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Cerebrospinal Fluid Lipids in Demyelinating Disease

II. Linoleic Acid as an Index of Impaired Blood-CSF Barrier

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Summary. The absolute linoleic acid concentration in CSF was determined and the findings of MS patients ($n = 10$) and controls ($n = 12$) were compared. The linoleic acid content of control CSF (1.6 ± 0.8 nMol/ml) is considerably lower than the corresponding serum value (2.5—4.1 μMol/ml). Although CSF from MS patients contains a significantly higher linoleic acid concentration than controls the close correlation between CSF linoleic acid and CSF albumin is maintained.

The high CSF concentration of cholesterol esters rich in linoleic acid, which are abundant in serum but represent only traces in CNS lipids, points towards an impaired BBB function as the cause of CSF linoleic increase. We are able to show that both albumin and linoleic acid are suitable as "serum markers" and also as reference parameters for the overproportional IgG concentration in the CSF of MS patients. On the basis of these results it can be assumed that changes in CSF linoleic acid content are an expression of dysfunction of the blood-CSF barrier in MS and not, as had previously been postulated, the result of altered myelin metabolism.

Key words: Cerebrospinal fluid – Demyelination – Fatty acid composition – Linoleic acid.

Zusammenfassung. Im Gegensatz zu früheren Untersuchungen wird erstmalig die absolute Linolsäurekonzentration im Liquor von MS-Patienten ($n = 10$) gegenüber Kontrollen ($n = 12$) verglichen. Hierbei zeigt sich, daß die gegenüber Serum normalerweise sehr viel niedrigere Liquorlinolsäurekonzentration in Liquores von MS-Patienten deutlich höhere Werte erreicht. Wie bei den Kontrollen wird hierbei jedoch eine sehr enge Korrelation zur Liquor-Albuminkonzentration beibehalten.

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Der hohe Anteil an linolsäurereichen Cholesterinestern im Liquor, die, wie die Linolsäure selbst, im ZNS nur in Spuren nachweisbar sind, dagegen aber reichlich im Serum, weist darauf hin, daß erhöhte Linolsäurewerte im Liquor Ausdruck der gestörten Schrankenfunktion sind. Wie wir in vergleichenden Untersuchungen zeigen konnten, sind Albumin und Linolsäure gleichermaßen als „Serummarker" verwertbar, auch als Referenzparameter zum Nachweis der bei der MS meist anzutreffenden überproportionalen IgG-Erhöhung im Liquor. Aufgrund dieser Befunde sind Änderungen in der Linolsäurekonzentration des Liquors Ausdruck der Schrankenstörung bei der MS und nicht — wie früher angenommen — Ausdruck eines gestörten Markscheidenstoffwechsels.

The majority of fatty acids (FA) in CSF are bound in ester-type linkage as glycerophosphatides and neutral lipids (cholesterol esters and triglycerides) [3, 12]; only a small fraction is amide-linked in the form of sphingolipids.

The first investigations of fatty acid profiles in CSF are closely related to the introduction of GLC techniques. Blomstrand [2] was the first to note an increased ratio of unsaturated fatty acids, particularly linoleic acid (C 18:2), in the CSF of three MS patients, with 25% as compared to controls with 4% a finding later confirmed by others [10, 13]. This change was interpreted as impaired myelin metabolism in the sense of demyelination [9, 10] whereby the concentration on C 18:2 increases in myelin lipids.

We correlated the absolute linoleic acid concentration in the CSF of MS patients and controls with the established parameters of albumin and IgG to test the hypothesis that CSF linoleic acid is primarily derived from serum rather than CNS lipids. To achieve this it was necessary to use an improved microanalytical technique with a single CSF specimen and to quantitate the absolute concentration of linoleic acid rather than the percent using C 17:0 as an internal standard.

Material and Methods

Control CSF was obtained from 12 patients (average age 40 years) with psychiatric or cerebrovascular disease without CSF abnormalities according to the usual criteria. These specimens were compared to CSF samples from ten patients with clinically definite MS (average age 37 years). All specimens were obtained by lumbar puncture, freed of cells by centrifugation, and subjected to the routine determinations (cell count, normomastix reaction, protein, electrophoresis, quantitative immunodiffusion of IgG and albumin).

Internal standard (10 nanomol C 17:0) was added to 1 ml cell-free CSF, total lipids were extracted with 5 ml chloroform/methanol 2:1 (v/v) and separated into two phases with 0.4 ml 0.1% KCl in H_2O. The lower layer was dried under nitrogen and infrared light, taken up with 1 ml 5% methanolic H_2SO_4, and transesterified for 4 h at 80°C in a water bath. After neutralization with 0.5 ml saturated bicarbonate of soda solution the resulting FAME were extracted with 3 times 1.5 ml hexane, the solvent evaporated, and the residue taken up in 10 µl toluol of which 1 µl was injected for GLC analysis as described in part I [11]. For statistical evaluation the mean value of double determinations (showing only about 5% variability) was used. IgG and albumin were determined by radial immunodiffusion in unconcentrated CSF [1, 14] with low concentration partigen plates (Behringwerke, Marburg).

Table 1. CSF basic data

	n	Cell count/mm^3	Protein (mg/l)	Albumin (mg/l)	IgG (mg/l)	Linoleic acid (nanomol/ml)
Normal	12	0— 1	$\bar{x}$ 384 SD 95	$\bar{x}$ 223 SD 76	$\bar{x}$ 27 SD 12.5	$\bar{x}$ 1.6 SD 0.8
MS	10	2—13	$\bar{x}$ 685 SD 470	$\bar{x}$ 502 SD 490	$\bar{x}$ 136 SD 97	$\bar{x}$ 3.9 SD 3.7

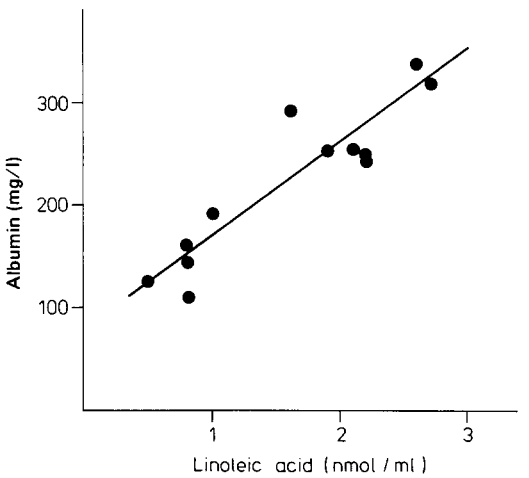

Fig. 1. Relationship between albumin and linoleic acid concentration in normal CSF

Results and Discussion

The results are shown in Table 1 giving mean ($\bar{x}$) and standard deviation (s). If linoleic acid (nanomol/ml) is related to the albumin concentration (mg/l) in normal CSF, a linear relationship evolves with $r = 0.92$ (Fig. 1) which is also maintained in MS-CSF ($r = 0.95$) although the mean albumin content has almost doubled.

Thus similar to the Q_G-quotient calculated according to the formula:

$$Q_G = \frac{\text{IgG (mg/l)} \times 100}{\text{albumin (mg/l)}} \quad \text{(upper normal limit: 20)}$$

we were able to form a Q_L-quotient:

$$Q_L = \frac{\text{IgG (mg/l)}}{\text{linoleic acid (nanomol/ml)}} \quad \text{(upper normal limit: 25)}$$

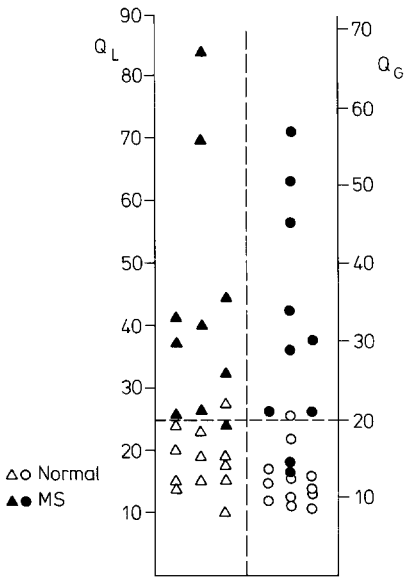

Fig. 2. Overproportional IgG increase in CSF of MS compared to controls using albumin (Q_G) and linoleic acid (Q_L) as serum reference parameter

The individual Q_G and Q_L values are displayed in Fig. 2, as expected from the Q_G values the Q_L values in MS-CSF were significantly higher than in controls. The mean Q_G values from normal CSF were 12.2 ± 3.6 and the Q_L values 18.2 ± 4.9. The comparable values from MS-CSF were Q_G 31.4 ± 15.2 and Q_L 42.4 ± 19.7. Both quotients can thus be used to define an overproportional IgG increase in the CSF from MS patients.

It has been known for some years [3, 7, 12, 13] that the FA profile of CSF lipids is very similar to serum with the exception of linoleic acid, which is only 4 relative percent compared to serum with 25 relative percent. Serum linoleic acid is mainly bound as esters in neutral lipids particularly cholesterol esters [6].

These are only present in very low concentrations in brain tissue while they are considerably more abundant in CSF [8] and it can be assumed that both cholesterol esters and linoleic acid in CSF are derived from serum. The close correlation to CSF albumin, which is exclusively synthesized in the liver and enters the CSF from the serum compartment, supports this assumption and CSF linoleic acid determined in absolute concentrations can be considered a suitable serum marker.

Since the straight line in Fig. 1 does not pass through the zero point it appears likely that cholesterol linoleate bound to lipoprotein rather than linoleic acid bound to albumin passes through the blood-CSF barrier. Lipoproteins are known to have a higher molecular weight and larger molecular volume than serum albumin [5].

Thus an increase in the absolute linoleic acid concentration of CSF can be taken as an indicator of dysfunction of the blood-CSF barrier. MS-CSF, while

retaining the close correlation between albumin and linoleic acid, shows an increase of CSF linoleic acid in proportion to albumin ($r = 0.95$). Patients with other inflammatory CNS diseases showed very high CSF linoleic concentrations [7] in addition to the often excessive increase of CSF protein. In this context the observation in a 79-year-old patient with pneumococcal meningitis is most impressive, there the CSF contained 6000 cells/mm^3, an albumin concentration of 2110 mg/l (representing $^1/_{20}$ of the serum value) and a linoleic acid content of 131 nanomol/ml (= $^1/_{20}$ of serum values).

Since the determination of albumin concentrations in CSF as serum marker became routinely possible this parameter has been introduced as the reference standard in comparison to IgG. Most patients with MS show an overproportional IgG increase in their CSF (expressed as Q_G) [4] and this points towards a local CNS synthesis of IgG in this disease. Using CSF linoleic acid concentration instead of albumin, a Q_L quotient can be calculated which is similarly suitable for demonstrating the overproportional IgG increase in CSF (Fig. 2). The increase of linoleic acid content in the CSF of MS patients is similar to the increase of albumin, an expression of the transsudative disturbance of the blood-CSF barrier between the blood and CSF compartments.

Alterations of the CSF lipid profile in MS patients—also demonstrated by the example of sphingolipid changes in part I [11]—are thus not an expression of a disturbance of myelin metabolism as had previously been postulated [9] but represent secondary phenomena dependent on an increase of CSF lymphocytes for sphingolipid increase and of dysfunction of the blood-CSF barrier for increased linoleic acid concentration. In the case of MS and similar demyelinating disease the usefulness of CSF lipid analysis for diagnostic purposes is limited at best to very special cases and in no sense a significant improvement of the routine CSF procedures commonly employed in the diagnosis particularly of MS.

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References

1. Augner, W.: Immunanalyse von Glycoproteinen. In: Proceedings, 12th colloqium on protides of the biological fluids, H. Peeters (ed.), pp. 363—371. Amsterdam: Elsevier 1965
2. Blomstrand, R., Dencker, S. J., Swahn, B.: The fatty acid profile of cerebrospinal fluid. Kungl. Fysiograf. Sällskap. Lund Förhandl. **30**, 21—26 (1960)
3. Blomstrand, R.: The fatty acid composition of cerebrospinal fluid lipids. Acta Chem. Scand. **14**, 775—776 (1960)
4. Eickhoff, K., Heipertz, R.: Discrimination of elevated immunoglobulin concentrations in CSF due to inflammatory reaction of the central nervous system and blood-brain barrier dysfunction. Acta Neurol. Scand. **56**, 475—482 (1977)
5. Felgenhauer, K.: Protein size and cerebrospinal fluid composition. Klin. Wschr. **52**, 1158—1164 (1974)
6. Karlsson, I., Alling, C., Svennerholm, L.: Major plasma lipids and their fatty acid composition in multiple sclerosis and other neurological diseases. Acta Neurol. Scand. **47**, 403—412 (1971)

7. Pazzagli, A., Arnetoli, G., Pepeu, I., Amaducci, L. A.: Fatty acid changes of cerebrospinal fluid in neurological disorders as an index of changes in the blood-brain barrier. Neurology **20**, 783—786 (1970)
8. Pedersen, H. E.: Cerebrospinal fluid cholesterol. Acta Neurol. Scand. **49**, 626—638 (1973)
9. Schrader, A., Schwarz, K.: Über den Gehalt an Metaboliten des Fettstoffwechsels im normalen und pathologischen Liquor. Münch. Med. Wschr. **105**, 2493—2500 (1963)
10. Seidel, D., Lindlar, F.: Über die Fettsäuren der Liquorlipide bei demyelinisierenden Erkrankungen. Z. Neurol. **198**, 223—236 (1970)
11. Seidel, D., Buck, R., Heipertz, R., Pilz, H.: Cerebrospinal fluid lipids in demyelinating disease. I. Sphingolipids. J. Neurol. **212**, 171—176 (1979)
12. Tichy, J., Alling, C., Dencker, S. J., Svennerholm, L.: Fatty acid profiles of cerebrospinal fluid lipids in normals and chronic alcoholics. Scand. J. Clin. Lab. Invest. **26**, 191—197 (1970)
13. Tuna, N., Logothetis, J., Kammereck, R.: The fatty acids of the human cerebrospinal fluid: their relation to the serum fatty acids. Neurology **13**, 331—335 (1963)
14. Weisner, B., Bernhardt, W.: Protein fractions of lumbar, cisternal, and ventricular cerebrospinal fluid. J. Neurol. Sci. **37**, 205—214 (1978)

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