Proinflammatory Cytokines and Glutamate in Acute Stroke

To the Editor:

I have with great interest read the article, “Proinflammatory Cytokines and Early Neurological Worsening in Ischemic Stroke,” by Vila et al.1 Bearing in mind the complexities of interleukin (IL)-6 effects, I would like to ask the authors the following questions:

Are the patients included in this study the same patients as in the earlier published studies2 from the same research group concerning glutamate?

If so, is there a relationship between IL-6 and glutamate that can be identified in the material?

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Glutamate, Interleukin-6, and Early Clinical Worsening in Patients With Acute Stroke

To the Editor:

We appreciate Dr Christensen’s interest in our work, recently published in Stroke.1 As we stated in the Subjects and Methods section of that article, the 231 patients analyzed in our study were selected from a larger cohort of 249 patients admitted consecutively between October 1992 and December 1996. Shrewdly, Christensen correctly guesses that this larger cohort includes the 128 patients that supported our report on glutamate excitotoxicity in acute ischemic stroke.2 Therefore, this larger series gives additional ground and further credit to the role of glutamate.

The concentrations of glutamate in plasma (309.6±64.8 versus 106.3±49.3 μmol/L; P<0.0001) and CSF (13.3±3.8 versus 6.4±4.2 μmol/L; P<0.0001) were significantly higher in patients with clinical deterioration than in patients who remained stable or improved during the first 48 hours. Glutamate and IL-6 levels were positively correlated in samples of both plasma (Spearman coefficient 0.66, P<0.001) and cerebrospinal fluid (CSF) (Spearman coefficient 0.49, P<0.001). As shown in the Table, variables that remained independently associated with early clinical deterioration on multivariate analysis included IL-6 >21.5 pg/mL in plasma or >6.3 pg/mL in CSF, glutamate >200 μmol/L in plasma or >8.2 μmol/L in CSF, admission Canadian Stroke Scale score, and serum glucose.

As pointed out by Dr Christensen, IL-6 has complex mechanisms of action that at present are not clearly understood. While we and others3 suggest proinflammatory properties in acute stroke, a recent article reported in this journal4 disputed these conclusions and found deleterious properties of IL-6. In this study, infarct size and neurological function at 24 hours were not different in animals deficient in IL-6 after transient cerebral ischemia. Moreover, other animal models5 have shown that the local infusion of IL-6 attenuated the neurotoxic effects of NMDA in striatal cholinergic neurons, thus suggesting neuroprotective rather than proinflammatory actions.

Glutamate and IL-6-disclosed related effects in our patients, in agreement with the overlap observed between inflammatory and excitotoxic pathways in experimental models. The increased expression of cytokine genes after injection of NMDA suggested that overactivation of NMDA receptor is one of the main stimuli to cytokine gene transcription in the ischemic brain.6 IL-1 may interact with AMPA and NMDA receptors in the striatum to stimulate cortical pathways that lead to neuronal death, probably through glutamate release.7 In contrast, IL-1 receptor antagonist blocks cerebral damage caused by both NMDA and AMPA receptor activation.8 Finally, IL-10 prevents apoptotic changes induced in response to glutamate release. Cortical cultures obtained in IL-10 knock-out mice are more vulnerable to NMDA injury in vitro, whereas the administration of IL-10 to this culture prevents neuronal death induced by excitotoxic stimuli.8,9

In summary, our results suggest that glutamate and IL-6 are important and independent contributors to neurological deterioration in patients with acute stroke. However, additional studies will be needed to clarify the mechanisms underlying neurological worsening, including the interaction between IL-6 and glutamate. Hopefully, this deeper understanding will result in more effective therapeutic strategies for this devastating condition.

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Factors Associated With Early Neurological Worsening

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>SE</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 &gt;21.5 pg/mL</td>
<td>2.8</td>
<td>0.7</td>
<td>16.5 (4.0–68.0)</td>
</tr>
<tr>
<td>Serum glucose, mg/dL</td>
<td>0.02</td>
<td>0.006</td>
<td>1.01 (1.01–1.03)</td>
</tr>
<tr>
<td>Canadian Stroke Scale score</td>
<td>0.4</td>
<td>0.19</td>
<td>1.5 (1.03–2.2)</td>
</tr>
<tr>
<td>Glutamate &gt;200 μmol/L</td>
<td>4.9</td>
<td>0.81</td>
<td>143.1 (29.1–701.3)</td>
</tr>
<tr>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 &gt;6.3 pg/mL</td>
<td>2.1</td>
<td>0.85</td>
<td>8.9 (1.7–47.8)</td>
</tr>
<tr>
<td>Serum glucose, mg/dL</td>
<td>0.03</td>
<td>0.01</td>
<td>1.03 (1.01–1.05)</td>
</tr>
<tr>
<td>Glutamate &gt;8.2 μmol/L</td>
<td>3.2</td>
<td>0.94</td>
<td>24.8 (3.9–158.8)</td>
</tr>
</tbody>
</table>

Variables included in the model were IL-6 (in plasma and CSF), tumor necrosis factor-α (in plasma and CSF), glutamate (in plasma and CSF), body temperature, serum glucose, fibrinogen, total leukocyte count, admission delay, and presence of early infarct signs on brain CT scan. Age and admission Canadian Stroke Scale score were forced.


Response

Drs Weih and Villringer estimated attributable risk for ACL, LA, APCR, and the prothrombin mutation in ischemic stroke. We would first like to clarify that our analysis\(^1\) was based on a systematic literature review and not a meta-analysis. Attributable risk estimates need to be calculated with multiple regression techniques so that each risk factor is adjusted for the others that may contribute to the outcome under consideration.\(^2\) However, the majority of the odds ratios used by Drs Weih and Villringer to calculate these estimates were not adjusted for traditional stroke risk factors. This can lead to an overestimation of attributable risk. Whisnant\(^3\) highlighted this problem during his 1997 Willis Lecture. He found that the unadjusted attributable risk for stroke in hypertension was 37% but decreased to 26% after adjustment for other risk factors and interactions in multiple regression modeling. In addition, O’Fallon and Sicks\(^3\) have shown that all stroke risk factors have a higher attributable risk in younger patients. This could lead to a further overestimation of the attributable risk associated with coagulopathies. Because of these concerns, we felt that we could not calculate the attributable risk associated with specific coagulopathies.

Our review focused on the prevalence of coagulopathies in ischemic stroke patients from case-control studies of highly selected patients. Population-based estimates of the prevalence of these coagulation disorders in the general population and in ischemic stroke patients, as well as the number of ischemic stroke patients without these coagulation disorders, are needed to provide the most valid assessment of population-attributable risk. Because of the low yield of specialized testing for coagulopathies, an assessment of traditional risk factors and etiologies is necessary before the diagnosis is pursued.

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### Estimated Attributable Risk for FVL/APCR, Prothrombin Mutation, ACL, and LA

<table>
<thead>
<tr>
<th>Coagulopathy</th>
<th>Age ≤50 y (95% CI)</th>
<th>All Ages (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL</td>
<td>19.4% (7.4–36.9)</td>
<td>7% (5.2–9.1)</td>
</tr>
<tr>
<td>FVL/APCR</td>
<td>9.5% (6.1–13.4)</td>
<td>2.9% (1.5–4.3)</td>
</tr>
<tr>
<td>LA</td>
<td>5.2% (0–24)</td>
<td>8.7% (1.5–22.8)</td>
</tr>
<tr>
<td>Prothrombin mutation</td>
<td>4.3% (1.5–9.1)</td>
<td>2% (0.1–4.3)</td>
</tr>
</tbody>
</table>

ACL indicates anticardiolipin antibodies; FVL/APCR, factor V Leiden/APC-resistance; and LA, lupus anticoagulant.

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data do not support a causative role for IgA-aCL and IgG-aCL but neither do the data justify dismissing IgM-aCL as an independent risk factor.

On multivariate adjustment for hypertension, diabetes mellitus, current cigarette smoking, and smokeless tobacco, the odds ratio for future stroke associated with IgM-aCL decreased from 1.34 to 1.24 and was no longer statistically significant. However, the 95% confidence interval was fairly narrow (0.98 to 1.56). It is highly likely that a type II error could explain this loss of statistical significance. The authors gave no indication of the power of the study to detect the independence of aCL as risk factors for stroke. The effective sample size would also have decreased because of missing values. Data from Table 1 of the article suggests that several patients and controls had no data on the covariates included in multivariate logistic regression model on which the authors based their conclusion. For example, 11 patients and 15 controls had no data on hypertensive status.

Smoking was not an independent risk factor in this study, either. However, it would be imprudent to suggest on the basis of this study that smoking is not a risk factor for stroke. As the authors indicate, this is the only prospective study to report an association between aCL and stroke. Furthermore, few studies have examined the role of IgM-aCL. In light of this, the consequences of a misleading conclusion are important.

Some of the risk associated with IgM-aCL was explained by other classic risk factors included in the regression model, and the antibodies could be the result of endothelial cell damage induced by these factors. On the other hand, they may have a causal role, and this needs to be further investigated. IgM-aCL increased the risk of stroke by only 24% in this study. If this is a true indication of the strength of the association, then future studies will need larger sample sizes to be able to assess the independence of IgM-aCL as a stroke risk factor.

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Response

We examined antibodies against cardiolipin as predictive factor for future stroke in an incident case-referent study nested within the MONICA and Västerbotten cohort project. According to our results, IgM ACL were associated with future stroke but not independent from the other risk factors hypertension, diabetes mellitus, serum cholesterol, and smoking.

Sargeant and coworkers have questioned this conclusion. We do agree that the title of the paper is a little provocative, and also that the power of the study should preferably have been stronger. In our study, we analyzed samples drawn an average of 34.1 months before the incident. The relative risks we report may thus have underestimated the long-term risk for stroke in healthy individuals having ACL. Nevertheless, this is the first truly prospective study that has analyzed all 3 isotypes of ACL. A possible association to IgM ACL would have been missed by the only previous study on IgG antibodies in healthy individuals and future stroke.

It is of interest to note our previous study on antibodies against oxidatively modified LDL in the same patient population as in the present study: there was no association of these antibodies to future stroke. Our earlier study on antibodies against cardiolipin and oxidatively modified LDL as predictors for myocardial infarction showed that raised levels of these antibodies at 50 years of age correlated positively with the incidence and mortality related to myocardial infarction 10 to 20 years later. In contrast to our study of stroke, only IgG and IgA antibodies against cardiolipin were associated with myocardial infarction. The predictive power of IgA and IgG antibodies was strong and largely independent of that of other strong risk factors.

It should finally be noted that the population we studied comes from the northern part of Sweden, an area that differs in many respects from that of the rest of the Swedish Caucasian population. There are genetic differences and also marked environmental and lifestyle differences that might affect the results of our study.

We thus agree with Sargeant et al that larger epidemiological studies are warranted to clarify the issue on the predictive value of antibodies against cardiolipin for stroke. In addition, since these antibodies are clearly heterogeneous, functional studies of ACL of different isotypes and specificities are necessary to understand their effect on the vessel wall.

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Revisiting the Question, “Is the Acetazolamide Test Valid for Quantitative Assessment of Maximal Cerebral Autoregulatory Vasodilation?”

To the Editor:

The letter of Derdeyn in response to the article by Demolis et al discusses the evidence against the use of acetazolamide for maximal cerebrovascular dilatation. This is important because (1) compared with CO2, acetazolamide is safer and more easily administered in assessing cerebrovascular reserve (CVR), and (2) CVR assessed by quantitative cerebral blood flow (CBF) with acetazolamide is predictive of increased stroke risk. If CVR proves to be equivalent to oxygen extraction fraction (OEF) in detecting Powers’ stage II compromised CVR, it would be more readily available, more easily done, and done at lower cost than PET OEF.

Demosil et al studied “CBF” changes in rats subjected to changes in arterial blood pressure with and without acetazol-
CO₂ caused further cerebrovascular dilation beyond that caused by acetazolamide. That CO₂ causes further cerebrovascular dilation beyond that caused by acetazolamide is not surprising. Acetazolamide decreases tissue and extracellular fluid buffering capacity, thus enhancing the effectiveness of CO₂ in lowering extracellular fluid pH, which does not invalidate the use of acetazolamide in detecting stage II compromised CVR. There is more to cerebrovascular autoregulatory dilation than brain pH.

True to the title of their study, Demolis et al2 studied the effect of acetazolamide on cerebrovascular autoregulation. However, it is not a model to assess the validity of acetazolamide in detecting compromised CVR. Thus, the extension of this work to the clinical realm may be questionable, but Derdeyn expressed concern about even suggesting a value of acetazolamide-based studies.

Citing Hauge et al, Derdeyn states that the vasodilatory effects of acetazolamide are complex and very likely caused by mechanisms other than PCO₂-induced vasodilation. The basis for this suggestion is purely speculative. Hauge et al noted a rapid effect of acetazolamide in causing cerebrovascular dilation, the magnitude of which they estimated would require an increase in brain PCO₂ of 2 kPa (15 mm Hg), which was thought highly unlikely since arterial PCO₂ was unchanged. Arterial PCO₂ has no bearing on whether brain PCO₂ may be elevated.

Derdeyn cites the work of Inao et al5 and Kazumata et al6 as showing striking discrepancies in the comparison between acetazolamide and hypercapnia. Inao et al5 studied 6 patients with unilateral “steno-occlusive lesion” and PET cerebral blood flow (CBF) measured during primary sensorimotor cortex activation (PSM) by bilateral hand clamping and after acetazolamide. The authors reported that with PSM activation, rCBF increased in both PSM regions. In contrast, their Figure 1 and Table 3 show that acetazolamide increased CBF markedly in the contralateral, unaffected hemisphere without increasing CBF in the ipsilateral hemisphere, suggesting that the lack of an increase with acetazolamide in the compromised region was probably due to interhemispheric steal. The comparison of PSM and acetazolamide is invalid. Indeed, in an earlier study,7 the same group concluded that both CO₂ and acetazolamide were correlated in CVR assessment and that acetazolamide was preferred over CO₂.

The study by Kazumata et al6 reported that acetazolamide identified patients with compromised CVR who had good responses to hypercapnia. However, the administration of CO₂ caused a significant increase in arterial blood pressure despite the authors’ statement that they could not find a correlation between change of blood pressure and CO₂ reactivity. Our regression analysis of their data shows that the change in blood pressure correlated linearly with the change in PacO₂ (P=0.0128), and more importantly, that the change in blood pressure and the change in CBF was significant in the ipsilateral (P=0.037) but not in the contralateral (P=0.159) hemisphere. Thus, the discrepancy between hypercapnia and acetazolamide can be explained by the effect of hypercapnia on blood pressure. Furthermore, only 4 of the 11 patients would have been identified with compromised CVR by our previously published criteria.8

Derdeyn cited methodological flaws in the study by Yonas et al7 that we believe were not flaws. The use of a mixed patient population would be a flaw if one were to use the qualitative method of OEF estimation used by Grubb et al.9 It requires “normal” contralateral hemispheres and patients without bilateral carotid disease, but it is not a requirement in quantitative CBF measurement of CVR. Yonas et al retrospectively defined hemodynamic thresholds to identify normal and abnormal acetazolamide responses because it was a hypothesis building study. On the basis of this information, the study was subsequently extended in a publication by Webster et al.10 which again identified a high-risk subgroup and should logically be followed by a larger multicenter study to validate or establish the CVR thresholds.

Although Derdeyn cited the study by Yokota et al11 as also indicating the lack of utility of acetazolamide as a cerebrovascular challenge, this study was flawed. They used the ratio of the affected side to that of the “normal” side for an asymmetry index or (ΔAI) based on qualitative SPECT measurements. As shown by Yonas et al,12 a CBF ratio approach fails to identify 50% of the patients with compromised CVR. Use of an average ΔAI index from a normative curve for comparison is an inferior statistical design that eliminates the power of repeated measures analysis. Quantitative CBF assessment makes no assumptions and simply assesses compromised CVR for a given region in response to acidosis induced by acetazolamide.

Finally, the relationships between changes in CBF, OEF, CMRO₂, and cerebral blood volume, to which we would add CVR, as illustrated by Powers,3 is based on the normal brain, and even then partly on speculation. We know little about the changes in these variables in the hemodynamically stressed brain. Despite the belief of Derdeyn et al13 that only OEF provides a measure of stage II hemodynamic stress, the use of the interhemispheric relative OEF ratio provides a view of only a highly select subgroup of patients, which may not be generalizable to the greater universe of patients with symptomatic occlusive vascular disease.
Coagulopathies in Ischemic Stroke
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The online version of this article, along with updated information and services, is located on the World Wide Web at:
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