ticlopidine-induced endothelial cell apoptosis is a provoking factor primary human microvascular endothelial cells, suggesting that exposure to both the pharmacologic levels of ticlopidine and

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Acknowledgment: This work was supported by a grant from Deutsche Krebshilfe, Dr Mildred Scheel Stiftung 12-2191 (K.H., H.K.).

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References


To the editor:

Complement factor H mutations are present in ADAMTS13-deficient, ticlopidine-associated thrombotic microangiopathies

The antiplatelet agent ticlopidine is associated with the rapid onset of a thrombotic microangiopathy (TMA) resembling thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic-uremic syndrome (aHUS). These disorders occur in 0.06% of individuals exposed to ticlopidine, usually within 1.5 to 6 weeks of exposure.\(^1\)\(^,\)\(^2\) The vast majority of ticlopidine TMAs are accompanied by antibody-mediated inhibition of the protease ADAMTS13, making them similar to idiopathic TTP.\(^3\)\(^,\)\(^4\) However, ADAMTS13 deficiency alone is not sufficient for the development of TMAs. Our group has shown that exposure to both the pharmacologic levels of ticlopidine and plasma from patients with ticlopidine TMAs induces apoptosis in primary human microvascular endothelial cells, suggesting that ticlopidine-induced endothelial cell apoptosis is a provoking factor in ticlopidine TMAs.\(^5\) In other TMAs such as aHUS, complement regulatory protein mutations represent another provoking factor for the development of these diseases.\(^6\) Complement mutations have not been studied in ticlopidine TMAs.

We obtained plasma samples from 4 consecutive patients with TMAs that occurred within 2.5 to 4 weeks of ticlopidine exposure (Table 1).\(^1\) ADAMTS13 activity and inhibitor titers were determined, as previously described by Bennett et al.\(^5\) All patients had thrombocytopenia, schistocytosis, markedly elevated levels of lactate dehydrogenase (LDH), renal impairment, and significantly decreased ADAMTS13 activity. Three of 4 had ADAMTS13 inhibitors. Plasma levels of C5a and C5b-9 (membrane attack complex) were measured by enzyme-linked immunosorbent assay.

Table 1. Clinical characteristics of 4 patients with ticlopidine TMAs

<table>
<thead>
<tr>
<th>Patient code</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Duration of ticlopidine (wk)</th>
<th>Creatinine (µmol/L)</th>
<th>Platelets pretherapy (x10^9)</th>
<th>Platelets posttherapy (x10^9)</th>
<th>LDH pretherapy (U/L)</th>
<th>LDH posttherapy (U/L)</th>
<th>Outcome</th>
<th>PEX sessions (N)</th>
<th>ADAMTS13 Activity (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>010</td>
<td>84</td>
<td>M</td>
<td>3</td>
<td>110</td>
<td>40</td>
<td>235</td>
<td>2555</td>
<td>NA</td>
<td>Death</td>
<td>8</td>
<td>&lt;5</td>
</tr>
<tr>
<td>022</td>
<td>77</td>
<td>M</td>
<td>4</td>
<td>150</td>
<td>5</td>
<td>131</td>
<td>1084</td>
<td>214</td>
<td>Survival</td>
<td>10</td>
<td>&lt;5</td>
</tr>
<tr>
<td>003</td>
<td>78</td>
<td>F</td>
<td>3.5</td>
<td>260</td>
<td>33</td>
<td>93</td>
<td>1006</td>
<td>1736</td>
<td>Death</td>
<td>3</td>
<td>&lt;5</td>
</tr>
<tr>
<td>012</td>
<td>42</td>
<td>F</td>
<td>2.5</td>
<td>110</td>
<td>13</td>
<td>323</td>
<td>790</td>
<td>170</td>
<td>Survival</td>
<td>30</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

Platelet values expressed as \(x10^9\).

F, female; M, male; NA, not available; PEX, plasma exchange.

*ADAMTS13 was assessed by both FRET-VWF assay (<5%) and immunoblot activity (<10%).
Table 2. Complement levels of 4 patients with ticlopidine TMAs and corresponding mutations

<table>
<thead>
<tr>
<th>Patient code</th>
<th>CSa (ng/mL) (normal range, 0.3-70)</th>
<th>sCSb-9 (ng/mL) (normal range, 100-300)</th>
<th>CFH polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>010</td>
<td>32.08</td>
<td>4862</td>
<td>Homozygous exon</td>
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<tr>
<td>022</td>
<td>51.21</td>
<td>6023</td>
<td>Heterozygous exon</td>
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<tr>
<td>003</td>
<td>27.74</td>
<td>5004</td>
<td>Heterozygous exon</td>
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<tr>
<td>012</td>
<td>50.96</td>
<td>6229</td>
<td>Heterozygous exon</td>
</tr>
</tbody>
</table>

Primer sequences used are as follows: forward primer for the first step: TAGACAGACAGACACCAGAAGG; reverse primer for the first step: AATTTATGAGTTAGTGAAACCTGAAT; forward primer for the second step: GGTACCACTTACACTTTG; reverse primer for the second step: GGTACCACTTACACTTTGAATGAAGA; forward primer for exon 18: TAGACAGACAGACACCAGAAGG; reverse primer for exon 18: AATTTATGAGTTAGTGAAACCTGAAT.

CFH, complement factor H.

(Quidel). Genomic DNA was isolated from plasma using the QIAamp kit (QIagen). To determine the presence of complement mutations, we selected primers to soluble complement factor H (CFH), complement factor I, and membrane-linked membrane cofactor protein, 3 complement regulatory factors with mutations that have been identified with high frequency in aHUS.4,5 We found substantially elevated levels of membrane attack complex despite normal C5a levels, and all 4 patients had CFH genetic abnormalities (Table 2). Two of the 3 polymorphisms are of known functional normal C5a levels, and all 4 patients had CFH genetic abnormalities substantially elevated levels of membrane attack complex despite developing aHUS.7,9 In conclusion, complement regulatory protein mutations may form the basis for TMA susceptibility and should be further studied.

References

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Contribution: J.C. performed DNA extraction and collected clinical information; H.-M.T. performed ADAMTS13 testing; S.E. and R.S. performed semi-nested PCR on all DNA samples; J.L. and J.C. analyzed the data and authored the manuscript; and all authors contributed equally to the editing and revision of this work.

Conflict-of-interest disclosure: The authors declare no competing financial interests.
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