-I/IGFBP3 ratios, measured within 6 hours after ischemic stroke onset, were associated with better neurological and functional outcome at 3 months. Both for IGF-I serum levels and the IGF-I/IGFBP3 ratio, there was an association with the NIHSS score and mortality at 3 months. The distribution of the mRS scores was significantly more favorable in the high IGF-I level group as compared with the low IGF-I level group. Baseline stroke severity, however, was similar between high and low IGF-I groups.

Our findings are in accordance with the smaller study of Denti and coworkers, who found in 88 elderly patients with ischemic stroke that low IGF-I levels correlated with poor outcome at 3 months. Our study is of pathophysiological interest, because it demonstrates what we hypothetically would expect from IGF-I. In the ischemic brain area, neurons and glial cells that are compromised by excessive glutamate receptor activation, calcium overload, oxygen radicals, or by mitochondrial and DNA damage can die by necrosis or apoptosis. Necrosis is the predominant mechanism occurring after ischemic injury, whereas apoptosis has been found only in the periphery of the ischemic lesion. This suggests that IGF-I may be beneficial in the central nervous system, particularly in the ischemic brain area, where it may protect neurons and glial cells from necrosis.

### Table 2. Stepwise Linear Regression Model Evaluating Factors Influencing NIHSS Score at 3 Months

<table>
<thead>
<tr>
<th>Coefficients of the Regression Line</th>
<th>Exp ($\beta$)</th>
<th>SE</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline NIHSS score</td>
<td>1.657</td>
<td>0.132</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Admission glucose level</td>
<td>1.120</td>
<td>0.012</td>
<td>0.044</td>
</tr>
<tr>
<td>IGF-I level</td>
<td>0.824</td>
<td>0.019</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline NIHSS score</td>
<td>1.659</td>
<td>0.134</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Admission glucose level</td>
<td>1.125</td>
<td>0.012</td>
<td>0.037</td>
</tr>
<tr>
<td>IGF-I/IGFBP3 ratio</td>
<td>0.872</td>
<td>0.307</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Variables included in Model 1: baseline NIHSS, admission glucose level, atrial fibrillation, previous stroke, and IGF-I. Variables included in Model 2: baseline NIHSS, admission glucose level, atrial fibrillation, previous stroke, and IGF-I/IGFBP3 ratio.

NIHSS indicates National Institutes of Stroke Scale; IGF-I, insulin-like growth factor I; IGFBP3, insulin-like growth factor binding protein 3.

* indicates the standardized partial regression coefficient.
in the ischemic core, whereas apoptosis contributes to delayed cell death in the ischemic penumbra.10

IGF-I displays strong antiapoptotic cell survival activities, and its receptor is present on most cells. Unbound bioavailable IGF-I, which has a short half-life of approximately 15 minutes,11 does not improve neuronal outcome when given before the induction of focal brain ischemia in rats. However, it becomes a very potent neuroprotective compound when administered shortly after stroke induction.12 IGF-I suppresses apoptosis through mechanisms involving activation of multiple protein kinase pathways, which are most likely to be important early in the latent phase of evolving programmed cell death.12 This may explain why we found no difference in baseline stroke severity between patients with high and low IGF-I serum levels.

Better functional outcome for the high IGF-I level group at 3 months was associated with a steady improvement of the neurological deficit on the NIHSS already starting from Day 3, probably reflecting an antiapoptotic effect in the penumbra. In addition, IGF-I may also provide beneficial effects after focal brain ischemia by its anti-inflammatory and regenerative properties.12 The latter might provide a mechanism to explain why higher serum levels of IGF-I in revalidating patients with stroke was associated with better improvement in functional and cognitive scores.6

Figure 3. Kaplan-Meier survival curves in (A) high and low serum IGF-I level groups and (B) high and low serum IGF-I/IGFBP3 ratio groups. IGF-I indicates insulin-like growth factor I; IGFBP3, insulin-like growth factor binding protein 3.
Some limitations of the present study should be taken into account. First, the patients in the LUB-INT-9 study were a selected group based on specified entry criteria, which excluded patients with a previous stroke with residual functional impairment, life-threatening concurrent illness, heart failure, recent myocardial infarction, ventricular arrhythmia, and second- or third-degree atrioventricular block. Thus, the findings may not be applicable to all patients with stroke. Second, serum IGF-I and IGFBP3 levels were only measured once after stroke onset, and additional measurements in the days thereafter would have been of interest. The strength of our study is that the study population is well suited for evaluating the effect of a neurotrophic factor on stroke outcome.

In contrast to the majority of the neuroprotective compounds that have been developed by the pharmaceutical industry and failed in Phase III studies, IGF-I is a compound that protects the whole neurovascular unit, which comprises neurons, glia, and the microvessels. Interestingly, treatment of patients with acute stroke with recombinant tissue-type plasminogen activator enhances the bioavailability of circulating IGF-I, suggesting that recombinant tissue-type plasminogen activator might also have neuroprotective properties in addition to its thrombolytic action. Enhancing serum IGF-I levels may be an interesting target to be considered in future therapeutic strategies for stroke.

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Disclosures

None.

References

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