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Elevated manganese levels in blood and CNS in human prion disease

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Prion disease or transmissible spongiform encephalopathies are neurodegenerative disorders of humans and other mammals. They are fatal and difficult to diagnose. Previous studies have suggested that some prion diseases cause elevation of manganese in the blood and brain. In the current study we analysed blood and brain samples from humans to determine whether elevation in manganese is a specific characteristic of Creutzfeldt–Jakob disease, the most common form of human prion disease. Analysis of manganese in the blood of normal humans showed that concentrations vary little with age or sex. Analysis of other diseases, including other neurodegenerative disease showed that only CJD showed an elevation in manganese and copper. Other diseases that showed elevated manganese included blood-brain barrier disorders and haemochromatosis. However, CJD could be easily distinguished from these diseases. This implies that increased blood manganese in prion disease is a highly specific characteristic of the disease.

Keywords: Prion; Manganese; CJD; Neurodegeneration

Introduction

Prion diseases or transmissible spongiform encephalopathies (TSEs) are neurodegenerative disorders that affect mammals (Prusiner, 1998). Prion diseases that affect humans fall into three groups, inherited forms such as Gerstemann–Sträussler–Scheinker syndrome (GSS), transmitted forms such as Kuru and sporadic forms such as Creutzfeldt–Jakob disease (CJD). Although sporadic CJD (sCJD) is the most common human TSE, there has been more concern about a variant form of CJD (vCJD) because it can be transmitted between individuals by blood transfusion (Llewelyn et al., 2004; Wroe et al., 2006) and may have transmitted to humans through BSE infected food (bovine spongiform encephalopathy). Although the number of vCJD cases is declining, the ability to diagnose vCJD or any prion disease before the onset of irreversible symptoms remains non existent. Diagnosis of human prion diseases currently occurs at a time when the patient is beyond treatment. Although some compounds such pentosan sulphate and quinacrine have been tested, there is currently no effective treatment (Haik et al., 2004; Whittle et al., 2006). Emergence of a treatment therefore requires an adequate diagnostic test.

Definite diagnosis of most forms of prion disease is currently only possible post mortem. Although a number of diagnostic criteria allow a possible diagnosis of the disease before death, a confirmation of this requires analysis of central nervous system tissue for the presence of the abnormal isoform of the prion protein (PrP^{Sc}). In some cases detection by tonsil biopsy has proven effective in diagnosis of prion disease (Hill et al., 1999; Wroe et al., 2006).

PrP^{Sc} is a protease resistant isoform of the normal cellular prion protein, PrP^{c}. This glycoprotein is expressed by neurons (Sales et al., 1998), particularly at the synapse and is a copper binding protein (Hornshaw et al., 1995; Brown et al., 1997, 2000; Thompsett et al., 2005). Although, there is strong evidence that this protein is an antioxidant (Brown et al., 1999, 2001), the finding is still disputed (Hutter et al., 2003; Jones et al., 2005). Conversion of PrP^{c} to PrP^{Sc} results in a conformational change from a helical form to one rich in beta sheet, increased protease resistance, altered metal binding and possible loss of function. It is the protease resistance of PrP^{Sc} that forms the basis of most diagnostic tests. Aggregates of PrP^{Sc} can be detected in brain sections or in Western blots. Digestion of PrP^{Sc} with proteinase K results in a shift in size of the resulting bands detected on the Western blot and ratios of the different glycoforms form the basis of determining the strain of type of the particular prion disease (Hill et al., 2003).

The two main avenues that are being explored for a potential diagnostic test include more sensitive detection of PrP^{Sc} and surrogate markers which could indicate presence of the disease during the pre-clinical asymptomatic incubation period. Amplification of the level of PrP^{Sc} in tissues has been successful accomplished by a cyclic method (Saborio et al., 2001). This method requires brain tissue from a disease free individual but is able to detect trace amounts of PrP^{Sc} in the brains of animals in the early stages of prion disease. A further study suggested that this method could also detect PrP^{Sc} in blood collected from the heart (Castilla et

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al., 2005). Unfortunately the application of this technique is limited by the need for normal brain tissue. Surrogate markers that have been investigated include erythroid differentiation-related factor, tau, uric acid, S100beta, neuron-specific enolase and 14-3-3 (Miele et al., 2001; Parveen et al., 2005; Van Everbroeck et al., 2005; Lekishvili et al., 2004). Only the protein 14-3-3 has been shown to be of benefit in detecting CJD (Hsich et al., 1996). Increased levels of this protein in the cerebrospinal fluid have been shown to be a relatively reliable confirmatory tool. As conversion of PrP^{c} to PrP^{Sc} results in a loss of copper binding to the protein, changes in metals in the brain have been investigated. In both scrapie infected mice and humans with CJD, the levels of manganese have been shown to be increased in the brain (Wong et al., 2001; Thackray et al., 2002). In parallel, there was a loss of copper binding to PrP and an increase in manganese associated with affinity isolated PrP. Binding of manganese to the PrP results in a change in its conformation (Brown et al., 2000; Tsenkova et al., 2004). In scrapie infected mice, an increase in blood levels of manganese have also been shown to occur with the increase notable before the onset of symptoms of the disease (Thackray et al., 2002). Recently we have shown that manganese is also elevated in both CNS tissue and blood of cattle with BSE and sheep with scrapie (Hesketh et al., 2007). In particular, elevation of manganese in blood was noted in experimental BSE and scrapie before the onset of clinical signs. This elevation was even noted in cases of experimental scrapie in resistant sheep where challenge with an infectious agent would not lead to clinical disease. This implies that the change in manganese correlates to the disease infection and not to the course of the disease pathogenesis or changes related to neurodegeneration.

In this study we re-investigated changes in manganese in the brains of human patients with CJD. We also examined blood manganese levels and compared the changes observed to changes in blood manganese levels in the normal population with age or with blood related illnesses. We found that CJD patients showed changes in brain and blood manganese and copper levels. Although other diseases showed different changes, the changes metal profile seen for sCJD in blood was unique. The only other diseases to show elevated manganese were haemochromatosis and disease of the blood-brain barrier.

Results

CJD brain metal levels

Previous studies have suggested that the concentrations of trace metals, particularly copper and manganese are altered in the brain

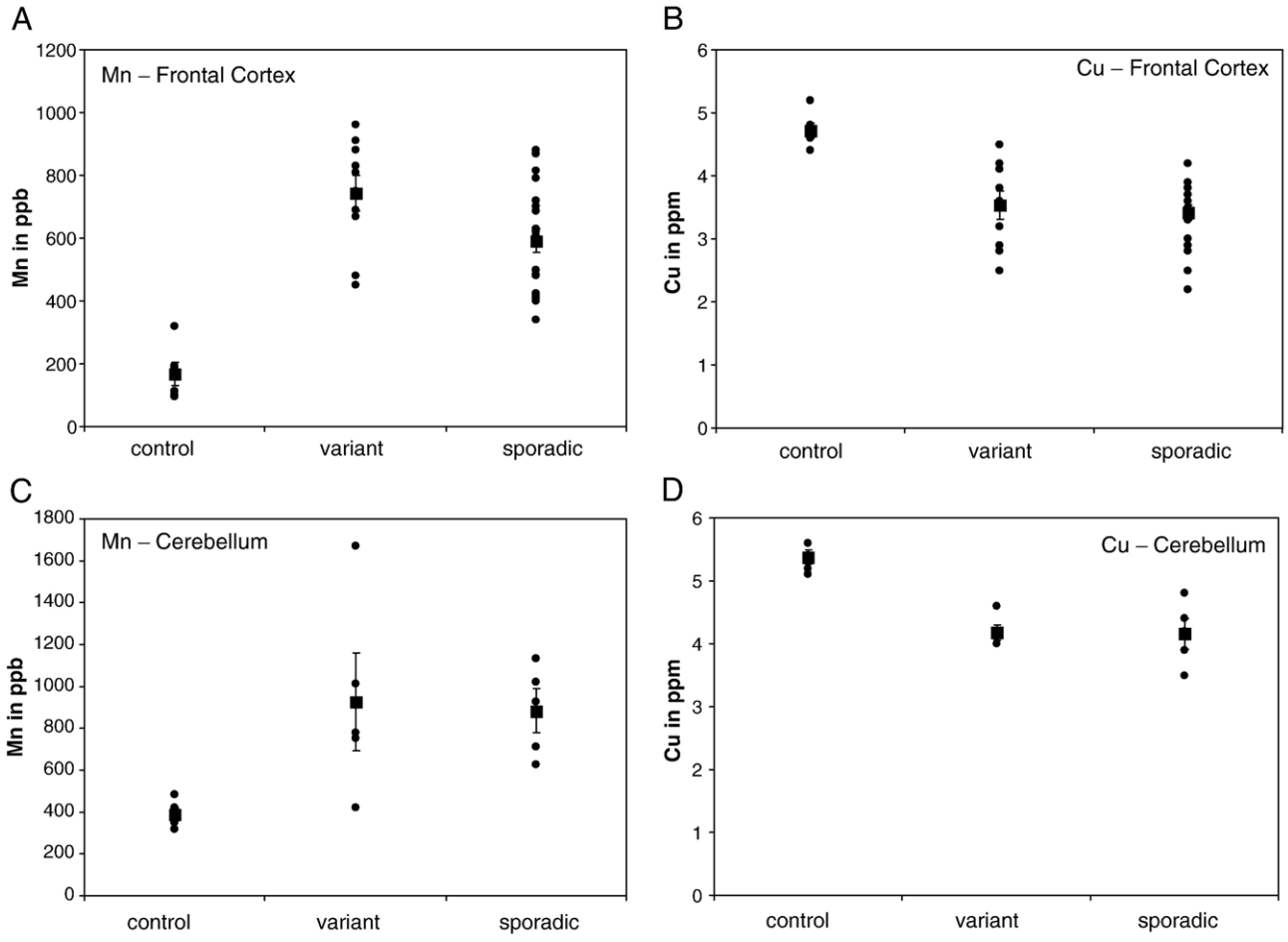

Fig. 1. Cu and Mn levels in sCJD brain. The concentrations of manganese (A, C) and copper (B, D) in both the frontal cortex (A, B) and the cerebellum (C, D) of patients with either vCJD (variant) or sCJD (sporadic) were determined and compared to those from patients with other neurodegenerative diseases (control). The individual values are shown as black circles. The mean and standard error for each group is shown as a black square. Values are in parts per million (ppm) or parts per billion (ppb).