Nervonic Acid and Demyelinating Disease

J. R. SARGENT*, K. COUPLAND† and R. WILSON‡

*Department of Biological and Molecular Sciences, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK, †Croda Universal Ltd, Hull, HU6 7PH, UK, ‡Cardiovascular Unit, Hugh Robson Building, University of Edinburgh, George Square, Edinburgh, EH8 9XF, UK.

Abstract — Demyelination in adrenoleukodystrophy (ALD) is associated with an accumulation of very long chain saturated fatty acids such as 26:0 stemming from a genetic defect in the peroxisomal beta oxidation system responsible for the chain shortening of these fatty acids. Long chain monoenoic acids such as erucic acid, 22:1(n-9), can normalise elevated serum levels of 26:0 in ALD by depressing their biosynthesis from shorter chain saturated fatty acids. Sphingolipids from post mortem ALD brain have decreased levels of nervonic acid, 24:1(n-9), and increased levels of stearic acid, 18:0. Increased levels of 26:0 are accompanied by decreased nervonic acid biosynthesis in skin fibroblasts from ALD patients. Sphingolipids from post mortem MS brain have the same decreased 24:1(n-9) and increased 18:0 seen in post mortem ALD brain. The 24:1(n-9) content of sphingomyelin is depressed in erythrocytes from multiple sclerosis (MS) patients. Defects in the microsomal biosynthesis of very long chain fatty acids including 24:1(n-9) in 'jumpy' and 'quaking' mice are accompanied by impaired myelination. An impairment in the provision of nervonic acid in demyelinating diseases is indicated, suggesting that dietary therapy with oils rich in very long chain monoenoic acid fatty acids may be beneficial in such conditions.

Introduction

Myelination of nerves in human brain starts at a gestational age of approximately 32 weeks, increases rapidly until and beyond birth, and is largely complete in the first 2 or 3 years of life (1-3). The rapidity of myelination is illustrated by the myelinating oligodendrocyte in the rat producing more than three times its weight of myelin per day (4). Myelin is unusual among biological membranes in that it contains more than 70% of its dry weight as lipid which is rich in sphingolipids, i.e. cerebrosides, sulphatides and sphingomyelin which comprise circa 23%, 4% and 8% of total myelin lipid respectively (5). These sphingolipids contain a high proportion of their total fatty acids as very long chain saturated and especially very long chain monounsaturated moieties exemplified by lignoceric acid, 24:0, and nervonic acid, 24:1(n-9), respectively. Moreover, the level of nervonic acid in human brain sphingolipids increases markedly from birth to reach a maximum at about 4 years after which it remains almost constant (6). Given such a large demand for relatively specialised fatty acids over a relatively short and critical period of neural development, an impaired provision of these fatty acids early in life could have serious consequences for neural performance, whether in the short or longer terms. The present article presents evidence for an association between demyelination and a substantially decreased content of nervonic acid in
Adrenoleukodystrophy

Recent years have seen an increased understanding of genetic disorders of lipid metabolism specifically associated with peroxisomes. The X-linked chromosomal condition adrenoleukodystrophy (ALD) is one such disorder apparently caused by a defect in the ligase that couples coenzyme A with very long chain saturated fatty acids such as 26:0, thereby reducing the oxidation of very long chain saturated acyl CoA derivatives by peroxisomes and causing the accumulation of these fatty acids in body lipids. Thus, the serum lipids of patients with ALD have circa 1.0% of their fatty acids as 26:0 compared with circa 0.3–0.4% in normal subjects (7). More important, neural tissues including the brains of ALD patients accumulate 26:0 in their lipids, particularly in their sphingolipids. Thus, sphingomyelin, cerebrosides and sulphatides from post mortem brains of ALD patients all have some 3% of their fatty acids as 26:0 compared to 1% in normal subjects (8). Cholesteryl esters are the final repository of excess 26:0 in ALD and in plaque tissue from ALD brain these esters have more than 12% of their fatty acids as 26:0 (8). The accumulation of very long chain fatty acids in ALD neural tissues is accompanied by destruction of myelin both centrally and peripherally, thus causing severe neural symptoms in young boys and ultimately death at an early age, usually 1 or 2 years after the onset of symptoms. Fibroblasts cultured from skin biopsies of ALD patients produce lipids with elevated levels of 26:0 compared to normal fibroblasts. Addition of oleic acid in 18:1(n-9) to the culture medium substantially decreases the levels and 26:0 in the cultured ALD cells (9) and erucic acid 22:1(n-9) is even more effective (10). Findings such as these are consistent with monounsaturated fatty acids inhibiting the biosynthesis of 26:0 with increasing efficiency in the order 18:1 <20:1 <22:1. They form the rational for a dietary therapy of ALD, currently under clinical trial, with a blend of glyceryltrioleate and glyceryltrierucate. This oil blend normalises the elevated levels of 26:0 in serum lipids of ALD patients (7) raising promise that, if administered sufficiently early to ALD patients, it may delay the appearance and/or decrease the severity of the severe neural symptoms expressed in the condition.

Multiple sclerosis

Multiple sclerosis (MS) has a much higher incidence than ALD and, despite many years of intensive research, there is still no effective therapy for this severely debilitating demyelinating condition which follows a relapsing and remitting clinical progression in the majority of patients. Moreover, the aetiology of MS remains unknown although its pathogenesis is generally believed to involve an autoimmune reaction to a component of myelin. A current plausible hypothesis is that the disease may result from an autoimmune response triggered by an environmental factor, possibly a non-specific viral infection, in a genetically susceptible individual (13). However, given the increased understanding of the causes and pathology of ALD, it is worthwhile reconsidering the role of lipids in the aetiology of MS.

Gerstl and his colleagues (14) originally noted from a very limited number of samples that sphingolipids in post mortem brain from MS patients had reduced levels of 24:1(n-9) accompanied by in-
creased levels of 18:0. These authors suggested that a defect in the biosynthesis of nervonic acid in MS leads to breakdown of myelin which triggers the onset of the autoimmune response (14). In view of these findings and our own findings with ALD we have recently analysed the sphingolipids in post mortem brains from 9 MS patients and 9 normal control individuals. The results (Table) clearly establish that in sphingomyelin, sulphatides and cerebrosides there is a marked decrease in 24:1(n-9) which is almost exactly balanced by an increase in 18:0 + 16:0 in MS individuals as compared to controls. This supports the contention of Gerstl et al (14) that nervonic acid biosynthesis is impaired in MS. The same result could, of course, be generated by a selective catabolism of sphingolipids in myelin shed from nerves by causes not related to impaired lipid metabolism. However, there is also evidence that sphingomyelin in erythrocytes from MS patients has significantly decreased and increased levels of 24:1(n-9) and saturated fatty acids respectively (15), albeit to a lesser extent than occurs in post mortem brain sphingolipids. This points to a generalised defect in nervonic acid biosynthesis in MS patients and supports the contention that the decreased nervonic acid content in brain sphingolipids in MS may contribute to demyelination rather than being a consequence of it. Recent evidence indicates that levels of 24:1(n-9) in erythrocyte sphingomyelin in premature infants may reflect levels of 24:1(n-9) in brain sphingomyelin and thus brain maturity (16).

That an impaired biosynthesis of 24:1(n-9) can be associated with dysmyelination is indisputable since the mutant mice 'jumpy' and 'quaking' both suffer from extensive demyelination and both have defects in the microsomal fatty acid elongase responsible for the biosynthesis of very long chain fatty acids including 24:1(n-9), specifically at the condensing enzyme reaction that couples malonyl CoA to the growing fatty acyl chain (17). The consequences of such an impairment will be most serious during the early stages of brain maturation when myelination is extensive and when the levels of 24:1(n-9) and 18:0 are increasing and decreasing respectively in myelin.

Table  Fatty acid compositions of sphingolipids of white matter from post mortem brains of normal individuals and multiple sclerosis patients

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Sphingomyelin</th>
<th>Sulphatides</th>
<th>Cerebrosides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>MS</td>
<td>Normal</td>
</tr>
<tr>
<td>16:0</td>
<td>7.4±2.1</td>
<td>11.2±4.4*</td>
<td>13.1±4.3</td>
</tr>
<tr>
<td>18:0</td>
<td>25.4±2.4</td>
<td>34.3±5.6***</td>
<td>9.4±2.8</td>
</tr>
<tr>
<td>19:0</td>
<td>1.1±0.6</td>
<td>0.9±0.2</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>20:0</td>
<td>1.0±0.1</td>
<td>1.1±0.3</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>22:0</td>
<td>1.5±0.1</td>
<td>1.6±0.3</td>
<td>1.6±0.2</td>
</tr>
<tr>
<td>23:0</td>
<td>1.6±0.2</td>
<td>1.6±0.3</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td>24:0</td>
<td>6.6±0.9</td>
<td>6.5±1.9</td>
<td>9.8±1.7</td>
</tr>
<tr>
<td>25:0</td>
<td>2.5±1.2</td>
<td>1.6±0.5</td>
<td>3.3±0.2</td>
</tr>
<tr>
<td>26:0</td>
<td>1.1±0.5</td>
<td>0.5±0.2**</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Total sats</td>
<td>48.2±2.5</td>
<td>59.3±7.5***</td>
<td>41.9±4.6</td>
</tr>
<tr>
<td>16:1</td>
<td>0.7±0.5</td>
<td>0.7±0.5</td>
<td>1.9±1.3</td>
</tr>
<tr>
<td>18:1</td>
<td>3.5±1.2</td>
<td>4.7±3.4</td>
<td>3.7±1.1</td>
</tr>
<tr>
<td>19:1</td>
<td>0.6±0.3</td>
<td>0.4±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>20:1</td>
<td>0.3±0.2</td>
<td>0.6±0.5</td>
<td>nd</td>
</tr>
<tr>
<td>22:1</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td>nd</td>
</tr>
<tr>
<td>23:1</td>
<td>0.8±0.2</td>
<td>0.8±0.2</td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>24:1</td>
<td>36.3±2.5</td>
<td>25.7±5.6***</td>
<td>36.2±3.7</td>
</tr>
<tr>
<td>25:1</td>
<td>5.3±0.6</td>
<td>4.1±0.9</td>
<td>8.6±1.3</td>
</tr>
<tr>
<td>26:1</td>
<td>4.3±0.7</td>
<td>3.6±1.0</td>
<td>7.0±1.8</td>
</tr>
<tr>
<td>Total mono unsats</td>
<td>51.8±2.5</td>
<td>40.7±7.5**</td>
<td>58.1±4.6</td>
</tr>
</tbody>
</table>

Total lipid was extracted by chloroform : methanol (2:1 vol:vol), separated by two dimensional thin layer chromatography and fatty acid methyl esters prepared by acid-catalysed transmethylation and analysed by high resolution gas-liquid chromatography. Values are means ± s.d. for separate determinations of post mortem brain samples from 9 normal individuals and 9 patients with multiple sclerosis. Monounsaturated fatty acids include all (n-9) and (n-7) isomers. nd signifies not detected. Where indicated (*), values of MS samples are significantly different from values of normal samples, \( p<0.05 \), **\( p<0.01 \), ***\( p<0.001 \).
sphingolipids (6,18). However, lack of myelination or demyelination may not necessarily be an immediate result of impaired nervonic acid biosynthesis in early brain development, especially if the impairment is only partial and leads to myelin with partially but not severely reduced stability. Thus, partially impaired nervonic acid biosynthesis could generate myelin with a significantly reduced stability, caused for example by incomplete compaction as occurs in the 'trembler' mouse mutant (19). Such a myelin would then have an enhanced susceptibility to damage by environmental stressors at any subsequent stage of life, causing it to be stripped from nerves and trigger the autoimmune response characteristic of the disease.

**Fatty acid elongase and the biosynthesis of very long chain fatty acids**

The proposition that a defect can occur in the biosynthesis of nervonic acid at first sight seems simplistic because it is commonly believed, on the basis that radioactivity is readily incorporated from 18:1(n-9) into 24:1(n-9) in brain (20,21), that nervonic acid biosynthesis proceeds readily in brain. However, what is at issue here is the extent to which such reactions proceed under all conditions, the extent to which they meet the quantitative requirements of the rapidly developing brain for very long chain fatty acids, and the extent to which they can be perturbed by internal or external factors. Current knowledge is far from definitive in these areas. For example, a recent review (22) on the microsomal fatty acid chain elongation system reveals how relatively uncharacterised this system is. Very few of the individual enzymes catalysing the various steps in very long chain fatty acid biosynthesis have been purified and little or nothing is known about how the overall reaction pathway is controlled. Evidence exists (22), however, that the reduction of beta-ketoacyl CoA in the overall microsomal reaction involves, not NADPH as is conventionally the case for other fatty acid synthetases, but the NADH-cytochrome b5 electron transfer system which is a key component of the fatty acid desaturases involved in the biosynthesis of monounsaturated fatty acids (delta-9 desaturase) and polyunsaturated fatty acids (delta-6 and delta-5 desaturases). This immediately raises the possibility that competitive effects could occur in the biosyntheses of very long chain monounsaturated fatty acids and (n-6) and (n-3) polyunsaturated fatty acids. There is also evidence (22) that the microsomal fatty acid elongation system can be induced by peroxisomal proliferators such as di(2-ethyl)hexyl phthalate. Since peroxisomal proliferators frequently induce the catabolism of very long chain fatty acids through the peroxisomal beta oxidation chain shortening system (see the foregoing section on ALD), a complex link between the biosynthesis and the catabolism of very long chain fatty acids, involving both the endoplasmic reticulum and peroxisomes, is indicated. Evidence for such a link now exists for the biosynthesis of docosahexaenoic acid, 22:6(n-3), which involves the microsomal chain elongation of 20:5(n-3) to 22:5(n-3) and thence to 24:5(n-3), followed by the conversion of 24:5(n-3) to 24:6(n-3) by the microsomal delta-6 fatty acid desaturase, and finally the conversion by chain shortening in peroxisomes of 24:6(n-3) to 22:6(n-3) (23). Thus, the whole area of very long chain fatty acid biosynthesis is proving much more complex than hitherto realised, a situation that is not made easier experimentally by the very hydrophobic nature of some of the fatty acids involved and especially the marked increase in hydrophobicity that occurs in saturated fatty acids of chain length >C20.

Against the foregoing it is difficult to state categorically that biosynthesis of very long chain fatty acids such as nervonic acid proceeds readily and generates the required amounts of end products in developing brain under all conditions. This is especially the case given the recent (and belated) realisation that the conversion of 18:3(n-3) to 22:6(n-3) may not proceed sufficiently rapidly in the developing foetus to provide the required amounts of 22:6(n-3) for optimal brain development before birth. Thus, premature infants fed formulae diets based on cow or soya milk supplemented with 18:3(n-3) but essentially lacking 22:6(n-3) have reduced visual acuity and probably also reduced mental performance later in life, as compared to premature infants fed either formulae diets supplemented with 22:6(n-3), or with mother's milk which naturally contains small but highly significant amounts of 22:6(n-3) (24–26). Human milk contains 24:1(n-9) as well as 22:6(n-3) at concentrations in the region of 0.25% and 0.20% respectively (mean values for the first 36 days postpartum) of total milk lipid fatty acids (27). This raises the intriguing possibility that, as the rate of conversion of 18:3(n-3) to 22:6(n-3) in the human infant may not always proceed sufficiently fast to meet the infant's optimal growth requirement, so the same may hold for the conversion of 18:1(n-9) to 24:1(n-9). A recent study has revealed a strong correlation between cow's milk consumption and the prevalence of MS in 27 countries and 29 populations world wide (28) and it is tempting to relate this to the very low levels of 24:1(n-9) in cow's milk reflected in very low levels of 24:1(n-9) in the small levels of sphingomyelin in cow's milk (29). The 24:1(n-9) necessary for human brain devel-
Development may plausibly arise partly from the diet and partly from internal biosynthetic activity, with the latter activity being directly influenced by other dietary factors, not least dietary intake of very long chain saturated fatty acids. The ratio of very long chain saturated fatty acids to 24:1(n-9) in cow's milk lipid is at least one order of magnitude greater than in human milk lipid (29,27).

A possible nutritional therapy for multiple sclerosis

An impaired biosynthesis of nervonic acid 24:1(n-9) in MS or any other demyelinating condition including ALD can, in principle, be readily rectified by dietary provision of nervonic acid or an immediate precursor of it. Experience with ALD suggest that erucic acid 22:1(n-9) in the form of glyceryl trierucate elevates the level of 24:1(n-9) in erythrocyte sphingomyelin (7). Borage oil, which contains low but significant amounts of both 22:1(n-9) and 24:1(n-9), has the same effect in normal subjects (12). Moreover, administration of glyceryl trierucate to ALD patients results in increased levels of 22:1(n-9) in brain lipids including sphingomyelin although most of the elevated 22:1(n-9) in brain is present in triacylglycerols (7). So far there is no published information on whether 24:1(n-9) is elevated in brain sphingomyelin in ALD patients treated with glyceryl trierucate. However, several factors point against the use of 22:1(n-9) in treating putative deficiencies of 24:1(n-9) biosynthesis and point instead to the desirability of using 24:1(n-9) itself, preferably as a highly purified triglyceride oil rich in this fatty acid. First, the availability of 24:1(n-9) as a natural triglyceride oil will allow smaller doses of dietary oil to be administered than is the case for glyceryl trierucate. Second, the use of 24:1(n-9) rather than 22:1(n-9) will avoid the probably undesirable build up of 22:1(n-9) in tissue lipids that can occur on feeding diets rich in erucic acid. Third, given that any step in the biosynthesis of 24:1(n-9) from 18:1(n-9) could be affected in the putative impairment of nervonic acid biosynthesis, including the final conversion of 22:1(n-9) to 24:1(n-9), it is prudent to administer the final end product of the pathway, 24:1(n-9) itself.

Natural oils rich in 24:1(n-9) are relatively rare but at least one has been identified during the course of preparing glyceryl trierucate for ALD treatment. This is the triglyceride seed oil of Lunaria biennis, the honesty plant, which contains 23% of 18:1(n-9), 46% of 22:1(n-9) and 23% of 24:1(n-9) (unpublished observation, Croda Universal Ltd, Hull, UK). Currently this oil is being prepared commercially in bulk quantities prior to refining it to a high degree of purity and thence preparing a triglyceride oil highly enriched in 24:1(n-9). The availability of such an oil will allow nutritional trials to be conducted, whether in patients with MS or in animal demyelinating models, to test the validity or otherwise of the hypothesis presented in this article. Care must clearly be taken in administering this oil to individuals not to perturb their essential fatty acid status since evidence exists that 22:1(n-9) added to cultured skin fibroblasts from ALD patients can depress the levels of 20:4(n-6) and 22:6(n-3) as well as the conversion of 18:0 to 26:0 in the cells (10). Such care is particularly pertinent in very young patients, given the importance of 22:6(n-3) and also 20:4(n-6) in neural development (30). It is interesting to note that a recent major clinical trial testing the effect of an oil rich in 20:5(n-3) and 22:6(n-3) in MS patients recorded some improvement in all of several clinical parameters tested, although in no case was the improvement statistically significant (31). There is every reason, therefore, to supplement a dietary oil rich in 24:1(n-9) with 20:5(n-3) and 22:6(n-3) prior to testing for beneficial effects on MS patients.

MS remains a basically intractable condition that seriously afflicts many individuals. We present the above hypothesis for the cause and amelioration of this distressing condition for two reasons. First, it is a simple and logical deduction from existing data in the literature. Second, it can simply be tested with minimal risk to those afflicted.

References

8. Wilson R, Sargent J R. Lipid and fatty acid composition of...
10. Wilson R, Tocher D R, Sargent J R. Effects of exogenous mo-
nounsaturated fatty acids on fatty acid metabolism in cultured
skin fibroblasts from adrenoleukodystrophy patients. J Neuro-
12. Barre D E, Holub B J. The effect of borage oil consump-
tion on the composition of individual phospholipids in human
Hypoth 1992; 37: 40–43.
golipids and their precursors in human brain (normal and MS).
Z Neurol 1972; 202: 104–120.
15. Navarro X, Segura R. Red blood cell fatty acids in multiple
16. Babir F, Sarda P, Limasset B et al. Nervonic acid in red blood
cell sphingomyelin in premature infants: an index of myelin
17. Suneja S K, Nagi M N, Cook L, Cinti D L. Decreased long
chain fatty acyl CoA elongation activity in quaking and jumpy
mouse brain: deficiency in one enzyme or multiple enzyme
18. Svennerholm L, Vanier M-T, Jungbjer B. Changes in fatty
acid composition of human brain myelin lipids during mat-
19. Ayers M M, McAnderson D R. Development of onion bulb
Neuropath 36: 137–152.
20. Fewster M E, Ihrig T, Mead J F. Biosynthesis of long chain
fatty acids by oligodendroglia isolated from bovine white mat-
154.
22. Cinti D L, Cook L, Nagi M H, Suneja S K. The fatty acid
chain elongation system of mammalian endoplasmic reticu-
23. Sprecher H. A reevaluation of the pathway for the synthesis
of 4,7,10,13,16,19-docosahexaenoic acid. Omega-3 News, 1–
Effect of dietary omega-3 fatty acids on retinal function of
very-low-birth weight neonates. Pediatric Research 1990; 28:
485–492.
Visual acuity development in healthy preterm infants: effect
of marine-oil supplementation. Am J Clin Nutr 1993; 58: 35–
42.
Breast milk and subsequent intelligence quotients in the children
27. Harzer G, Haug M, Dieterich I, Gentner P R. Changing pat-
terns of human milk lipids in the course of the lactation and
between milk and dairy product consumption and multiple
sclerosis prevalence: a worldwide study. Neuroepidemiology
29. Patton S, Jensen R G. Lipid metabolism and membrane func-
tions of the mammary gland. Progr Lipid Res 1975; 14: 163–
277.
Significance. The Report of the British Nutrition Foundation’s
controlled trial of long chain n-3 polyunsaturated fatty acids
in the treatment of multiple sclerosis. J Neurol Neurosurg Psy-