Cerebrospinal fluid folate, ascorbate, and tetrahydrobiopterin deficiency in superficial siderosis: a new potential mechanism of neurological dysfunction?

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Dear Editor,

Superficial siderosis of the central nervous system (CNS) is a disabling but rare disease caused by chronic bleeding in the subarachnoid space of the brain and spinal cord [1,2,3]. This chronic slow leakage of red blood cells into the CSF, causes an accumulation of haem. In response, microglia and Bergmann glial cells release hemeoxygenase-12 [**Fig 1, Panel A (i)**] which breaks down haem into free iron (ferrous iron, Fe^{2+}) and biliverdin. Ferritin binds the free iron to form hemosiderin. Deposits of hemosiderin can be detected by MRI, allowing in vivo diagnosis of superficial siderosis [4]. After prolonged low level bleeding (sometimes over decades) it is presumed that the homoeostatic system of the glial cells is overwhelmed, leading to the release of potentially neurotoxic free iron species into the CSF. Free iron is a potent oxidant and can produce free radicals which damage cellular components including cofactors and vitamins, e.g. tetrahydrobiopterin (BH_4) and 5-methyltetrahydrofolate (5MTHF) [5,6].

Case report

In view of their lability, we assessed CSF BH_4 plus its oxidised catabolite, dihydrobiopterin (BH_2) and 5MTHF in a 34 year old female patient who had presented with progressive balance and hearing impairment, and a myelopathy. MRI showed low signal on blood-sensitive sequences over the surface of the brainstem, cerebellum, craniocervical junction and spinal cord [**Fig 1, Panel B**]., leading to a diagnosis of superficial siderosis of the CNS. Because of the key cofactor role BH_4 plays in monoamine metabolism, we quantified dopamine and serotonin metabolites (homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5HIAA)). Additionally we determined ascorbate status because it is a key antioxidant and a cofactor for the downstream metabolism of dopamine to noradrenaline.

CSF ferritin was **126** ng/ml (reference range <16 ng/ml), consistent with active or recent haemorrhage into the subarachnoid space. BH_4 (**<4** nmol/L (reference range 9-39 nmol/L), ascorbate (**26** nmol/L (reference range 118-246 nmol/L) and 5MTHF (**<10** nmol/L (reference range 46-160 nmol/L) concentrations were all markedly below their respective reference ranges. BH_2, an oxidation product of BH_4 catabolism, was elevated (**15.9** nmol/L (reference range 0.4-13.9 nmol/L).

Dopamine turnover and/or availability appeared increased (HVA **566** nmol/L (reference range 71-565 nmol/L), which was further supported by the elevated HVA to 5HIAA ratio (**4.4** (reference range 1-3.7)). In contrast, serotonin metabolism was not affected (5HIAA 130 nmol/L (reference range 58-220 nmol/L).

Previous studies have described CSF pterin and monoamine analysis in other neurological diseases, including Parkinson's disease [5], cerebrovascular diseases and subarachnoid haemorrhage [6]. These studies have described a deficit of tetrahydrobiopterin/total biopterin in both Parkinson's disease and subarachnoid haemorrhage [6] but have not analysed CSF ascorbate or folate. To the best of our knowledge, this is the first report of the CSF status of BH_4, 5MTHF, HVA, 5-HIAA and ascorbate in superficial siderosis of the CNS. Our findings are consistent with oxidative stress, due to free iron species, and raise the possibility that this is a potential mechanism of neurological disability in superficial siderosis. Both BH_4 and 5MTHF are highly susceptible to oxidative catabolism *in vitro* [**Fig 1, Panel A (ii)]**[7,8]. A reduced level of BH_4, via oxidative breakdown, is supported by the increased concentration of one of its oxidation products, BH_2. Our finding of a very low CSF ascorbate provides further evidence for oxidative stress, as this antioxidant may become depleted by increased generation of reactive oxygen species [8]. The markedly low 5MTHF CSF levels reported here might also be expected to impair metabolic pathways that are dependent upon folate cofactors including the one-carbon metabolic pathways, responsible for the methylation of DNA, RNA

and proteins. Further work is therefore required to ascertain the potential biochemical effects of this deficiency in superficial siderosis.

Both ascorbate and 5MTHF are vitamins and must be constantly replaced by dietary intake. In contrast, BH_4 levels can be maintained within the cell by *de novo* synthesis or recycling. Although CSF BH_4 levels were low in this patient, the CSF profile reported here does not suggest a deficiency of BH_4 within dopaminergic or serotonergic cells as HVA and the HVA:5-HIAA ratio were elevated. The synthesis of noradrenaline [**Fig 1, Panel A (iii)**] from dopamine requires the enzyme dopamine-ß-hydroxylase (DßH), for which ascorbate is an essential cofactor. Decreased ascorbate due to iron-mediated oxidative stress exceeding dietary replenishment, may thus lead to reduced dopamine-ß-hydroxylase activity, leading to an accumulation of the substrate dopamine, and increased turnover to HVA. It should be noted that, although serotonin and it's metabolites are generally considered to be relatively labile in aqueous solutions and in some biological matrices, it has been shown that 5HIAA is stable in 'control' CSF for up to 48 hours at room temperature [9]. This may explain why serotonin turnover is not affected in this patient. However, it is possible that excessive iron accumulation could eventually result in degradation of other labile species in the CNS, such as serotonin and it's metabolites.

The CSF findings of this patient with superficial siderosis support a role for oxidative stress in this disease. This is a plausible pathogenic mechanism of neurological disability that merits further exploration. In addition, central 5MTHF and ascorbate status might be potentially treatable with supplementation, although caution is required since folate supplementation can aggravate B12 deficiency and ascorbate might act as a pro-oxidant as well as an antioxidant [10]. Whether this treatment strategy can help reduce progression of this disabling disease will require controlled clinical trials.

Appendix 1

Authorship

Jack Belsten – responsible for biochemical analysis, interpretation of data and drafted and revised manuscript for intellectual content.

David Werring – responsible for acquisition and interpretation of clinical data and drafted and revised manuscript for intellectual content.

Howell Jones – responsible for acquisition and interpretation of clinical data and revised the manuscript for intellectual content.

Simon Heales – responsible for study design, interpretation of data and drafted and revised manuscript for intellectual content.

Simon Pope - responsible for biochemical analysis, interpretation of data and drafted and revised manuscript for intellectual content.

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Figure legend

Figure 1.

Panel A

(i) The production of hemosiderin and free iron from a dural leak in the central nervous system (CNS).

(ii) The iron based Fenton chemistry for the production of the hydroxyl radical from hydrogen peroxide.

(iii) The metabolism of serotonin, dopamine and noradrenaline. BH_4 is a cofactor for the synthesis of dopamine and serotonin. Ascorbate is a cofactor for DßH in the conversion of dopamine to noradrenaline. Dopamine and serotonin are metabolised to HVA and 5HIAA, respectively. HVA and 5HIAA can be used as markers of dopamine and serotonin turnover.

Panel B

Axial T2-weighted MRI showing low signal consistent with haemosiderin deposition around the superior cerebellar vermis and brainstem (white arrows).

BH_2: dihydrobiopterin, BH_4: tetrahydrobiopterin, 5HIAA: 5-hydroxyindoloacetic acid, HVA: homovanillic acid, DßH: dopamine-ß-Hydroxylase.