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A hypercoagulable state in activated protein C resistant patients with ischemic stroke

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Dahlback et al. [1] have demonstrated a new mechanism of hereditary thrombophilia characterized by a poor response to activated protein C (APC). This alteration, named APC resistance, is due in most cases to a factor V (FV) gene defect in exon 10, changing Arg 506 to Gln [2]. APC resistance is a strong and common risk factor for venous thrombosis. Heterozygosity for the FV gene defect is associated with a five- to ten-fold increased risk for thrombosis, whereas homozygosity is associated with a 50- to 100-fold increased risk [3]. The association of APC resistance and arterial thrombosis is controversial, and little is known about the frequency of APC resistance in cerebrovascular arterial thromboses. Therefore we investigated the prevalence of APC resistance in patients with previous ischemic stroke.

We studied 50 consecutive unrelated patients with a diagnosis of ischemic stroke (38 males, 12 females, mean age±SD=65±13 years). All patients satisfied the World Health Organization definition for the diagnosis of ischemic stroke. The cerebral ischemic episode occurred in all subjects 12–15 months before the study. One hundred healthy subjects matched for sex and age served as a control group. None of the patients and controls was on oral anticoagulant treatment or contraceptive pills.

Routine coagulation tests (PT) prothrombin time, (APTT) activated partial thromboplastin time, (Fg) fibrinogen were performed by standard laboratory techniques. Natural anticoagulants antithrombin III (ATIII), protein C (PC), total and free protein S (PS), fibrinolytic proteins (plasminogen activator inhibitor-1, tissue-type plasminogen activator), and activation peptides (F1+2, D-dimer) were assayed by commercial kits (Behringwerke AG, Marburg, Germany). The sensitivity of plasma to APC was measured by a modified APTT, as previously described [4], and expressed as normalized APC ratio [2]. All patients and healthy subjects found to be resistant to APC by the functional assay were tested for the R506Q mutation in the FV gene as previously described by Bertina et al. [2].

In stroke patients, coagulation screening, inhibitory anticoagulant proteins, and fibrinolytic proteins were not significantly different from controls (data not shown). However, the number of subjects with PC or PS deficiency was significantly higher in patients than in controls (4% vs. 1%, P<0.001 and 6% vs. 1%, P<0.0001, respectively, Fisher exact test). Increased plasma levels of Fg and D-dimer were found in stroke patients compared with healthy subjects (Table 1). An APC ratio lower than 0.84 (i.e., a value indicating a resistance to the anticoagulant effect of APC) was detected in 11 of 50 patients (22%) (Table 1). A MnlI restriction profile of the resistant patients revealed that 9 patients were heterozygotes, while 2 patients, with the lowest APC ratios, were homozygotes for the FV R506Q mutation. In the control group, 2 of 100 volunteers (2%) had a normalized APC sensitivity ratio below 0.84 and were homozygous for the R506Q mutation. The plasma concentration of F1+2, a marker of prothrombin activation, was significantly higher in patients with ischemic stroke (Table 1). Notably the highest levels of F1+2 were found in patients with the FV R506Q mutation (1.55±0.25 nM vs. 1.05±0.35 nM) (Fig. 1).

In agreement with Halbmayer et al. [5], our data suggest an increased prevalence of APC resistance, due to the
Fig. 1 Plasma F1+2 levels in healthy subjects, in patients without activated protein C (APC) resistance, and in APC-resistant patients. Data are mean±SD. Statistical significance was assessed by the Mann-Whitney test.
* P<0.001 vs. controls.
* * P<0.001 vs. patients without APC resistance (FV factor V)

Table 1 Selected laboratory data in patients with ischemic stroke and in healthy subjects (APC activated protein C)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=100)</th>
<th>Patients (n=50)</th>
<th>Statistical significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>244.9±53.3</td>
<td>335.0±65.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>D-dimers (ng/ml)</td>
<td>153±65</td>
<td>275±45</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>F1+2 (nmol/l)</td>
<td>0.45±0.24</td>
<td>1.01±0.56 (32)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Normalized APC ratio</td>
<td>1.07±0.15 (2)</td>
<td>0.88±0.15 (11)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

a Values represent mean±SD
b The number of patients and controls with abnormal results are in parentheses

R506Q mutation in FV, in cerebrovascular ischemic disease. The observation that the extent of in vivo blood clotting activation, as assessed by F1+2 determination, is higher in patients carrying this defect than patients with a normal APC response, indicates that APC resistance may contribute to the hypercoagulable state in this clinical condition.

References
3. Dahlback B. Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in the factor V gene. Haemostasis 1994; 24: 139.