Peroxiredoxin 5 (PRX5) Is Correlated Inversely to Systemic Markers of Inflammation in Acute Stroke

Allison Kunze, BS; Dannielle Zierath, BS; Patricia Tanzi, BSN; Kevin Cain, PhD; Kyra Becker, MD

Background and Purpose—Peroxiredoxins are endogenous antioxidants that function as peroxide and peroxynitrite scavengers. Extracellular peroxiredoxins, however, are shown to initiate inflammation within the ischemic brain through activation of Toll-like receptors. Based on this observation, we hypothesized that plasma peroxiredoxin concentrations in ischemic stroke would correlate biomarkers of inflammation and predict poor outcome.

Methods—In a prospective study of patients with ischemic stroke, plasma peroxiredoxin 5 (PRX5) concentrations and inflammatory biomarkers at day 3 after stroke onset were correlated and the association between PRX5 at day 3 and outcome at 3 months assessed.

Results—PRX5 concentrations were available for 98 patients and were lower in those with more severe strokes ($P=0.001$). PRX5 was inversely correlated to biomarkers of inflammation at day 3 after stroke and did not predict 3-month outcome.

Conclusions—Plasma PRX5 is decreased in severe stroke and inversely correlated to biomarkers of systemic inflammation. These data suggest that PRX5 is not a proinflammatory mediator in acute stroke. Moreover, the inverse relationship between PRX5 and stroke severity suggests that PRX5 is either consumed or its production is impaired in severe stroke. Further study is needed to define the potential role of PRX5 in stroke. (Stroke. 2014;45:608-610.)

Key Words: inflammation ■ outcome measures ■ stroke

Peroxiredoxins are endogenous antioxidants that function as peroxide and peroxynitrite scavengers. There are 6 isoforms of peroxiredoxin and evidence suggests a protective role for peroxiredoxins in neurological diseases in which oxidative stress and inflammation are thought to contribute to pathology.1 A recent study by Shichita et al,2 however, found that extracellular peroxiredoxins initiate inflammation within the ischemic brain through activation of Toll-like receptor-2 and Toll-like receptor-4. Of the different isoforms of the peroxiredoxins, peroxiredoxin 5 (PRX5), in particular, seems to function as a danger signal to initiate inflammation.2 We previously showed that the endogenous danger signal high-mobility group box protein-1 was associated with circulating biomarkers of inflammation but was not independently predictive of stroke outcome.3 Based on the Shichita data, we hypothesized that plasma PRX5 concentrations in this same cohort of subjects with ischemic stroke would correlate with high-mobility group box protein-1 and other biomarkers of inflammation and that increases in PRX5 would be predictive of poor outcome.

Methods

The patient cohort has been described elsewhere.1 Briefly, patients with ischemic stroke admitted to Harborview Medical Center from September 2005 to May 2009 who were ≥18 years were enrolled within 72 hours of symptom onset. PRX5 concentrations were determined at day 3 in 98 of these patients. The study was approved by the institutional review board; all patients or their surrogates provided informed consent.

Clinical Data

Clinical and demographic data were collected on all patients. Stroke severity was determined by the National Institutes of Health Stroke Scale score at stroke onset and outcome by the modified Rankin Scale at 3 months.

Laboratory Studies

All laboratory tests (including PRX5 determinations) are from the same blood draw on day 3 after stroke onset. White blood cell count and differential, as well as the concentrations of high-sensitivity C reactive protein, were determined by the clinical laboratories using standard methodologies. Plasma concentrations of PRX5 were determined by enzyme linked immunoassay (USCN Life Science Inc); the sensitivity of the assay is 0.34 ng/mL. Interleukin (IL)-6, IL-10, IL-2, tumor necrosis factor-α, and IL-1 receptor antagonist were measured with a cytometric bead-based system (Fluorokine MAP; R&D Systems). The lower limits of detection were 1.1, 0.30, 2.23, 1.5, and 10.91 pg/mL, respectively. Plasma concentrations of high-mobility group box protein-1 were determined by enzyme linked immunoassay (IBL International); the sensitivity of the assay was 0.20 ng/mL.3

Statistics

Descriptive data are presented as median and interquartile range; group comparisons were performed using the Kruskal–Wallis H test. Correlations are presented using Spearman ρ. Logistic regression was used to assess the contribution of PRX5 to poor outcome (modified
Results

Details of the study design and patient characteristics are provided elsewhere. Patients from the parent study were divided into tertiles based on stroke severity; patients with more severe strokes had decreased concentrations of PRX5 in comparison with patients with less severe stroke (Table 1). In contrary to our initial hypothesis, plasma PRX5 was correlated inversely with multiple markers of systemic inflammation (Table 2). Logistic regression, controlled for known predictors of stroke outcome, showed no effect of PRX5 at day 3 after stroke onset on 3-month outcome (Table 3).

Discussion

The peroxiredoxins are a family of endogenous antioxidants that are capable of sensing redox states and scavenging peroxides. Intracellular peroxiredoxins are thought to protect against diseases characterized by oxidative stress. Despite evidence for a proinflammatory role of extracellular PRX5 in an animal model of stroke, our data show that systemic PRX5 is correlated inversely with biomarkers of inflammation. Our data, however, do not address the local actions of extracellular PRX5 within the brain. Furthermore, the source of PRX5 detected in the peripheral circulation in this study is unknown; PRX5 in the periphery may reflect the contents of necrotic cells, the results of active secretion by healthy cells, or a combination of both.

The decrease in PRX5 in subjects with severe stroke suggests that either PRX5 is consumed in proportion to stroke severity (and hence the degree of oxidative stress) or it is not produced in the setting of severe stroke. Uric acid is another endogenous antioxidant that is capable of stimulating inflammation through activation of Toll-like receptors. Despite the potential for uric acid to initiate the innate immune response, serum uric acid levels are correlated inversely to stroke severity in most studies and higher concentrations independently predict better outcome—especially in those who are treated with thrombolytics. It is possible that PRX5 behaves similarly to uric acid in stroke; it is also that the effects of intra- and extracellular uric acid, peroxiredoxins, and other endogenous antioxidants differ; and although we did not find peroxiredoxin to be predictive of stroke outcome in this study, power was limited by the relatively small sample size. Given the conflicting data about the role of endogenous antioxidants such as peroxiredoxin and uric acid, as both initiators of inflammation and as potent antioxidants, more data are needed on their role in acute stroke. Furthermore, it is unclear whether the inverse relationship between PRX5 and systemic inflammation is merely an association or whether PRX5 is acting as an immunomodulator. Given that inflammation after stroke is presumed to be detrimental, it is possible that PRX5 may represent a unique stroke therapeutic with both antioxidant and anti-inflammatory properties. The timing of immunomodulatory therapies, however, seems to be critical, as the immune response also seems to be important in the repair/recovery process.

Acknowledgments

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Sources of Funding

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Disclosures

None.

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**Table 1. Plasma Concentration of PRX5 (ng/mL) 3 Days After Stroke Onset**

<table>
<thead>
<tr>
<th>NIHSS≤5 (n=37)</th>
<th>NIHSS 6–16 (n=31)</th>
<th>NIHSS≥17 (n=30)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.8 (40.5, 76.3)</td>
<td>48.0 (36.5, 88.6)</td>
<td>37.6 (28.5, 45.3)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

NIHSS indicates National Institutes of Health Stroke Scale; and PRX5, peroxiredoxin 5.

**Table 2. Correlations Between PRX5 and Biomarkers of Inflammation at 3 Days After Stroke Onset**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spearman ρ</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-mobility group box protein 1</td>
<td>-0.14</td>
<td>P=0.17</td>
</tr>
<tr>
<td>White blood cells</td>
<td>-0.38</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>-0.41</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.09</td>
<td>P&gt;0.20</td>
</tr>
<tr>
<td>Monocytes</td>
<td>-0.36</td>
<td>P=0.001</td>
</tr>
<tr>
<td>C-Reactive protein</td>
<td>-0.26</td>
<td>P=0.02</td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td>-0.25</td>
<td>P=0.02</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>-0.28</td>
<td>P=0.006</td>
</tr>
<tr>
<td>Interleukin-10</td>
<td>-0.33</td>
<td>P=0.006</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>-0.35</td>
<td>P=0.001</td>
</tr>
<tr>
<td>Interleukin-1 receptor antagonist</td>
<td>-0.17</td>
<td>P=0.20</td>
</tr>
</tbody>
</table>

PRX5 indicates peroxiredoxin 5.

**Table 3. Plasma PRX5 at Day 3 After Stroke Does Not Predict Poor Outcome (Modified Rankin Scale>3) at 3 Months**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRX5 (per 10 ng/mL)</td>
<td>0.77 (0.57–1.03)</td>
<td>0.08</td>
</tr>
<tr>
<td>PRX5 (per 10 ng/mL)</td>
<td>0.93 (0.71–1.21)</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>PRX5 (per 10 ng/mL)</td>
<td>0.94 (0.74–1.21)</td>
<td>&gt;0.20</td>
</tr>
</tbody>
</table>

NIHSS indicates National Institutes of Health Stroke Scale; OR, odds ratio; and PRX5, peroxiredoxin 5.
References
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