Toxicology

Iron and copper in progressive demyelination – New lessons from Skogholt’s disease

Klaus Thanke Aspli, Trond Peder Flaten, Per M. Roos, Trygve Holmøy, Jon H. Skogholt, Jan Aaseth

A Department of Neurology, Innlandet Hospital Trust, Lillehammer Hospital Division, Lillehammer, Norway
b Department of Chemistry, Norwegian University of Science and Technology, Trondheim, Norway
c, d Department of Neurology, Division of Clinical Neurophysiology, Oslo University Hospital, Oslo, Norway
e, f Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
g Department of Neurology, Akershus University Hospital, Norway
h Institute of Clinical Medicine, University of Oslo, Norway
i Innlandet Hospital Trust, Kongsvinger Hospital Division, Kongsvinger, Norway

ARTICLE INFO

Article history:
Received 1 September 2014
Accepted 4 December 2014

Keywords:
Copper
Demyelination
Iron
Multiple sclerosis
Skogholt disease

ABSTRACT

The pathophysiological mechanisms of progressive demyelinating disorders including multiple sclerosis are incompletely understood. Increasing evidence indicates a role for trace metals in the progression of several neurodegenerative disorders. The study of Skogholt disease, a recently discovered demyelinating disease affecting both the central and peripheral nervous system, might shed some light on the mechanisms underlying demyelination. Cerebrospinal fluid iron and copper concentrations are about four times higher in Skogholt patients than in controls. The transit into cerebrospinal fluid of these elements from blood probably occurs in protein bound form. We hypothesize that exchangeable fractions of iron and copper are further transferred from cerebrospinal fluid into myelin, thereby contributing to the pathogenesis of demyelination. Free or weakly bound iron and copper ions may exert their toxic action on myelin by catalyzing production of oxygen radicals. Similarities to demyelinating processes in multiple sclerosis and other myelinopathies are discussed.

Introduction

The knowledge of causal mechanisms in demyelination is incomplete [1]. Several studies indicate that iron (Fe), copper (Cu) and other trace metals are involved in the pathogenesis of various neurodegenerative disorders [2–5] including the demyelinating disorder multiple sclerosis (MS) [6,7]. The potential neurotoxicity of Fe has been actualized by recent findings of Fe deposits in affected brain regions in Parkinson’s disease [8,9] and in Friedreich’s ataxia [10]. Copper neurotoxicity is illustrated by the neuropsychiatric symptoms characterizing Wilson’s disease (WD) [11]. In WD, brain areas with Cu accumulation may be affected by demyelination [11]. Copper has been found in significantly elevated concentrations in cerebrospinal fluid (CSF) from patients with amyotrophic lateral sclerosis [12]. Recent studies have also indicated a pathogenetic role for Cu in Alzheimer’s disease [13–16], evident by the copper-enrichment of the amyloid-beta-peptide plaques in the nervous tissue of Alzheimer patients [17,18], and in Parkinson’s disease, evident by the specific Cu catalyzed oxidation of synuclein in Parkinson patients [19]. To what extent metal dyshomeostasis or elevated nerve metal concentrations can precipitate demyelination is however still unknown.

In a previous study [20] we found high levels of Fe and Cu in the CSF from patients with a disease which we named Skogholt disease. We have now made a clinical re-evaluation of that material, including new records of patient histories, resulting in exclusion of one patient, who had got his neurological symptoms from excessive drug abuse and who did not present demyelinated plaques when investigated with magnetic resonance imaging. The present updated discussion of the remaining nine patients is focusing on a possible pathogenetic role of Cu and Fe, in light of new insights from recent literature on Fe and Cu neurotoxicity.

Skogholt disease presents with demyelination of both the central and the peripheral nervous system. It was first described in 1998 by Dr. Jon Skogholt [21] in a region of Hedmark county in Norway. Clinically, it is characterized by a slowly progressing distal sensory loss, distal muscle weakness, unsteady gait and dysarthria.
Onset varies from the third to sixth decade in affected family members. As the disease does not conform to known hereditary demyelinating disorders it is considered a distinct disease entity [21], Protein levels in CSF from these patients are substantially elevated [20], and a blood–brain barrier (BBB) dysfunction may be suspected.

The re-evaluation reported in the present paper confirms the previously reported large increase in intrathecal levels specifically of Fe and Cu in the Skogholt patients. CSF concentrations of the other elements analyzed were essentially similar to or marginally altered compared to those in the control group. We propose that Fe and Cu contribute to demyelination by oxidative mechanisms.

**Materials and methods**

**Subjects**

After a clinical reassessment of patients in our previous study [20], one patient was reclassified as he did not suffer from this disease. This resulted in 9 patients in the Skogholt group, 4 females and 5 males, mean age 47.1 years (Table 1). The control group consisted of 13 individuals without demyelinating neurological disorders [20], 8 females and 5 males, mean age 49.4 years. The project has been approved by the Regional Committee for Medical Research Ethics in Norway (Region East, ref. no. 556-04224).

**CSF and blood sampling**

Cerebrospinal fluid was collected by lumbar puncture with a standard Spinocan 0.9 mm × 88 mm syringe and transferred to metal free plastic tubes. Blood samples were collected from the cubital vein into heparinized vacutainer tubes specially designed for trace element analyses (Becton Dickinson).

Analytical methods have been described in detail previously [20] and are briefly outlined here. Metals were determined in CSF and blood plasma using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). The accuracy of the method was checked by analyzing SeroNorm Serum Level 1 and Level 2 reference material (Sero, Norway), showing values within 85–115% of the certified concentrations. The precision of the method, checked by repetitive analyses of the same sera, showed coefficients of variation lower than 10%.

Proteins in blood serum and CSF were quantified colorimetrically [20] and fractionated by electrophoresis. Concentrations of albumin were determined by an immunoturbidimetric assay (Tinaquant Albumin, Roche) analyzed on a Cobas e-501 instrument with CV 6%. IgG was quantified by the same method with a CV of 7%. The CSF IgG/total protein ratio and the CSF/serum protein ratio were calculated.

**Results**

The present re-evaluation demonstrated an almost fourfold increase in both Fe (mean 115.2 μg/L) and Cu (mean 73.8 μg/L) concentrations in CSF from patients with Skogholt disease compared to Fe (mean 30.5 μg/L) and Cu (mean 21.7 μg/L) from controls (Table 2, Fig. 1). In CSF from Skogholt disease patients, Fe and Cu levels were correlated, but not very strongly (correlation coefficient, r = 0.697, cf. Fig. 1), which might indicate similar groups of carriers across BBB, e.g. peptides or proteins (see below).

CSF sulfur concentrations were substantially increased in Skogholt disease patients compared to controls, whereas a less pronounced increase was found for CSF zinc (Table 2). A modest increase in the levels of phosphorus and rubidium was observed, presumably not of independent pathogenetic significance. Only minor or negligible differences in CSF levels of the other elements, including manganese, lead and mercury, were found between Skogholt disease patients and controls (Table 2). The blood plasma levels for all measured elements including Fe and Cu in the Skogholt group did not differ from the control values (data not shown), suggesting a specific transfer of Fe and Cu into the CSF compartment. Concomitantly increased CSF Cu and S concentrations were found in the patient group compared to controls (r = 0.937) (Fig. 2), indicating that S-containing molecules, e.g. sulfur proteins, act as Cu transporters from blood to CSF in this group [20]. The same