SHORT REPORT

Folate deficiency in cerebrospinal fluid associated with a defect in folate binding protein in the central nervous system

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Abstract

An adult male patient of Dutch ancestry has a slowly progressive neurological disease characterised by a cerebellar syndrome, distal spinal muscular atrophy, pyramidal tract dysfunction, and perceptive hearing loss. A severe folate deficiency state was found in CSF in combination with a normal serum and red cell folate state. Two unknown abnormal metabolites were present in CSF. The concentration of immunoreactive folate binding protein in CSF was unusually low, whereas the concentration of the protein measured with radioligand (\(^{3}H\)-folate) binding was unusually high. The transfer of folate over the choroid plexus seems to be disturbed, potentially reflecting a defect in the choroid plexus folate binder.

Methods

Folate and cobalamin were measured with a radioassay (Biorad Quantaphase Nr 1911001, Richmond, California, USA).\(^{11}\) Boiling was used to inactivate endogenous binding proteins. Reduced folate and its analogues are stabilised by dithiothreitol during heating. Immobilised affinity purified porcine intrinsic factor and folate binding protein are used in this assay. Results on folate for our patient were confirmed with another radioassay (Nr KBDS1, Diagnostic Products Corporation, Los Angeles, USA). Folate binding protein in CSF was measured quantitatively with an enzyme linked immunosorbent assay (ELISA), with rabbit antibodies against purified human milk folate binding protein.\(^{7}\) Radioligand (\(^{3}H\)-folate) binding of folate binding protein in CSF and Aca 44 gel filtration chromatography were performed as described earlier.\(^{4}\)

Case report

An 18-year-old man was seen at the neurological outpatient department because of difficulties in using his hands and feet. He was born after an uncomplicated pregnancy and delivery. Motor and mental development had been normal. He went through common childhood infections without complications or sequelae. At the age of 9 he developed a
rapidly progressive bilateral sensorineural hearing loss. When he was 18 years old he noticed difficulties in writing and in using his feet when playing the organ. Family history revealed that his parents were consanguineous, in having the same ancestors eight generations ago. His father had died from a myocardial infarction at age 48, whereas his mother and brother were healthy. On neurological examination we saw a leptomeningeal young man with normal cognitive functions. There was dysarthria, dysgraphia, an action tremor of the right hand, and slight gait ataxia. Muscle tone and sensation were normal. Except for pronounced Achilles reflexes, the reflex pattern was unremarkable. EEG showed a dominant α-frequency of 9-10 Hz. X-ray films of the skull, ossa petrosa, cranio-vertebral region, and cervical spine were normal, as was cranial CT. Extensive laboratory studies were negative (see later). A neurodegenerative disorder of unknown cause was suspected. Two years later he complained of low back pain, radiating to both legs. The radiological evidence of bilateral sacroiliitis and the presence of HLA B27 led to the diagnosis of ankylosing spondylitis. At the age of 21 he noticed exercise related muscle cramps in his legs. Findings on neurological examination were unchanged. When he was 26 years, a slowly progressive weakness of the distal extremities became apparent. Neurological examination showed mild distal paresis of both legs, moderate atrophy and fasciculations in the distal musculature of arms and legs, a broad-based gait, intention tremor of both hands, and dysdiadochokin. There was generalised hyperreflexia—except for normal Achilles reflexes—and extensor plantar responses. Muscle tone was not increased. Ancillary examinations including erythrocyte sedimentation rate, white blood cell count, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ globulin, immunoferritin, serum creatinine, sedimentation rate, rheumatoid factor, antinuclear antibodies, and toxoplasma were normal or negative. He had a normal haematological state (Hb/Ht, mean corpuscular volume) and normal serum concentrations for iron, iron binding capacity, transferrin, ferritin, haptoglobin, and carnitine (free and bound). Amino acid analysis in serum was unremarkable. There were no abnormalities in organic acids, amino acids, purines, and pyrimidines in urine. Appropriate investigations ruled out endocrine and ophthalmological disorders. The EEG showed a slowly progressive diffuse slowing of the background activity (dominant α-frequency 6-7 Hz vs 9-10 Hz 10 years earlier). Cranial CT showed cerebral and cerebellar atrophy and bilateral hypodensities in the basal ganglia. There was no contrast enhancement. On electromyography (EMG) normal sensory and motor conduction velocities and conduction times were seen in upper and lower extremities. The lower leg and hand muscles showed a single unit pattern with giant potentials (>5 mV). Somatosensory evoked potentials were normal. Histological and histochemical examination of a quadriceps muscle biopsy was normal; there were no ragged red fibres. Until now, symptoms and signs have worsened slowly but steadily. In summary, our patient suffered from a rapidly progressive sensorineural hearing loss, followed by a slowly progressive neurological syndrome consisting of cerebellar and pyramidal tract dysfunction and spinal muscular atrophy. Cranial CT findings and EEG indicated a progressive encephalopathy.

Results

Analysis of CSF

No significant biochemical abnormalities were found in serum or urine (case report). Total protein in CSF was raised (table 1). A transudatory phenomenon was a constant finding. High CSF concentrations of neuron-specific enolase, lactate dehydrogenase, and aspartate aminotransferase were found indicating persistent neuronal damage. Serum lactate dehydrogenase and aspartate aminotransferase were normal. The CSF always had a xanthochromic aspect over the 3-year follow up period (absorbance at 415 nm ranged between 0.64 and 104 mAbs). There were no indications for an immunological response within the CNS and an infectious aetiology was ruled out. Analysis of amino acids in CSF according to Gerrits et al12 revealed high amounts of two o-phthalaldehyde positive metabolites that have not been identified. One of the metabolites elutes just before methionine, the other immediately after phenylalanine. Only the first could be hydrolysed under strongly acidic conditions. Both peaks are always present but in much lower, almost undetectable concentrations in CSF samples of patients with other neurological diseases. They could not be shown in our patient’s serum or urine. Analysis of organic acids and purines and pyrimidines in CSF showed no abnormalities.

Folate and cobalamin

Folate in CSF was very low on several occasions when serum folate was normal (table 2). Similar results were obtained when a different commercially available radioimmunoassay

Table 1 Analysis of CSF

<table>
<thead>
<tr>
<th>Component</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (mg/l)</td>
<td>1428</td>
</tr>
<tr>
<td>Leucocytes (×10⁶)</td>
<td>2</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>3.1</td>
</tr>
<tr>
<td>Lactate (µM)</td>
<td>1460</td>
</tr>
<tr>
<td>LD (U/l)</td>
<td>157</td>
</tr>
<tr>
<td>ASAT (U/l)</td>
<td>89</td>
</tr>
<tr>
<td>Astrocyte protein (µg/l)</td>
<td>1.9</td>
</tr>
<tr>
<td>Neuron specific enolase (µg/l)</td>
<td>21</td>
</tr>
<tr>
<td>S100 (µg/l)</td>
<td>1.7</td>
</tr>
<tr>
<td>IgG (mg/l)</td>
<td>108</td>
</tr>
<tr>
<td>IgG index</td>
<td>0.45</td>
</tr>
<tr>
<td>IgA index</td>
<td>0.36</td>
</tr>
<tr>
<td>IgM index</td>
<td>0.26</td>
</tr>
<tr>
<td>γ sub-bands on isoelectric focusing</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not detectable.
was used and with the *Lactobacillus casei* assay. A mixing experiment with CSF from our patient and another patient (1:1) resulted in an intermediate folate concentration. Red blood cell folate in our patient was in the low normal range (371, reference range 303–1937). Without any additional vitamin supplementation the concentration of cobalamin in CSF was significantly above our reference range but the concentration in serum was normal.

### Folate binding protein

We have found a very low concentration of immunoreactive folate binding protein in CSF of our patient as determined by ELISA. Results for two different CSF samples were 0·08 and 0·09 nmol/l (reference range 0·14–0·38 nmol/l; n = 20). A similar result (0·083 nmol/l) was found after incubation of CSF by a factor 10 before the assay. Other CSF samples that were sent simultaneously from The Netherlands to Denmark for control purposes had normal concentrations of folate binding protein, thus excluding an artefact resulting from shipping the samples.

The relative molecular mass (Mr) of folate binding protein in CSF was estimated by gel filtration on Ultrogel AcA 44 after preincubation with [3H]-folate (fig. 4). The figure illustrates the profound deficiency of immunoreactivity in both of the known CSF peaks.

### Table 2: Folate (nmol/l) and cobalamin (pmol/l) in CSF and serum on two occasions

<table>
<thead>
<tr>
<th>Folate</th>
<th>Cobalamin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>Serum</td>
</tr>
<tr>
<td>I</td>
<td>2·6</td>
</tr>
<tr>
<td>II</td>
<td>1·4</td>
</tr>
</tbody>
</table>

Our results indicate that folate binding protein of CSF of patient was similar to that of folate binding protein in pooled serum samples (data not shown). The results indicate that his CSF contains above normal amounts of a non-immunoreactive folate binding protein form of apparent Mr normal M, and normal folate binding characteristics.

Experiments were performed to establish whether ligand binding would change the immunoreactivity of folate binding protein in CSF. The immunoreactivity of folate binding protein in CSF saturated with folate was similar to that of the unsaturated form of the protein both in CSF of our patient as well as in a CSF pool. Mixing CSF from our patient with a CSF pool resulted in the expected intermediate folate binding protein value when measured by ELISA.

### Discussion

Low CSF folate concentrations have been found in Kearns–Sayre syndrome and inborn errors of folate metabolism. In some Kearns–Sayre cases the combination of normal plasma folate with very low CSF folate as found in our patient has been described. A similar finding was made in human immunodeficiency virus infection. Both diagnoses can be ruled out in our patient. The combined findings of extremely low CSF folate concentration, low relative immunoreactivity for folate binding protein in CSF, and normal serum folate concentration suggest a defective folate transport through the choroid plexus into the CNS, and a causative role for CNS folate deficiency in the development of the neurological syndrome.

In established inherited disorders of folate metabolism, like methylene tetrahydrofolate reductase deficiency and glutamate formiminotransferase deficiency, prominent neurological features are developmental delay, motor and gait abnormalities, seizures, and psychiatric manifestations. On pathological examination patients with these enzyme
deficiencies had low brain weight, cortical atrophy, and dilated ventricles. One patient with hereditary folate malabsorption had calcification of the basal ganglia.17 By analogy, the cerebral and cerebellar atrophy and bilateral hypodensities in the basal ganglia, as found in our case, may well result from folate deficiency within the CNS. Considering the clinical heterogeneity in inherited disorders of folate transport and metabolism, the symptoms and signs in our patient seem compatible with an inherited disorder causing the folate deficiency state in the CNS.

Twelve patients have been identified with hereditary gastrointestinal malabsorption of folate.1 Among these are the cases of Luhby et al18 and Lanzkowsky19 where the defect in the gastrointestinal absorption of folic acid was combined with a defect in the transport of folate from plasma into the CSF. Most of these patients showed progressive neurological dysfunction, including mental retardation, ataxia, atheletic movements, and convulsions. Remarkably, the only boy reported with this disorder had no signs of mental retardation.19 Our patient has normal serum and red blood cell folate reflecting adequate dietary intake and undisturbed intestinal absorption. By contrast with the patients described by Luhby18 and Lanzkowsky19 our patient has an isolated defect in the transport of folate over the choroid plexus. In this respect we have demonstrated extremely low relative immunoreactivity (immunoreactivity/radioligand binding capacity) for folate binding protein in CSF. Hardly any immunoreactivity was seen in the gel filtration profile where the folate binding proteins normally elute. Instead immunoreactive material eluted immediately after the void volume in fractions not associated with any folate binding activity. Similar immunoreactive material without folate binding capacity was found in urine of our patient and is not detectable in urine of healthy volunteers (data not shown). Furthermore the non-immunoreactive fractions eluting in the position of folate binding protein peak II were able to bind high amounts of radiolabelled folate, thus proving the presence of normal molecular size folate binding protein in our patient’s CSF. Radioligand binding affinity of this folate binding protein was normal, indicating an intact folate binding site. The low relative immunoreactivity can be explained by assuming a molecular defect in folate binding protein in CSF. In view of the proposed interrelation between folate binding protein in CSF and the choroid plexus,8-10 it is possible that choroid plexus folate binding protein in our patient shares these characteristics. This putative defect may explain the observed inadequate transport of folate from blood to CSF. Potentially it defines an abnormality in folate accumulation not previously recognised. The sporadic occurrence of this case and the consanguinity of the parents suggest an autosomal recessive mode of inheritance.

Additional interesting observations were the two unknown metabolites in CSF amino acid analysis, the transudation, increased CSF concentration of neuron specific enolase, and an increased concentration of cobalamin in CSF. Explanations must be speculative, but these abnormalities are likely to reflect secondary changes.

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