Age-Related Deficiency of the Synthesis of Platelet Activating Factor by Leukocytes From Zellweger Patients


Ca²⁺-ionophore A23187-induced synthesis of the alkoxyether lipid platelet activating factor (PAF) by leukocytes from Zellweger patients was undetectable in two patients studied at 3 and 4 weeks of age, reduced in a third patient studied at 2 months of age, and in the low normal range in four patients studied between 4 months and 5 years of age. We have previously reported that plasmalogen-type phosphatidylethanolamine (PE) levels of erythrocytes are reduced in Zellweger patients up to 20 weeks of age, but normal in older patients. These levels were reduced in the three patients with abnormal PAF synthesis, and normal in the other four patients. The results suggest a close relationship between the age of the patients at sampling, and both the A23187-induced capacity of leukocytes to synthesize PAF and the plasmalogen PE levels in their erythrocytes. © 1987 by Grune & Stratton, Inc.

The cerebro-hepato-renal syndrome of Zellweger is a rare disease with an autosomal recessive mode of inheritance. It is characterized clinically by a typical craniofacial dysmorphism, hepatomegaly, severe hypotonia and psychomotor retardation, failure to thrive, and usually an early death as 80% of the patients die in the first 6 months of life. In this syndrome disturbances of bile acid biosynthesis, very-long-chain fatty acid oxidation, and alkoxyether lipid biosynthesis have been reported, which may all be caused by the absence of ultrastructurally detectable peroxisomes in tissues of these patients. For instance, the activities of dihydroxyacetone phosphate acyltransferase (DHAP-AT) and alkyl-DHAP synthase, two enzymes involved in the biosynthesis of alkoxyether lipids, and shown to be localized in (micro)peroxisomes of rodent brain and liver, were severely reduced in liver, brain, and fibroblasts of Zellweger patients. In accordance, plasmalogen-type alkoxyether lipids were shown to be virtually absent in several tissues of Zellweger patients, and reduced in their erythrocytes and fibroblasts. However, the phosphatidylethanolamine (PE) plasmalogen content of erythrocytes of Zellweger patients was subsequently found to be reduced in patients less than 20 weeks of age, but normal in older Zellweger patients.

The alkoxyether lipid platelet activating factor (PAF), 1-0-alkyl-2-acetyl-sn-glycerol-3-phosphocholine, is synthesized by several cell types, including endothelial cells, leukocytes, and platelets. It induces both platelet and leukocyte aggregation and degranulation, and is implicated in several pathological processes, such as inflammation, asthma, anaphylaxis, hypotension, and bacterial endotoxin-induced hypotension and thrombocytopenia. Cells and tissues with a deficient PAF synthesis would provide a useful tool to study the mechanisms involved in PAF-mediated processes in vitro. In the present study we provide evidence that the Ca²⁺-ionophore A23187-induced PAF synthesis by leukocytes of Zellweger patients may be an age-related deficiency similar to the plasmalogen content of their erythrocytes.

Materials and Methods

Patients. All patients demonstrated typical clinical and biochemical features of the Zellweger syndrome (Table 1). Informed consent was obtained from their parents and the study was approved by the medical ethical committee of the Academic Medical Center.

Methods. Leukocytes enriched in polymorphonuclear leukocytes (PMN) were isolated, and PAF synthesis measured as described previously. Briefly, 0.4 ml of I x 10⁷ leukocytes/mL in a Tris-Tyrode buffer (137 mmol/L NaCl, 2.6 mmol/L KCl, 0.36 mmol/L NaH₂PO₄, 5.5 mmol/L glucose, 2.0 mmol/L CaCl₂, 1.0 mmol/L MgCl₂, 10 mmol/L Tris, 0.25% albumin, pH 7.35) were stimulated for 45 minutes with 10 μmol/L A23187. PAF was isolated by extraction with ethanol and chloroform, further purified by thin layer chromatography, and quantified by aggregation measurements of gel-filtered rabbit platelets.

The isolation of erythrocytes and determination of their plasmalogen content, and the measurements of DHAP-AT activity, plasmalogen content, percentage of particle-bound catalase, and de novo plasmalogen biosynthesis of cultured skin fibroblasts were performed as described before.

Results

The results of the analyses of cells of the Zellweger patients are presented in Table 2. The analyses of fibroblast parameters, i.e., DHAP-AT activity, catalase latency test, PE plasmalogen content (with the exception of the oldest patient tested), and de novo phosphatidylcholine (PC) and PE plasmalogen synthesis, were abnormally reduced in all patients tested and thus confirmed the diagnosis of Zellweger syndrome in patients no. 1 and 3 through 7. The diagnosis in patient no. 2 was confirmed by DHAP-AT measurement of the thrombocytes (patient no. 2, 0.95; eight controls 1.97 to 3.41 nmol · 30 minutes⁻¹ · mg protein⁻¹). PAF synthesis was undetectable in leukocytes from two patients studied at 23 and 29 days of age and reduced in a third patient studied at 62 days of age. In fact, in this third patient PAF synthesis by leukocytes collected at 58 days of age could not be demonstrated. The PAF synthesis was in the
AGE-RELATED DEFICIENCY OF THE SYNTHESIS OF PAF

Table 1. Clinical and Biochemical Characteristics of the Zellweger Patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craniofacial dysmorphia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tapetoretinal degeneration/cataract</td>
<td>nd</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hepatomegaly/liver dysfunction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Psychomotoric retardation</td>
<td>+</td>
<td>nd</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Picrocric acid in serum and/or urine</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>T- and DHCA in serum and/or urine</td>
<td>↑</td>
<td>nd</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>p-OH-phenylactate</td>
<td>↑</td>
<td>↑</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Cysts in kidney</td>
<td>+</td>
<td>nd</td>
<td>+</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

Cause of death

B U P SA U P SA

Abbreviations: +, present; -, absent; nd, not determined; ↑, increased; SA, still alive; P, pneumonia; B, brain hemorrhage; U, unknown; T- and DHCA, tri- and dihydroxycoprostanic acid.

Patient demonstrated hypotonia.

low normal range in four patients tested between 4 and 61 months of age. The three patients with the abnormal PAF synthesis died before 3 months of age, and the other patients were still alive after 32 months.

The patients with abnormal PAF synthesis also showed a significantly reduced PE plasmalogen content of the erythrocytes, whereas this parameter was virtually normal in the other patients.

DISCUSSION

The first steps of the de novo alkoxyether lipid biosynthesis, which are dependent on DHAP-AT and alkyl-DHAP synthase, are localized in the peroxisome.78 Subsequently, in several extra-peroxisomal steps 1-0-alkyl-2-acylglycerol-3-phosphocholine and 1-0-alkyl-2-acylglycerol-3-phosphoethanolamine are formed. These molecules may finally be oxidized to the 1-0-alkenyl compounds, ie, plasmalogens. However, 1-0-alkyl-2-acylglycerol-3-phosphocholine may also be stored in the cellular membrane and, upon cell stimulation, converted into the 2-acyl compound, PAF.14

The impairment of de novo alkoxyether lipid synthesis in Zellweger patients is clearly established, ie, DHAP-AT and alkyl-DHAP synthase activities as well as plasmalogen content and de novo plasmalogen synthesis33 are reduced or virtually absent in several tissues and cells of these patients.914 However, evidence was put forward that the plasmalogen content of erythrocytes of these patients reaches normal levels if blood is collected after 3 to 5 months of age.15 In the present study we have provided evidence that deficient PAF synthesis by the leukocytes of Zellweger patients may also be an age-related phenomenon limited to the first 3 months of life. Several explanations for these findings are possible. First, with age the patients may partly overcome the deficient alkoxyether lipid synthesis. Plasmalogens, DHAP-AT, and alkyl-DHAP synthase activities are never completely absent in tissues of Zellweger patients,14 and a residual capacity for de novo plasmalogen biosynthesis is clearly present in their cultured skin fibroblasts.33 These residual enzyme activities may be due either to the presence of a few microperoxisomal structures,35 or to a reduced activity of peroxisomal enzymes that are synthesized on free polyribosomes and remain in the cytosol instead of being incorporated in pre-existing peroxisomes.34,37 A possible mechanism for the presumed improvement in PAF synthesis and erythrocyte plasmalogen content might be the positive selection with age of progenitor cells in the bone marrow with slightly more residual alkoxyether lipid synthesis capacity. A second possible explanation for the age-related PAF synthe-

Table 2. Alkoxy Ether Lipid Parameters in the Zellweger Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Age Controls</th>
<th>Adult Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at sampling</td>
<td>23 d</td>
<td>39 d</td>
<td>62 d</td>
<td>48 mo</td>
<td>30 mo</td>
<td>54 mo</td>
<td>61 mo</td>
<td>&lt;3 mo</td>
<td>—</td>
</tr>
<tr>
<td>Age at death</td>
<td>50 d</td>
<td>33 d</td>
<td>91 d</td>
<td>&gt;38 mo</td>
<td>&gt;60 mo</td>
<td>&gt;60 mo</td>
<td>&gt;60 mo</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PAF synthesis</td>
<td>&lt;2</td>
<td>&lt;3</td>
<td>731</td>
<td>232</td>
<td>273</td>
<td>572</td>
<td>249-2757 (n = 4)</td>
<td>291-5433 (n = 5)</td>
<td></td>
</tr>
<tr>
<td>Erythrocyte PLG*</td>
<td>2.38</td>
<td>21.0</td>
<td>4.8</td>
<td>41.8</td>
<td>34.7</td>
<td>57.3</td>
<td>41.0</td>
<td>37.6-47.5 (n = 9)</td>
<td>36.9-53.1 (n = 66)</td>
</tr>
<tr>
<td>Fibroblast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHAP-AT§</td>
<td>0.38</td>
<td>—</td>
<td>0.81</td>
<td>0.97</td>
<td>0.34</td>
<td>0.88</td>
<td>0.48</td>
<td>—</td>
<td>5.4-19.8 (n = 27)</td>
</tr>
<tr>
<td>Catalase latency</td>
<td></td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>—</td>
<td>—</td>
<td>50-81 (n = 9)</td>
</tr>
<tr>
<td>PLG†</td>
<td>26.4</td>
<td>—</td>
<td>21.7</td>
<td>34.4</td>
<td>31.6</td>
<td>26.1</td>
<td>48.6</td>
<td>—</td>
<td>46.4-62.4 (n = 13)</td>
</tr>
<tr>
<td>De novo synthesis A‡</td>
<td>2.2</td>
<td>—</td>
<td>0.8</td>
<td>1.2</td>
<td>1.4</td>
<td>2.9</td>
<td>1.7</td>
<td>—</td>
<td>13.6-30.4 (n = 19)</td>
</tr>
<tr>
<td>De novo synthesis B‡</td>
<td>57.9</td>
<td>—</td>
<td>26.9</td>
<td>49.5</td>
<td>40.4</td>
<td>72.1</td>
<td>55.0</td>
<td>—</td>
<td>84.3-93.4 (n = 19)</td>
</tr>
</tbody>
</table>

*PAF synthesis is expressed as picomoles PAF/10⁶ PMN.† The plasmalogen content (PLG) is expressed as the percent plasmalogen of PE relative to the total PE.‡Blood for erythrocyte PLG collected at 42 days of age. §Fibroblast DHAP-AT activity is expressed as nanomoles · 2 h⁻¹ · mg protein⁻¹. ||Catalase latency of fibroblasts is expressed as the percent particle-bound catalase.‡De novo plasmalogen synthesis of the fibroblasts is expressed as the percent [¹⁴C]-hexadecanol uptake in plasmalogen of either PC relative to the incorporation in the total PC (A), or PE relative to the incorporation in the total PE (B).
sis deficiency would be the cellular uptake of alkoxyether lipids or precursors from the diet, but this possibility is less likely as the plasmalogen content of erythrocytes of patients suffering from the rhizomelic type of chondrodysplasia punctata remains severely reduced even up to 16 years of age. However, a third explanation cannot be excluded: Possibly the PAF synthesis in the patients tested at 4 months to 5 years of age has also been normal earlier in life. A PAF synthesis deficiency would then be present only in the more severely affected patients who die within a few months of age. Unfortunately, this possibility could not be tested as the older patients were not investigated at an early age, and the patients with the deficient PAF synthesis died before 3 months of age.

PAF has been ascribed an important role in inflammation. Interestingly, Zellweger patients frequently suffer from pneumonia and it is a frequent cause of their death. Of course, severe neurological impairment, malfunction of several organs, and possibly the reduced cellular plasmalogen content may cause the frequent pneumonia, but it is tempting to speculate that the deficient PAF synthesis may also be a pathogenetic factor.

In summary, we conclude that the deficiency of PAF synthesis by leucocytes of Zellweger patients may be an age-related phenomenon, and this deficiency may be of etiological importance for some of the complications in these patients. However, a relation of the deficient PAF synthesis and the severity of the disease cannot be excluded.

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