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Cerebrospinal fluid kynurenines in multiple sclerosis; relation to disease course and neurocognitive symptoms

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ABSTRACT

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the central nervous system, with a high rate of neurocognitive symptoms for which the molecular background is still uncertain. There is accumulating evidence for dysregulation of the kynurenine pathway (KP) in different psychiatric and neurodegenerative conditions. We here report the first comprehensive analysis of cerebrospinal fluid (CSF) kynurenine metabolites in MS patients of different disease stages and in relation to neurocognitive symptoms.

Levels of tryptophan (TRP), kynurenine (KYN), kynurenic acid (KYNA) and quinolinic acid (QUIN) were determined with liquid chromatography mass spectrometry in cell-free CSF. At the group level MS patients (cohort 1; $n = 71$) did not differ in absolute levels of TRP, KYN, KYNA or QUIN as compared to non-inflammatory neurological disease controls ($n = 20$). Stratification of patients into different disease courses revealed that both absolute QUIN levels and the QUIN/KYN ratio were increased in relapsing-remitting MS (RRMS) patients in relapse. Interestingly, secondary progressive MS (SPMS) displayed a trend for lower TRP and KYNA, while primary progressive (PPMS) patients displayed increased levels of all metabolites, similar to a group of inflammatory neurological disease controls ($n = 13$). In the second cohort ($n = 48$), MS patients with active disease and short disease duration were prospectively evaluated for neuropsychiatric symptoms. In a supervised multivariate analysis using orthogonal projection to latent structures (OPLS-DA) depressed patients displayed higher KYNA/TRP and KYN/TRP ratios, mainly due to low TRP levels. Still, this model had low predictive value and could not completely separate the clinically depressed patients from the non-depressed MS patients. No correlation was evident for other neurocognitive measures. Taken together these results demonstrate that clinical disease activity and differences in disease courses are reflected by changes in KP metabolites. Increased QUIN levels of RRMS patients in relapse and generally decreased levels of TRP in SPMS may relate to neurotoxicity and failure of remyelination, respectively. In contrast, PPMS patients displayed a more divergent pattern more resembling inflammatory conditions such as systemic lupus erythematosus. The pattern of KP metabolites in RRMS patients could not predict neurocognitive symptoms.

1. Introduction

The kynurenine pathway (KP) comprises a chain of steps starting with the breakdown of the essential amino acid tryptophan (TRP), which through subsequent enzymatic reactions results in the formation of a number of neuroactive metabolites that include kynurenic acid (KYNA) and quinolinic acid (QUIN). KYNA is mainly produced in astrocytes, at low concentrations (IC50 ≈ 8–15 μM), it blocks the strychnine-insensitive glycine site of the glutamatergic receptors N-metyl-D-aspartate (NMDA) (Birch et al., 1988; Kessler et al., 1989; Parsons et al., 1997) and the cholinergic α7 nicotinic receptors (α7-NAChR; IC50 ≈ 7 μM) (Grilli et al., 2006). At higher concentrations, it antagonizes the glutamate recognition site of the NMDA receptor (IC50 ≈ 0.2–0.5 mM) and at even higher, millimolar, concentrations it blocks kainate and α-amino-3-hydrox y-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Perkins and Stone, 1982). Moreover, KYNA is as an endogenous agonist of

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the GPR35 receptor (Wang et al., 2006), and a ligand of the aryl hydrocarbon receptor (AhR) (DiNatale et al., 2010). An alternative metabolic pathway will generate QUIN, which is produced mainly by resident microglia and acts as an agonist of NMDA receptors (IC50 ≈ 180 μM) (Stone, 1993), but can also increase glutamate transmission directly by stimulating glutamate release from neurons and inhibiting astrocyte glutamate removal (Tavares et al., 2002, 2000; Ting et al., 2009). Therefore the balance between different metabolites is likely to be of importance, as KYNA can be neuroprotective (Stone, 1993) by counteracting the potentially excitotoxic effects of QUIN (Sapko et al., 2006; Schwarcz et al., 2010). In addition, QUIN can also induce neurotoxic effects through lipid peroxidation mediated by oxidative stress (Behan et al., 1999; Rios and Santamaria, 1991; Santamaria et al., 2001) and by promoting disruption of the blood brain barrier (Reynolds and Morton, 1998; St'astny et al., 2000; Steiner et al., 2012).

Alterations in the KP pathway have been described in a number of psychiatric conditions, including schizophrenia (Erhardt et al., 2001; Nilsson et al., 2005), bipolar disorder (Olsson et al., 2012) and suicidality (Bay-Richter et al., 2015; Erhardt et al., 2013), but also in neurodegenerative conditions like Alzheimer's (AD), Parkinson's, Huntington's (HD) diseases and amyotrophic lateral sclerosis (ALS) (Schwarcz et al., 2012). Thus, in psychosis, KYNA and its precursor KYN are increased in cerebrospinal fluid (CSF) and post mortem brain tissue (Erhardt et al., 2001; Sathyasaikumar et al., 2011; Schwarcz et al., 2001). In suicide attempters, often suffering from major depression, increased CSF levels of QUIN have been observed (Bay-Richter et al., 2015; Erhardt et al., 2013). Conversely, lowered levels of CSF KYNA have been observed in AD and HD (Heyes et al., 1992; Vecsei et al., 2013), which could promote the neurodegenerative processes of these conditions. Moreover, increased expression of indoleamine 2,3-dioxygenase (IDO) and QUIN has been observed in microglia of ALS patients (Chen et al., 2010). Notably, IDO, which is a rate-limiting enzyme that catalyzes the first step in the pathway, is activated by pro-inflammatory cytokines (Campbell et al., 2014; Mandi and Vecsei, 2012). In addition, in a recent landmark study, chronic mild stress was shown to alter systemic KP metabolism, in turn leading to inflammatory activation in the hippocampi and depression-like behavior in mice (Agudelo et al., 2014).

In light of an increasing body of evidence demonstrating that the KP pathway becomes activated in inflammatory conditions and can mediate neurocognitive symptoms it is of relevance to explore to what extent a dysregulation of the KP may be present in MS, a condition where neuroinflammatory activation and neurocognitive symptoms are hallmarks.

So far, alterations in CSF KYNA levels in MS have been reported for two smaller groups of MS patients, with lower levels compared to controls in the first study (Rejdak et al., 2002), and higher levels in patients experiencing clinical exacerbation (Raison et al., 2010; Rejdak et al., 2007). The KP has also been studied in blood of MS patients (Amirkhani et al., 2005) but the relevance of changes occurring in the periphery regarding intrathecal effects is uncertain since the correlation between KP blood and CSF levels is limited and varies depending on the KP metabolite measured (Raison et al., 2010).

The aims of this study were to (i) investigate if different MS courses are reflected in changes of KP metabolites and (ii) to examine the relationship between kynurenines and neurocognitive symptoms in MS patients.

2. Material and methods

2.1. Clinical material

This study includes two cohorts of patients and controls attending the Neurology Clinic, Karolinska University Hospital, Solna. Cohort 1 ($n = 71$) consists of patients where CSF samples have been obtained during diagnostic workup or for other clinical purposes and includes both untreated MS patients ($n = 38$) and patients with other neurological conditions (OND; $n = 20$), as well as a group of patients with inflammatory OND (iOND; $n = 13$). The OND group consisted of patients with normal magnetic resonance imaging (MRI) scans and lacking signs of inflammatory activity in CSF (pleocytosis or signs of intrathecal IgG production); unspecific sensory symptoms $n = 14$, vertigo $n = 1$, syringomyelia $n = 1$, anxiety $n = 1$, postcommotio syndrome $n = 1$, alcohol-related spastic paraparesis $n = 1$ and neurasthenia $n = 1$. The iOND group consisted of patients sampled during disease flares; neurosarcoidosis $n = 2$, herpes encephalitis $n = 1$, systemic lupus erythematosus (SLE) $n = 8$, unspecified demyelinating disease $n = 1$ and progressive multifocal leukoencephalopathy $n = 1$. iOND patients were not treated with immunomodulatory drugs, except for 7 out of 8 SLE patients who were on treatment with chloroquine and/or low dose prednisolone. CSF cell counts, immunoglobulin content and electrophoresis were performed at the time of sampling at the Department of Clinical Chemistry, Karolinska University Hospital according to clinical routines. Cohort 2 ($n = 48$) consisted exclusively of RRMS patients that were newly diagnosed or who had short disease duration (<5 years), and who also underwent a psychiatric evaluation in conjunction with CSF sampling performed in clinical routine. Patients in cohort 2 were untreated ($n = 39$) or were receiving interferons ($n = 7$), rituximab ($n = 1$) or glatiramer acetate ($n = 1$). Exclusion criteria were a known pre-existing psychiatric disorder or other major somatic disorders, as well as medication with psychiatric drugs or glucocorticoids. Written informed consent was obtained from all patients and the study was approved by the Regional Ethical Vetting Board of Stockholm (Diary Numbers: 2009/2107-31-2 and 2012/352-31/4). Clinical neurological examinations were performed by specialists in neurology, and all patients diagnosed with MS fulfilled the McDonald criteria (McDonald et al., 2001). All MS patients were evaluated clinically at time of sampling with the Expanded Disability Status Scale (EDSS) and the subsequent calculation of Multiple Sclerosis Severity Scale (MSSS).

RRMS patients were subdivided into patients in remission and relapse, respectively. The latter group was defined as an increase of $\geqslant 1$ EDSS point with duration of at least one week, not longer than 3 months before sampling, where systemic infection had been ruled out. SPMS was defined as >12 months of continuous worsening of neurological function ($\geqslant 0.5$ EDSS point) not explained by relapses and PPMS as patients with progression of disability from onset, with no, or only occasional and minor, remissions and improvements.

A comprehensive neuropsychiatric evaluation was made on MS patients of cohort 2, which included The Montgomery-Asberg Depression Rating Scale (MADRS-S) (Svanborg and Asberg, 1994) and the Modified Fatigue Impact Scale (MFIS) (Fisk et al., 1994), both administered as self-rating instruments, and a structured diagnostic interview (The Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998)), performed by a psychiatrist, for diagnosis of major depressive disorder (MDD). Depression was defined as meeting criteria for major depressive disorder in the M.I.N.I. or a self-rated MADRS-S score >11, and analyzed as a dichotomous variable. MADRS-S were further divided into vegetative (sleep, fatigue, appetite, ability to concentrate) and cognitive items which were analyzed separately. Fatigue was defined as a score $\geqslant 38$ on the MFIS. All evaluations were performed within 90 days of CSF sampling. For patient characteristics see Tables 1 and 2.

2.2. Multiplex analyses of TRP, KYN, QUIN and KYNA using targeted mass spectrometry

CSF samples were centrifuged immediately after sampling and stored frozen at −80 °C until analysis. All samples were handled