Diagnosis of Peroxisomal Disorders by Analysis of Phytanic and Pristanic Acids in Stored Blood Spots Collected at Neonatal Screening

Herman J. ten Brink,1,4 Cornelia M. M. van den Heuvel,1 Ernst Christensen,2 Claude Largillière,3 and Cornels Jakobs1

Concentrations of phytanic acid and pristanic acid were measured in stored dried blood spots collected at neonatal screening from patients with peroxisomal disorders, and compared with concentrations in control blood spots. In blood spots from two patients with Zellweger syndrome both phytanic acid and pristanic acid concentrations were increased but their concentration ratio was normal. In the blood spot from a patient with rhizomelic chondrodysplasia punctata, the concentration of phytanic acid was increased, whereas pristanic acid was within the control range, resulting in a low pristanic acid/phytanic acid ratio. In the blood spot from a patient with X-linked adrenoleukodystrophy, the concentrations of the acids and their ratio were normal. These findings are consistent with results for these acids in plasma from such patients. Measurement of phytanic acid and pristanic acid and their ratios in stored dried blood collected at neonatal screening can therefore be used in the diagnosis of peroxisomal disorders, especially for those cases in which, owing to early death of the patient, no other material is available for biochemical investigations.

Indexing Terms: pediatric chemistry · heritable disorders · mass spectroscopy

Peroxisomes play an important role in several metabolic pathways such as the synthesis of ether phospholipids (plasmalogens) and bile acids, and the catabolism of very-long-chain fatty acids (VLCFA), phytanic and pristanic acids, glyoxalic acid, and pipecolic acid (see I, 2 for reviews).5 Defects in these pathways usually result in the accumulation in tissues and body fluids of one, a few, or all of the pathway-related metabolites, according to the number of functional disturbances. These accumulations are used for (differential) biochemical diagnosis of the so-called peroxisomal disorders, a new category of metabolic diseases involving absence or dysfunction of peroxisomes. Three different groups are recognized, according to the number of peroxisomal functions involved: disorders with: (a) general loss of peroxisomal functions—Zellweger syndrome, neonatal adrenoleukodystrophy (NALD), infantile Refsum’s disease, hyperpipolic acidemia; (b) loss of multiple peroxisomal functions—rhizomelic chondrodysplasia punctata (RCDP), Zellweger-like syndrome; and (c) loss of a single peroxisomal function—X-linked adrenoleukodystrophy (X-ALD), acyl-CoA oxidase deficiency, bifunctional protein deficiency, 3-oxoacyl-CoA thiolase deficiency, hyperoxaluria type I, acatalasemia.

Except for hyperoxaluria type I and acatalasemia, the clinical features of all peroxisomal disorders include severe neurological dysfunction. Most peroxisomal disorders are further clinically characterized by a variable degree of cranial facial dysmorphism, severe psychomotor retardation, eye dysfunction, and hearing loss presenting in the first year postpartum. Severe hypotonia is present early in life, with the exception of RCDP (see 3, 4 for reviews).

Children with the more severe forms of peroxisomal dysfunction (Zellweger syndrome, Zellweger-like syndrome, bifunctional protein deficiency, and peroxisomal 3-oxoacyl-CoA thiolase deficiency) rarely survive beyond the first year (3, 4). Because these disorders are rare, they may be overlooked because of the pediatrician’s unfamiliarity with them. Consequently, biochemical investigations may not be performed before the death of the child and, therefore, no diagnosis is available. Sometimes the suspicion of a peroxisomal disorder is raised later, during counseling in the next pregnancy, when the presence or absence of a peroxisomal defect in the index case needs to be confirmed or excluded. In such a case the only material left for investigations may be the blood spot collected at the neonatal screening for phenylketonuria.

Plasma analysis of phytanic acid and pristanic acid (5), next to VLCFA (6) and bile acids (7), is an important component in the biochemical diagnosis of peroxisomal disorders; especially for RCDP there is no alternative, because VLCFA and bile acids do not accumulate in such patients. Analyses of bile acids (8), plasmalogens (8), and VLCFA (9) in stored dried blood collected at neonatal screening are already being applied to the diagnosis of Zellweger syndrome. We performed this study to investigate whether concentrations of phytanic acid and pristanic acid in stored dried blood samples collected at neonatal screening could be used in the diagnosis of peroxisomal disorders, notably RCDP.

Materials and Methods

Filter-paper blood spots from the patients were collected in 1981 (X-ALD patient), in 1987 (RCDP patient),
and in 1989 (Zellweger patient 1) according to the Danish routine neonatal screening program (disk size: 14 mm diameter); a blood spot from a second Zellweger patient was collected in 1989 according to the French program (disk size: 10 mm diameter). Control blood samples from healthy children were available from 1981 and 1987 collected in the Danish program. Diagnosis of peroxisomal disease in the patients had been previously established based upon clinical symptomatology and was confirmed biochemically.

Disks of the filter paper containing blood spots from the patients and the controls (corresponding to 25–50 μL of blood, depending on the disk sizes on the filter paper) were cut along the black circle and soaked in water (600 μL). After shaking the samples for 20 min, we added [3-methyl-2H3]phytanic acid (2.0 nmol) and [2-methyl-2H3]pristane acid (0.2 nmol) as internal standards (10). The sample preparation procedure thereafter and the stable isotope dilution mass fragmentographic analysis were according to ten Brink et al. (5). A calibration curve was established by cutting out disks of the same size from the same filter paper and adding authentic phytanic acid and pristanic acid in increasing amounts. These disks followed the same preparation and analytical procedure as described for blood-soaked disks. Linear regression analysis was used to calculate pristane acid and phytanic acid concentrations, which were expressed in nanomoles per blood spot.

Results

The calibration curves passed through the origin, indicating that neither phytanic acid nor pristanic acid was present in the filter paper. The concentrations of pristane and phytanic acids and their ratios in blood spots from controls and patients are shown in Table 1. There was no significant difference in the control ranges for pristane acid and phytanic acid nor in the ratios derived from the blood spots collected in 1981 and 1987, indicating a good stability of these acids during storage. From this we conclude that these control ranges can be used to compare the concentrations determined in the Zellweger patients' blood spots, which were collected in 1989.

The blood spots from the Zellweger patients showed increased concentrations of both phytanic and pristanic acid, but with ratios within the control range. (We point out that the disk size from the French paper is smaller than that from the Danish paper.)

In the blood spot from the RCDP patient, the concentration of phytanic acid was increased and the pristanic acid concentration was within the control range, giving a low pristanic acid/phytanic acid concentration ratio.

Phytanic acid and pristanic acid concentrations in the blood spot from the X-ALD patient were both within the control range, as was the pristanic acid/phytanic acid concentration ratio.

Discussion

Apparently, phytanic acid and pristanic acid concentrations—as well as plasmalogen, bile acid, and VLCFA concentrations—in blood spots collected at neonatal screening are diagnostic. The Zellweger syndrome is the prototype of the generalized peroxisomal disorders. Because of the absence of peroxisomes, all biochemical pathways that involve these organelles are disturbed. This disturbance is also reflected in the defective phytanic acid α-oxidation and pristanic acid β-oxidation, leading to accumulation of both acids in tissues and body fluids; however, the plasma concentration ratios are similar to those in control plasma (5). Phytanic acid in humans is exogenous and, in control subjects younger than 2 years, plasma values are age dependent (5). In plasma from Zellweger patients the accumulation of phytanic acid also is age dependent, and early diagnosed patients do not always show already increased plasma concentrations of phytanic acid (11). It is therefore surprising to find the clearly above-normal values in the blood spots collected between days 6 and 10 postpartum. We cannot exclude that part of the phytanic acid measured originates from blood cells.

In RCDP the biochemical defect is restricted to the phytanic acid α-oxidation, de novo plasmalogen biosynthesis, and peroxisomal 3-oxoacyl-CoA thiolase appearing in an unprocessed form (2, 12). Because the metabolism of VLCFA and bile acids is not disturbed in this disorder, the determination of phytanic and pristanic acids, and possibly of plasmalogens, is of great importance. In RCDP the impairment of phytanic acid α-oxidation is more severe than in the Zellweger syndrome, and higher plasma concentrations of phytanic acid are measured at an earlier stage of life. This is also reflected in the finding of a higher phytanic acid concentration in the blood spot from the RCDP patient than in those from the patients with Zellweger syndrome. As with the results obtained for plasma from RCDP patients (5), the low pristanic acid/phytanic acid ratio in stored blood from such a patient indicates a disturbed phytanic acid α-oxidation, whereas pristanic acid β-oxidation is normal.

<table>
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<tr>
<th>Table 1. Concentrations of Pristanic Acid (PrA) and Phytanic Acid (PhA) and Their Ratios in Blood Spots from Control Subjects and Patients</th>
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<td>Conc, nmol/blood spot</td>
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<tr>
<td>Controls</td>
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<td>1981, n = 15</td>
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<td>1987, n = 15</td>
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<td>Patients</td>
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<td>Zellweger, (1989, Denmark)</td>
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<td>Zellweger, (1989, France)</td>
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<td>RCDP</td>
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<td>X-ALD, (1981, Denmark)</td>
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In X-ALD a single enzyme defect exists, at VLCFA acyl-CoA synthetase (2). Patients with this disorder accumulate neither pristanic acid nor pristanic acid in plasma. The finding of normal pristanic acid and phytanic acid concentrations in the blood spot from such a patient is in accordance with the absence of a disturbance in the phytanic acid/pristanic acid metabolism.

Plasma pristanic and phytic acids are increased in infantile Refsum disease and in NALD, which, like Zellweger syndrome, belong to the group of generalized peroxisomal disorders (5, 13). It is unknown whether the milder phenotype encountered in the two former disorders means a less severe defect in phytanic acid $\alpha$-oxidation and pristanic acid $\beta$-oxidation and consequently less of a chance to accumulate these acids in early life. However, these patients usually live beyond the age of 1 year, so that more time for biochemical diagnosis is available. In plasma from patients with a defect in peroxisomal $\beta$-oxidation at the level of bifunctional protein or 3-oxoacyl-CoA thiolase, pristanic acid and, to a lesser extent, phytic acid accumulate in addition to VLCFA and bile acids (5). It is reasonable to expect also that in blood spots collected at neonatal screening from such patients at least pristanic acid will accumulate.

It is difficult to compare the absolute concentrations in blood spots with those in plasma because the absolute amount of blood assayed in a blood spot is not known exactly and may vary with possible differences in filter paper material and thickness. However, the ratios of pristanic and phytic acid concentrations are not dependent on these considerations.

The low concentrations in the controls and the small amount of blood available made the sensitive stable isotope dilution method indispensable. This approach to measure pristanic and phytic acid concentrations in blood spots collected at neonatal screening can be used for limited epidemiological studies to establish the frequency of certain peroxisomal disorders. However, the criteria for mass screening are not fulfilled: the frequency of most of the peroxisomal disorders has not yet been established; in all cases except X-ALD no therapy is available; and the method presented here is not cost effective. However, the method is certainly of value in individual cases in which a diagnosis is lacking and no further tissues or body fluids are available.

References