role or contribution of specific cells or factors in the development of PVR. Dispase is an inexpensive and readily available enzyme, and the intravitreal injection procedure is easy to perform and produces little trauma. The anterior chamber remained clear, and the pupil usually dilated widely. Because the lens remained free of opacity in most cases, a clear view of the fundus was obtained. Neither mechanical vitrectomy nor gas compression vitrectomy was performed, thus reducing risks such as gas-induced cataract. The time course of development of PVR and the histologic appearance of the cellular membranes that form correspond to observations made in the clinical setting.

Future work on the dispase model will include the characterization of cell types within the PVR membranes, a histologic examination of the progression of PVR, and the determination of the effectiveness of dispase in other animals. Studies on the prevention and treatment of PVR will be enhanced by the availability of this new model of PVR.

Acknowledgments

The authors thank Gaurav Shah and Anil Synghal for assistance with taking the fundus photographs, Rudy Padua for preparing the histology samples, and Susann Remington and Christine Sodering for critically reading and improving the manuscript.

References


Polyunsaturated Fatty Acids Are Lower in Blood Lipids of Usher’s Type I but Not Usher’s Type II

Maureen B. Maude1,4,5 Elizabeth O. Anderson4 and Robert E. Anderson1,2,3,4,5

Purpose. Previous studies have shown that persons with retinitis pigmentosa and Usher’s syndrome have lower blood levels of long-chain polyunsaturated fatty acids (PUFAs). In this study, the fatty acid composition of phospholipids from plasma and red blood cells (RBCs) was compared in persons with Usher's syndrome type I; Usher's syndrome type II; or no retinal disease (control subjects).

Methods. Blood was drawn from fasting volunteers and separated into plasma and RBCs by centrifugation. Lipids were extracted and phospholipids were obtained by thin-layer chromatography. Fatty acid methyl esters were prepared and analyzed by gas-liquid chromatography.

Results. There were no differences in plasma or RBC phospholipid fatty acid composition between control subjects (n = 54) and persons with Usher’s syndrome type II (n = 20). However, all 20- and 22-carbon PUFA levels from RBCs of persons with Usher’s syndrome type I were lower than those from control subjects and persons with Usher’s Syndrome type II. Likewise, plasma levels of 20:3n-6, 20:5n-3, and 22:6n-3 were lower in Usher’s syndrome type I compared with the control group. In contrast, plasma levels of 18:1n-9 and RBC levels of 16:0 and 18:1n-9 were higher in the group with Usher’s syndrome type I.

Conclusions. Plasma and RBCs from Usher’s syndrome type I, but not type II, have lower levels of long-chain PUFAs than plasma and RBCs from control subjects. (Invest Ophthalmol Vis Sci. 1998;39:2164–2166)

Numerous studies have shown that some humans and animals with inherited retinal degenerations have lower plasma and red blood cell (RBC) levels of long-chain polyun-
saturated fatty acids (PUFAs), especially docosahexaenoic acid (22:6n-3; reviewed in Reference 1), the major fatty acid of retinal rod outer segments. In addition to having lower plasma levels of 22:6n-3, men with Usher’s syndrome have recently been shown to have lower levels of 22:6n-3 in their sperm. In the present study, total phospholipid fatty acid composition of plasma and RBCs was studied in persons with Usher’s Syndrome type I and type II.

**METHODS**

Fasting blood was drawn from persons with Usher’s syndrome type I (n = 35; average age, 39.5 years; 17 women, 18 men), Usher’s syndrome type II (n = 20; average age, 40.1 years; 15 women, 5 men), or no retinal disease (n = 54; average age, 35.8 years; 34 women, 20 men) at meetings of the Foundation Fighting Blindness (held in Orlando, FL) and the American Association of the Deaf-Blind (held in Los Angeles, CA) and separated into plasma and RBCs by centrifugation. The blood products were placed immediately on dry ice and shipped frozen to the laboratory. All donors signed an informed consent and affected persons gave consent to contact their ophthalmologists to confirm a diagnosis of Usher’s syndrome type I or type II. Those persons in whom a definitive diagnosis could not be established were eliminated from the study (n = 5). All protocols were approved by the Institutional Review Boards of Baylor College of Medicine (Houston, TX) and the University of Oklahoma Health Sciences Center (Oklahoma City).

Lipids were extracted from plasma and RBCs. An aliquot from each was placed on a thin layer plate, and neutral lipids were separated from phospholipids by a neutral lipid solvent system. The phospholipids that remained at the origin were scraped from the plate, converted to methyl esters, and analyzed by gas liquid chromatography. The values are given as relative mole percent ± SD. Details of these procedures were published in Aguirre et al.

The disease state of all blood donors was not known until all the analyses were completed. Statistical significance of differences between groups was determined by a two-tailed Student’s t-test.

**RESULTS**

The plasma phospholipid fatty acid compositions of the three groups are given in Table 1. Except for the minor fatty acids 16:1n-7 and 18:3n-6, there were no significant differences between control subjects and Usher’s type II donors. However, Usher’s type I donors had significantly lower plasma levels of 20:5n-3, 20:5n-3, and 22:6n-3 and higher levels of 18:1n-7 than control subjects. Interestingly, the levels of the two major fatty acids of the n-6 family (18:2 and 20:4) were not different between these two groups. There were no statistical differences between plasma phospholipid fatty acid levels from Usher’s type I and type II donors.

The differences between the group with Usher’s type I and the other two groups were more dramatic in the RBC phospholipid fatty acids (Table 2). The levels of all C-20 and C-22 PUFAs were significantly lower in the group with Usher’s type I compared with those with type II or control subjects. Palmitic acid (16:0) and 18:1n-7 were significantly higher in the group with Usher’s type I than in the control subjects or the group with Usher’s type II. Of the two major n-6 fatty acids in RBCs (18:2n-6 and 20:4n-6), only 20:4n-6 was reduced in Usher’s type I. There were no differences between values for control subjects and the group with Usher’s type II values, except for 16:1n-7.

**DISCUSSION**

These results confirm and extend the earlier work of Bazan et al. and demonstrate a lower level of long-chain PUFA in persons with Usher’s syndrome type I than in control subjects or Usher’s type II donors. The difference between the two types of Usher’s Syndrome is particularly interesting because of the difference in phenotype. Type I has an early onset of hearing and vision loss that often proceeds rapidly to blindness and deafness, whereas type II disease is less severe.

Connor et al. recently reported lower levels of 22:6n-3 in the sperm of six persons with Usher’s type II than in control subjects or persons with other forms of inherited retinal degenerations. They did not report blood levels of 22:6n-3 for their Usher’s patients but did show that the RBC level of 22:6n-3 was lower in 26 patients with retinitis pigmentosa (RP) (including 6 with Usher’s type II and 2 with Usher’s type I) than in control subjects. Their finding of lower sperm levels of 22:6n-3 in type II patients may seem at odds with our report of no differences in blood levels of 22:6n-3. Their patient pool was male and our 20 type II patients were 15 women and 5 men. When the RBC values of 22:6n-3 for our type II men were analyzed separately, no significant difference was found between them (1.8 ± 0.9) and control subjects (2.8 ± 1.9), although the trend was for the affected men to have lower 22:6n-3. The values for type II (2.8 ± 1.0) and control (2.6 ± 0.9) women were indistinguishable. The difference between men with Usher’s type I (1.5 ± 0.8) and men in the control group and women with Usher’s type I (1.9 ± 0.9) and women in the control group was significant, as expected from the
Acid that men may show the phenotype more dramatically than type II genes have been mapped and one has been cloned seems unlikely. Hoffman and Birch found that RBC phospho-(iWi). We do not know the genotype of the affected persons RBCs tended to be lower in men than in women with type I combined results (Table 2). However, the 22:6n-3 levels in 22:5n-6 levels of 22:6n-3. One of the intriguing findings in the present study was that the low level of 22:6n-3 was found only in persons with Usher’s type I.

How a mutation in a gene expressed in the retina or the ear can be related to lower blood 22:6n-3 is a mystery. Nevertheless, the high level of significance of the differences in long-chain PUFA between control and type I, but not type II, disease clearly shows that these differences are real. Because multiple genotypes are involved, it seems reasonable to suggest that some common convergent pathway leads to the reduction of blood levels of 22:6n-3. One possibility is that the different mutations produce a metabolic stress that provokes structural and biochemical changes in photoreceptor cells and their rod outer segments. In albino rats raised in 800 lux cyclic light, reduced rod outer segment levels of 22:6n-3 and increased antioxidant defenses were observed compared with rats raised in 5 lux cyclic light (reviewed by Penn and Anderson). The 800 lux animals were not damaged by acute constant light of 2000 lux, but 2000 lux completely destroyed the retinas of the 5 lux animals. However, the daily stress of bright cyclic light did result in the loss of about 40% more of their photoreceptors over a 12-week period than in the 5 lux rats. It may be possible that many inherited retinal degenerations have in common a metabolic stress that slowly kills cells, much like that which occurred in the rat study just discussed. If the stress is oxidant, the retina could downregulate 22:6n-3 and upregulate antioxidant defenses. How such a stress could lead to changes in blood levels of 22:6n-3 is not understood; however, Scott and Bazan have proposed that the retina communicates with the liver to control 22:6n-3 delivery to the retina. We are currently testing the metabolic stress hypothesis by repeating some of our earlier studies to determine whether bright cyclic rearing causes changes in blood levels of 22:6n-3 in albino rats.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Control (n = 54)</th>
<th>Usher’s Type I (n = 35)</th>
<th>Usher’s Type II (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>28.8 ± 3.2</td>
<td>31.6 ± 3.9</td>
<td>28.2 ± 2.9</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>0.68 ± 0.28</td>
<td>0.73 ± 0.51</td>
<td>0.52 ± 0.22</td>
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<tr>
<td>18:0</td>
<td>17.0 ± 1.3</td>
<td>17.5 ± 1.9</td>
<td>17.6 ± 1.4</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>16.8 ± 1.9</td>
<td>18.1 ± 2.3</td>
<td>16.1 ± 1.7</td>
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<tr>
<td>18:1n-7</td>
<td>1.9 ± 0.5</td>
<td>2.1 ± 0.6</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>13.0 ± 1.8</td>
<td>12.7 ± 3.2</td>
<td>13.0 ± 2.1</td>
</tr>
<tr>
<td>18:3n-6</td>
<td>0.05 ± 0.05</td>
<td>0.04 ± 0.06</td>
<td>0.05 ± 0.05</td>
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<tr>
<td>18:3n-3</td>
<td>0.19 ± 0.16</td>
<td>0.14 ± 0.24</td>
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</tr>
<tr>
<td>20:3n-6</td>
<td>1.4 ± 0.3</td>
<td>1.1 ± 0.3c</td>
<td>1.4 ± 0.5</td>
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<tr>
<td>20:4n-6</td>
<td>12.9 ± 3.0</td>
<td>10.7 ± 3.5</td>
<td>13.5 ± 2.6</td>
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<td>20:5n-3</td>
<td>0.35 ± 0.29</td>
<td>0.21 ± 0.15</td>
<td>0.32 ± 0.16</td>
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<tr>
<td>22:4n-6</td>
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<td>22:5n-6</td>
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<td>22:5n-3</td>
<td>1.4 ± 0.5</td>
<td>1.0 ± 0.5a</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>2.7 ± 1.3</td>
<td>1.7 ± 0.9a</td>
<td>2.5 ± 1.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD. *P < 0.005 for control and type II; †P < 0.05 for control and type I vs type II; ‡P < 0.02 for control and type II vs type I.

combined results (Table 2). However, the 22:6n-3 levels in RBCs tended to be lower in men than in women with type I (1.5 versus 1.9) or type II (1.8 versus 2.8) disease, suggesting that men may show the phenotype more dramatically than women.

At the present time, five Usher’s type I and three Usher’s type II genes have been mapped and one has been cloned (FVII). We do not know the genotype of the affected persons who donated blood for our study, so it is impossible to determine whether only one mutation is involved. However, this seems unlikely. Hoffman and Birch found that RBC phospholipids from patients with X-linked RP have significantly less 22:6n-3 and other PUFAs than control subjects. X-linked RP has been mapped to seven different locations on the X-chromosome, and two of the genes have been cloned (FVII). Clearly, in both Usher’s Type I and X-linked RP, there are multiple genotypes that have the low 22:6n-3 phenotype. Also, numerous studies in persons with autosomal dominant and recessive RP and in dogs and cats with inherited retinal degenerations have reported lower plasma or RBC levels of 22:6n-3 than control subjects. Interestingly, not all families with RP or dogs with inherited retinal degenerations have low blood levels of 22:6n-3. One of the intriguing findings in the present study was that the low level of 22:6n-3 was found only in persons with Usher’s type I.

How a mutation in a gene expressed in the retina or the ear can be related to lower blood 22:6n-3 is a mystery. Nevertheless, the high level of significance of the differences in long-chain PUFA between control and type I, but not type II, disease clearly shows that these differences are real. Because multiple genotypes are involved, it seems reasonable to suggest that some common convergent pathway leads to the reduction of blood levels of 22:6n-3. One possibility is that the different mutations produce a metabolic stress that provokes structural and biochemical changes in photoreceptor cells and their rod outer segments. In albino rats raised in 800 lux cyclic light, reduced rod outer segment levels of 22:6n-3 and increased antioxidant defenses were observed compared with rats raised in 5 lux cyclic light (reviewed by Penn and Anderson). The 800 lux animals were not damaged by acute constant light of 2000 lux, but 2000 lux completely destroyed the retinas of the 5 lux animals. However, the daily stress of bright cyclic light did result in the loss of about 40% more of their photoreceptors over a 12-week period than in the 5 lux rats. It may be possible that many inherited retinal degenerations have in common a metabolic stress that slowly kills cells, much like that which occurred in the rat study just discussed. If the stress is oxidant, the retina could downregulate 22:6n-3 and upregulate antioxidant defenses. How such a stress could lead to changes in blood levels of 22:6n-3 is not understood; however, Scott and Bazan have proposed that the retina communicates with the liver to control 22:6n-3 delivery to the retina. We are currently testing the metabolic stress hypothesis by repeating some of our earlier studies to determine whether bright cyclic rearing causes changes in blood levels of 22:6n-3 in albino rats.

NOTE

A list of cloned and/or mapped genes causing retinal degeneration or related diseases has been compiled by Dr. Steven Daiger and can be accessed on the World Wide Web on RetNet, http://www.sph.uth.tmc.edu/RetNet.

Acknowledgments

The authors thank the staffs of the Foundation Fighting Blindness (Hunt Valley, Maryland) and the American Association of the Deaf-Blind for assistance in making the arrangements for blood collection. The authors also thank the many people who donated blood for this study.

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