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Cerebrospinal fluid ATP metabolites in multiple sclerosis

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Abstract

Increased axonal energy demand and mitochondrial failure have been suggested as possible causes for axonal degeneration and disability in multiple sclerosis.

Our objective was to test whether ATP depletion precedes clinical, imaging and biomarker evidence for axonal degeneration in multiple sclerosis.

The method consisted of a longitudinal study which included 21 patients with multiple sclerosis. High performance liquid chromatography was used to quantify biomarkers of the ATP metabolism (oxypurines and purines) from the cerebrospinal fluid at baseline. The Expanded Disability Status Scale, MRI brain imaging measures for brain atrophy (ventricular and parenchymal fractions), and cerebrospinal fluid biomarkers for axonal damage (phosphorylated and hyperphosphorylated neurofilaments) were quantified at baseline and 3-year follow-up.

Central ATP depletion (sum of ATP metabolites $>19.7\,\mu$mol/litre) was followed by more severe progression of disability if compared to normal ATP metabolites (median 1.5 versus 0, $p < 0.05$). Baseline ATP metabolite levels correlated with change of Expanded Disability Status Scale in the pooled cohort ($r = 0.66$, $p = 0.001$) and subgroups (relapsing–remitting patients: $r = 0.79$, $p < 0.05$ and secondary progressive/primary progressive patients: $r = 0.69$, $p < 0.01$). There was no relationship between central ATP metabolites and either biomarker or MRI evidence for axonal degeneration.

The data suggests that an increased energy demand in multiple sclerosis may cause a quantifiable degree of central ATP depletion. We speculate that the observed clinical disability may be related to depolarisation associated conduction block.

Keywords

Neurofilament phosphoforms, multiple sclerosis, prognosis, disease subtype, disability, axonal injury

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Introduction

Progression of disability in multiple sclerosis (MS) is hypothesized to be ultimately linked to axonal loss.[1] One of the mechanisms driving axonal degeneration has been suggested to result from virtual hypoxia.[2] There is some evidence that the increased expression of sodium channels along demyelinated axons requires an increased amount of ATP even at a resting state.[3] This places an increased energy demand and may result in an additional energy penalty of neuroelectrical conduction in the damaged axons. It has been proposed that the exhaustion of ATP stores results in loss of protective ion pump function leading to intracellular Ca^{2+} overload which then activates a 'death cascade'.[2,4] Consequently, the disintegrating axons release their cellular content into the adjacent body fluid compartment, the extracellular fluid from were they diffuse into the cerebrospinal fluid (CSF).[5] The editorial by

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Waxman[4] refers to a landmark paper by Kapoor et al. on axonal vulnerability. Kapoor et al. demonstrated convincingly how electrically active axons degenerate in a biochemically hostile environment. Axonal loss and neurodegeneration are currently understood to be followed by brain atrophy and disability progression.[1,6]

Energy available to cells depends crucially on the availability of ATP. In MS there is accumulating evidence for a mismatch between energy production and energy consumption which has been linked genetically and histologically to mitochondrial dysfunction.[7–10] Immunohistochemical and histochemical studies suggested that particularly complex IV of the respiratory chain may be affected in demyelinated axons.[8] These authors also showed that the inhibition of complex IV augmented glutamate mediated axonal injury,[8] a mechanism well recognized from cell biology.[11] Mitochondrial failure is known to be enhanced by oxidative stress,[12] for which there is convincing postmortem evidence in MS.[7,9] The biochemical consequence of mitochondrial failure is a metabolic imbalance between ATP consumption and ATP production.[13–15] There is a correlation between ATP depletion and concomitant increase of ATP breakdown products (purines and nucleosides).[16–19]

Therefore, exhaustion of ATP results in an increase of the end products of the metabolic pathway.[16–19] The dominant end products of this pathway are oxypurine (uric acid, hypoxanthine, and xanthine) and purine nucleosides (inosine, adenosine, and guanosine).[16–19] Higher CSF concentrations of total purine nucleosides and total oxypurines in patients with MS compared to control subjects provide indirect evidence for increased ATP consumption in MS.[20] This finding raises the question whether increased *in vivo* ATP consumption in patients with MS could precede axonal degeneration as suggested by the energy insufficiency/virtual hypoxia hypothesis.[2]

In this prospective longitudinal study, we tested if ATP depletion at baseline would predict the development of axonal degeneration assessed by three methods: a clinical scale for disability (the Expanded Disability Status Scale, EDSS), MRI brain atrophy measures, and protein biomarkers for axonal damage.

Materials and methods

This study was approved by the Institutional Review Board (IRB) and written informed consent was obtained from all patients.

Two CSF samples (baseline and three year follow-up) were available from 21 patients of a previously reported cohort[21] with clinically definite MS.[22] The EDSS score[23] was recorded at baseline and 3-year follow-up. Progression of disability was calculated as ΔEDSS = follow-up EDSS - baseline EDSS. MRI examinations were performed at 1.0 T or 1.5 T at baseline and follow-up. In brief, as measures for brain atrophy we used (1) the parenchymal fraction (PF), calculated as the ratio of the whole brain parenchyma to the intracranial volume and (2) the ventricular fraction (VF) calculated as the ratio of the ventricular volume to the whole brain parenchyma.[24] The change between baseline and follow-up was calculated as ΔPF = follow-up PF - baseline PF and ΔVF = follow up - VF - baseline VF.

Samples of CSF were obtained by routine lumbar puncture. Aliquots of CSF were centrifuged to remove cellular debris and immediately stored at -70°C until assayed. CSF levels of ATP metabolites (uric acid, hypoxanthine, xanthine, inosine, adenosine, and guanosine) were quantified by high performance liquid chromatography (HPLC).[20] The sum of the ATP metabolite levels was considered high if above the range observed in the control population (mean 13.87, SD 5.83; cut-off >19.7 µmol/litre).[20] CSF biomarker levels for axonal damage, the phosphorylated neurofilament heavy chain (NfH^{SMI35}) and hyperphosphorylated neurofilament heavy chain (NfH^{SMI34}) were quantified by enzyme-linked immunosorbent assay (ELISA)[25] at baseline and 3-year follow-up. The change between baseline and follow-up was calculated as ΔNfH = follow-up NfH - baseline NfH.

Data analysis

All statistical analyses and graphs were carried out using SAS software (version 9.1.3, SAS Institute, Inc., Cary, North Carolina, USA). Because of non-Gaussian distribution, the median values and the 25–75% interquartile range (IQR) were shown. Independent variables were compared using the non-parametric Kruskall–Wallis test. Analyses of covariance were performed with general linear models. The linear relationship between continuous variables was evaluated using the Spearman's correlation coefficient.

Results

The baseline characteristics of the patients are summarized in Table 1. One-third (7/21) of the MS patients had normal CSF ATP metabolite levels (<19.7 µmol/litre). Two-thirds (14/21) of the MS patients showed evidence of ATP depletion (Table 1). There was no significant difference between the two groups in terms of age, gender distribution, disease duration, or treatment with disease modifying drugs. The median EDSS appeared to be slightly higher in patients with normal ATP stores, but data distribution was overlapping and no statistically significant difference was found (Table 1).

Table 1. Baseline characteristics of MS patients. Patients were classified at baseline according to their central ATP metabolism. The median (IRQ) is shown

	MS (all)	MS (normal ATP)	MS (depleted ATP)	*p*-value
Age (years)	45 (38–51)	49 (40–55)	43 (35–48)	NS
Gender (F:M)	12:9	4:3	8:6	NS
Disease duration (years)	13.9 (9.5–19.1)	19.1 (12.6–22.0)	13.45 (7.4–17.0)	NS
Interferon (IFN)	8 (38%)	3 (43%)	5 (34%)	NS
EDSS	4.0 (2.0–6.0)	6.0 (2.5–6.5)	2.25 (1.5–5.5)	NS
MRI PF	0.81 (0.79–0.83)	0.80 (0.77–0.83)	0.82 (0.79–0.83)	NS
MRI VF	0.03 (0.02–0.04)	0.03 (0.03–0.04)	0.03 (0.02–0.04)	NS
CSF NfH^{SMI34}	9 (7–13)	11 (7–19)	9 (7–12)	NS
CSF NfH^{SMI35}	78 (17–155)	139 (31–190)	575 (110–123)	NS
Number	21	7	14	

CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; IFN, interferon; IRQ, interquartile range; NfH, neurofilament heavy chain; NS, not significant; PF, parenchymal fraction; VF, ventricular fraction.

Table 2. Change from baseline to 3-year follow-up. The median (IRQ) is shown

	MS (all)	MS (normal ATP)	MS (depleted ATP)	*p*-value
Δ EDSS	0.05 (0–1.5)	0 (−0.5–1.0)	1.5 (0–2.0)	0.035
Δ MRI PF	0.015 (−0.005–0.015)	0.02 (−0.03–0.02)	0.01 (0–0.04)	NS
Δ MRI VF	−0.005 (−0.01–0)	−0.01 (−0.01–0)	0 (−0.01–0)	NS
Δ CSF NfH^{SMI34}	19 (−3–51)	28 (10–95)	2 (−7–51)	NS
Δ CSF NfH^{SMI35}	0 (−78–87)	−11 (−91–68)	41 (−78–104)	NS

CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; IRQ, interquartile range; NfH, neurofilament heavy chain; NS, not significant; PF, parenchymal fraction; VF, ventricular fraction.

This observation was, however, taken into account for a post hoc covariate analysis (see below). Likewise, there was no statistically significant difference between MRI atrophy measures (PF, VF) and axonal damage biomarkers (NfH^{SMI34}, NfH^{SMI35}).

Three years later, those patients with evidence for central ATP depletion progressed significantly more on the EDSS (median + 1.5 points) compared to those with normal central ATP stores (median 0 points, $p = 0.035$, Table 2). No difference was found for either MRI atrophy measures (ΔPV, ΔVF) or axonal damage biomarkers (ΔNfH^{SMI34}, ΔNfH^{SMI35}).

Importantly, CSF ATP metabolites correlated significantly with the degree of disease progression on the EDSS in the entire cohort ($r = 0.67$, $p = 0.001$, Figure 1). This correlation was not influenced by the disease subtype. In fact, the subgroup analysis revealed that significance was retained in relapsing–remitting (RR) disease ($r = 0.79$, $p < 0.05$) and secondary progressive/primary progressive (SP/PP) disease ($r = 0.69$, $p < 0.01$).

In a post hoc analysis the baseline EDSS was identified as a relevant covariate for the statistical analyses on disease progression. Controlling for this covariate reduced the difference for CSF ATP metabolites below noise ($F = 2.54$, $p = 0.12$).

No correlations were found between CSF ATP metabolite levels and the patients' age, disease duration, MRI atrophy measures, or axonal damage biomarkers (data not shown).

Discussion

The main finding of this prospective longitudinal study was that patients who suffer from MS and have indirect biomarker evidence for depletion of ATP energy stores also suffered from significantly more disability progression over the following 3 years compared to patients who had normal ATP energy stores. This finding is consistent with the concept that a central energy penalty may possibly contribute to disability progression in MS.[2]

The conclusions derived from the data on ATP metabolites need to be circumspect because numbers were small and an unexpected finding of this study was that central ATP depletion did not lead to any detectable degree of axonal damage either by MRI atrophy measures or axonal damage biomarkers. This biochemical–radiological

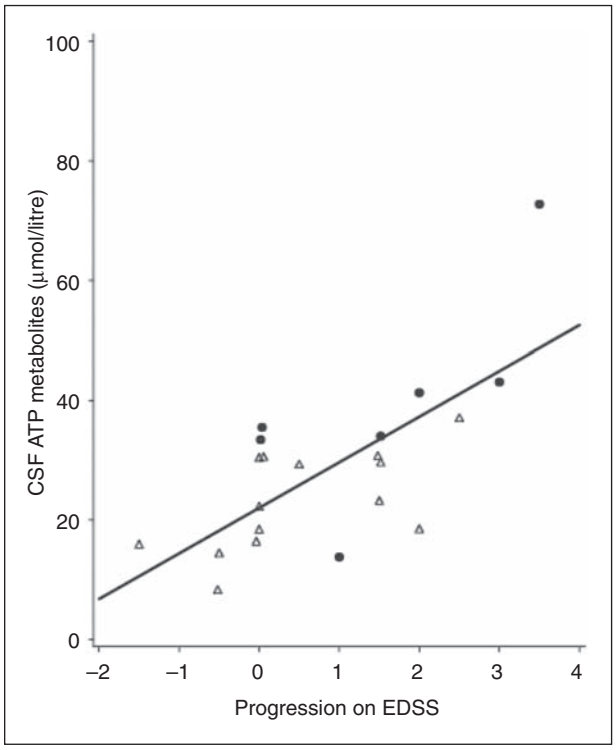

Figure 1. Baseline cerebrospinal fluid (CSF) ATP metabolite levels correlated with the subsequent progression on the Expanded Disability Status Scale (EDSS) over the 3-year observation period in the pooled cohort ($r = 0.67$, $p = 0.001$). Patients with relapsing–remitting (RR) disease are indicated by closed circles and patients with secondary progressive/primary progressive (SP/PP) disease by open triangles. Significance of the correlation was retained in the subgroup analyses (SP/PP disease: $r = 0.69$, $p < 0.01$; RR disease: $r = 0.79$, $p < 0.05$).

paradox is in line with a previous observation by Narayana et al. who also found that a significant drop of *N*-acetyl aspartate (NAA) could just be transient and was not necessarily followed by axonal loss.[26] The findings of Narayana's and our study may possibly be linked because both NAA and ATP are highly concentrated in mitochondria.[27] The synthesis of NAA depends on ATP. Thus, the indirect evidence for an impaired ATP metabolism from our study could be related to a decrease of neuronal NAA concentrations. Decreased NAA peaks were correlated to disability in a number of studies.[28] It could therefore be speculated that a decrease of ATP may also indicate dysfunction of the mitochondrial energy metabolism, reduced NAA synthesis, and disability. As mentioned above, the present biomarker data is only indirect and such a hypothesis would need to be tested prospectively combining CSF and serial proton magnetic resonance spectroscopic imaging in another, much larger cohort of MS patients. It needs also to be borne in mind that the present data like other clinical in vivo studies on this hypothesis [29], was only correlative. A statistically significant correlation is by no means a proof of causality. This is further emphasized by the differences of the EDSS between the groups at baseline, which are also not statistically significant, may still have influenced disability progression. Proof of causality will need to be provided experimentally.

How can an ATP energy penalty lead to disability progression without quantifiable evidence (MRI and protein biomarkers) for neuroaxonal loss? One possible explanation is that physiologically disability may result from neuroelectrical conduction block.[30] Biochemically, it has been shown that synaptic mitochondria are considerably more vulnerable to an ATP deficit than non-synaptic mitochondria.[31,32] It may therefore be possible that the observed increase of ATP metabolites which we interpret in this context as indirect biomarker evidence for ATP depletion may suffice to block transmission at the synaptic level. Admittedly, this conclusion is speculative. An alternative explanation is that conduction could be blocked due to the enhanced energy demand following increased expression of sodium channels.[3] Finally, conduction may be blocked on a ganglionic level. Rush et al. showed for voltage-gated sodium channels (Na1.7) that a single mutation (L858H) can cause hypopolarization (increased threshold and attenuated repetitive firing) in one cell population and hyperpolarization in another cell population.[33] Crucially, the polarization pattern depended on the presence of Na1.8 channels.[33] Experimentally, it has been shown that Na1.8 channels were up-regulated in Purkinje cells of an animal model of MS.[34] In addition, there is human post-mortem evidence for altered expression of Na1.2 and Na1.6 channels in MS plaques.[35] It has therefore been proposed that MS has features of an acquired channelopathy.[36]

To summarize, the present data provides indirect evidence for an impaired ATP metabolism in multiple sclerosis. There is some evidence that an impaired ATP metabolism may be related to depolarization and conduction block on at least three anatomically distinct levels (synaptic, axonal, and ganglionic). This would explain the relationship between loss of function and indirect biomarker evidence for an impaired ATP metabolism without indirect evidence for substantial axonal loss on a brain imaging or CSF protein biomarkers level. An important limitation of this argument is that conduction block is understood to be reversible. On the background of our current understanding of conduction block it would therefore be difficult to explain how depolarization caused an impaired ATP metabolism could possibly lead to sustained disability. In fact, in a post hoc analysis the degree of baseline disability was identified as an important covariate for the statistical results. Therefore, any future study aiming to test the validity of the proposed model would need to compare groups of MS patients which

are matched for their disease characteristics and possibly also their co-morbidity prospectively. Another limitation of this study is that the PF/VF measurements were performed before semi-automated protocols such as SIENA had been validated.[37] Future, better powered studies could include more detailed and localized atrophy rates in combination with MR spectroscopy on NAA and possibly also emerging biomarkers such as optical coherence tomography of the retinal nerve fibre layer.

Taken together, our data suggests that an impaired ATP metabolism may be associated with disability progression in MS, possibly due to depolarization related neuroelectrical conduction block. Considering the possibility of conduction block may be found useful in refining the virtual hypoxia hypothesis.[2]

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