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Research Article

Heme Deficiency in Alzheimer's Disease: A Possible Connection to Porphyria

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Mechanisms that cause Alzheimer's disease (AD), an invariably fatal neurodegenerative disease, are unknown. Important recent data indicate that neuronal heme deficiency may contribute to AD pathogenesis. If true, factors that contribute to the intracellular heme deficiency could potentially alter the course of AD. The porphyrias are metabolic disorders characterized by enzyme deficiencies in the heme biosynthetic pathway. We hypothesize that AD may differ significantly in individuals possessing the genetic trait for an acute hepatic porphyria. We elaborate on this hypothesis and briefly review the characteristics of the acute hepatic porphyrias that may be relevant to AD. We note the proximity of genes encoding enzymes of the heme biosynthesis pathway to genetic loci linked to sporadic, late-onset AD. In addition, we suggest that identification of individuals carrying the genetic trait for acute porphyria may provide a unique resource for investigating AD pathogenesis and inform treatment and management decisions.

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INTRODUCTION

AD is a progressive and invariably fatal neurodegenerative disease and the leading cause of senile dementia [1]. Synaptic and neuronal loss best correlates with cognitive decline [2]. Metabolic imbalance in diseased neurons may contribute to neuropsychiatric symptoms that include delusions and hallucinations, anxiety, mood disorder, and sleep disturbance that are common in AD [3]. Mechanisms that cause AD are unknown. Recently we proposed a hypothesis that explains why elevated plasma homocysteine is a risk factor for AD [4, 5]. Implicit in that hypothesis is development of neuronal heme deficiency, and evidence of heme deficiency in AD brains has been reported [6]. Here, we extend this theme by considering the possible impact of porphyria on AD. The porphyrias are metabolic disorders characterized by enzyme deficiencies in the heme biosynthesis pathway. We propose that an understanding of porphyria may provide novel insights into AD pathogenesis.

GENERAL CONSIDERATIONS

Molecular and biochemical aspects of the porphyrias and their diagnosis and treatment are the subject of several excellent reviews [7–13]. Eight enzymes are required for *de novo* heme biosynthesis. With the exception of 5-aminolevulinic acid synthase [ALAS, EC 2.3.1.37], the initial and rate-limiting enzyme of the heme biosynthesis pathway, deficiency in one of the other seven enzymes is associated with a specific form of inherited porphyria [10]. Four of the hepatic porphyrias, so-called because liver is the major site of expression of the enzymatic defect in heme biosynthesis, are designated "acute" porphyrias because clinical expression of the disease is associated with an acute neurologic syndrome (the acute attack or porphyric crisis) [8, 11]. These are the extremely rare Doss porphyria (deficiency of ALA dehydratase, EC 4.2.1.24), acute intermittent porphyria (deficiency of porphobilinogen deaminase, EC 4.3.1.8), hereditary coproporphyria (deficiency of coproporphyrinogen oxidase, EC 1.3.3.3), and variegate porphyria (deficiency of protoporphyrinogen oxidase, EC 1.3.3.4). Acute intermittent porphyria is generally the most common form of acute hepatic porphyria encountered. Significantly, enzyme deficiencies are present in other organs, including the brain, and the enzyme deficiency is life-long.

Acute neurologic syndrome associated with clinical attacks of acute hepatic porphyria can include both neuropsychiatric symptoms and neurodegenerative change [8, 11, 12]. Neuropsychiatric symptoms that include anxiety, insomnia, confusion, hallucinations, agitation, and paranoia (so-called

porphyric encephalopathy-8) underscore CNS involvement. Autonomic neuropathy may underlie severe abdominal pain and cardiovascular symptoms. In severe cases, a peripheral neuropathy resembling Guillain-Barre syndrome can develop [8, 11]. Clinical attacks of acute porphyria can be induced in latent individuals by a variety of environmental factors including many common medications, nutritional factors, restricted carbohydrate and calorie intake, smoking, and hormones such as progesterone; lists of safe and unsafe drugs are available [9, 11, 12]. A common mechanism of inducing agents is believed to be greatly increased hepatic heme demand. Thus, biosynthesis of cytochrome P450 enzymes that utilize heme as a prosthetic group can be induced as much as 40–50-fold in liver by drugs such as barbiturates [11]. Increased heme demand results in the induction of ALAS and increased synthesis of the heme precursor, 5-aminolevulinic acid [ALA]. In individuals who have inherited a partial deficiency in one of the enzymes of the heme biosynthesis pathway, that enzyme and not ALAS is the rate-limiting step in heme biosynthesis. Then, ALA and other heme precursors can accumulate. Moreover, heme biosynthesis is insufficient to meet demand and heme deficiency is unresolved. Acute attacks are treated with infusions of glucose and hemin [9, 12]. Hemin restores the regulatory heme pool. This suppresses hepatic ALAS induction and the overproduction of ALA and other heme precursors. Glucose infusion may also suppress ALAS but by a different mechanism. Fasting, which can induce the acute attack, appears to activate transcriptional coactivator PGC-1α (via a cAMP/CREB pathway) and PGC-1α greatly increases hepatic ALAS expression by activating transcription factors NRF-1 and FOXO1 [14, 15]. In addition, ALAS may respond directly to cAMP [14, 15]. Glucose appears to antagonize both pathways. Abdominal pain and psychotic symptoms resolve quickly upon timely treatment of the acute attack but peripheral neuropathy can require months to resolve and recovery is often incomplete [11].

The pathogenesis of nervous system dysfunction in the acute attack remains unclear. There are two predominant hypotheses [8, 11]. One suggests functional heme deficiency develops during the acute attack, in liver and possibly in neural tissues, and impairs critical cell processes dependent on hemoproteins such as energy production by the mitochondrial electron transport chain. Studies utilizing mice deficient in porphobilinogen deaminase, an experimental model of acute intermittent porphyria, underscore the importance of functional heme deficiency in nervous tissue in the development of motor neuropathy [16–18]. The second hypothesis suggests that heme precursors and their metabolites accumulate to toxic levels during the acute attack. ALA, in particular, is implicated because it is produced excessively in all the acute hepatic porphyrias and may have neurotoxic properties [8, 11]. Excessive ALA production occurs in lead poisoning due to lead-mediated inhibition of ALA dehydratase, and also in hereditary infantile tyrosinemia (type I) in which the enzyme defect leads to endogenous production of the ALA dehydratase inhibitor, succinylacetone [19]. In both diseases, neuropsychiatric symptoms that resemble those of the acute attack occur [8, 11]. While recent clinical studies underscore the potential importance of excessive hepatic production of heme precursors as the primary cause of the neurologic complications in the acute porphyric attack [20, 21], induced elevation of plasma ALA in a human volunteer, by itself, did not produce symptoms of porphyria [22]. Clearly, many details are unresolved [8, 11].

DOES PORPHYRIA OFFER INSIGHT ON AD?

We hypothesize that heme deficiency is important in AD pathogenesis and that AD may differ significantly in individuals possessing the genetic trait for an acute hepatic porphyria because there is the potential to develop more severe heme deficiency. Figure 1 schematically depicts this hypothesis.

AD-related factors may create an imbalance in neuronal heme supply and demand. In AD, heme supply may be reduced. Aging is the greatest risk factor for development of AD, and at least in rat brain, heme biosynthesis declines in normal aging [25]. Nutritional factors could be important. Pyridoxine deficiency in the aged could contribute to age-related decrease in heme biosynthesis because pyridoxal phosphate is a cofactor for ALAS [10]. Glycoxidation reactions are prominent in AD brain [26], and glycoxidation reactions might inactivate enzymes required for heme biosynthesis [27]. Moreover, heme biosynthesis requires mitochondrial integrity and mitochondrial damage is prominent in AD [28]. In AD, heme demand may be increased. Mitochondrial damage would necessitate increased mitochondrial turnover and *de novo* synthesis of heme-containing proteins such as cytochromes. Moreover, mitochondrial damage may itself be caused by heme deficiency [29, 30] thus creating a vicious cycle further impairing heme biosynthesis. Glycoxidation reactions could promote degradation of heme proteins [31]. Heme degradation may be favored over heme biosynthesis in AD neurons because of chronically elevated HO-1 [32, 33]. Factors unique to AD could also increase heme demand. Thus, amyloid-β [Aβ] binds heme, which may contribute to development of a functional heme deficiency [6] and affect Aβ toxicity by inhibiting Aβ aggregation [34, 35]. We speculate that the reduced capacity to synthesize heme in individuals with porphyria could exacerbate such an imbalance in heme supply and demand.

In addition, the two proposed mechanisms of nervous system dysfunction in the acute porphyric attack, functional heme deficiency and toxic accumulation of ALA, have cellular effects that could be important in AD pathogenesis. Inhibition of heme biosynthesis produced senescence-associated changes in gene expression in cultured mouse cortical neurons [36] and was proapoptotic in NGF-induced PC12 cells [37]. Increased oxidative stress, which is one of the earliest observed events in AD pathogenesis [38], and heme deficiency may help explain several pathophysiological features of AD including mitochondrial abnormalities and impaired energy metabolism, cell cycling and cell signaling abnormalities, neuritic pathology, and abnormal expression of iron regulatory protein 2 (IRP2) [5]. ALA is a source of

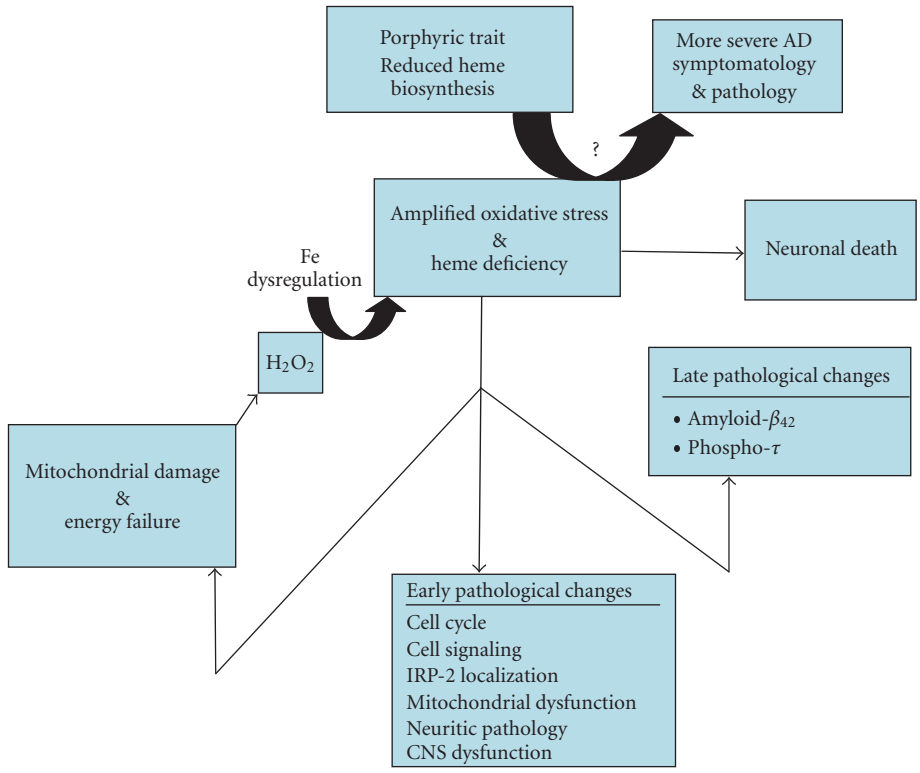

Figure 1: AD and porphyria. Oxidative stress and free radical damage occur early in AD [23]. Disruption in iron homeostatic mechanisms contributes to oxidative damage in AD [24]. A consequence of oxidative stress predicted by the ferric cycle hypothesis is heme deficiency [4, 5]. Moreover, AD-related factors such as accumulation of amyloid-β may limit heme bioavailability [6]. We hypothesize that reduced capacity for cells to synthesize heme, in individuals with the genetic trait for acute hepatic porphyria, contributes to development of heme deficiency (and possibly oxidative stress). AD-related pathological change and neuropsychiatric and behavioral symptoms associated with AD may be more severe in these individuals.

oxygen free radicals in the presence of heavy metals such as iron [39]. The product of iron-catalyzed oxidation of ALA, 4, 5-dioxovaleric acid, is an effective alkylating agent of guanine moieties in DNA *in vitro* [40, 41], and ALA-induced mitochondrial and nuclear DNA damage has been shown in several cell lines including PC12 cells [42]. Moreover, ALA may disrupt normal iron sequestration by ferritin. It released iron from ferritin *in vitro* [43, 44] and caused oxidative damage to the ferritin molecule [45]. As in the toxic mechanism proposed for homocysteine in AD pathogenesis [4, 5], ALA may make available a catalytic metal that can promote oxidative stress.

DOES PORPHYRIA INCREASE THE RISK OF AD?

Clinically overt acute hepatic porphyria (predominantly acute intermittent porphyria) is relatively rare with a prevalence of perhaps 5 per 100,000 [12]. However, the prevalence of the genetic trait for acute porphyria is far greater because perhaps 90% of affected individuals are clinically latent [7, 12]. In a Finnish population the estimated prevalence of porphobilinogen deaminase deficiency, the biochemical defect in acute intermittent porphyria, was 1 per 500 [46]. Using gene analysis to supplement enzymatic analysis, the estimated prevalence of porphobilinogen deaminase deficiency in a French population was 1 per 1675 [47]. Consistent with the possibility that deficiency in heme biosynthesis could increase susceptibility for AD is the intriguing observation that the chromosomal location of genes encoding enzymes in the heme biosynthesis pathway correlate with genetic loci linked to sporadic, late-onset Alzheimer's disease (maximum lod score $\geqslant 1$) [48] (Table 1). However, the significance of this observation is unclear. In cases such as deficiency in porphobilinogen deaminase, heme deficiency alone may be insufficient to cause AD but could contribute to disease progression when superimposed on other disease processes. However, effects may be indirect and not related to heme levels. For example, the proximity of the ALA dehydratase gene to an AD-related locus is noted in Table 1. ALA dehydratase-porphyria is an extremely rare form of acute hepatic porphyria. Moreover, ALA dehydratase activity is far in excess of the activities of other enzymes in the heme biosynthetic pathway and for that reason > 95% loss of activity is needed before clinical symptoms of porphyria develop [12]. However, ALA dehydratase is also a high K_m enzyme. Under AD-associated conditions, toxic levels of ALA may possibly accumulate and contribute to AD pathogenesis. While interesting, any relationship between heme deficiency

Table 1: Chromosomal locations of genes encoding enzymes of the heme biosynthesis pathway and genes linked to development of late-onset Alzheimer's disease.

Heme biosynthetic enzymes	Location[1]	Closest AD-related loci[2]
ALAS-1 (EC 2.3.1.37)	3p21	3p14, 3p26
ALA-dehydratase (EC 4.2.1.24)[3]	9q34	9q34
Porphobilinogen deaminase (EC 4.3.1.8)[3]	11q23.3	11q25
Uroporphyrinogen III synthase (EC 4.2.1.75)	10q25.3	10q21–10q25
Uroporphyrinogen decarboxylase (EC 4.1.1.37)	1p34	1p31–1p36
Coproporphyrinogen III oxidase (EC 1.3.3.3)[3]	3q12	3q28
Protoporphyrinogen oxidase (EC 1.3.3.4)[3]	1q22	1q23, 1q24
Ferrochelatase (EC 4.99.1.1)	18q21.3	18q22

[1] from Meissner et al [10].
[2] From Table 1 by Kamboh in [48].
[3] A deficiency is associated with a specific form of acute hepatic porphyria.

and AD is speculative. If the genetic trait for one of the acute hepatic porphyrias is a risk factor for AD, why has this relationship gone unnoticed? The answer simply may be that the majority of individuals with the biochemical defects of acute porphyria are clinically latent, and that many genetic and environmental factors likely contribute to the development of sporadic, late-onset AD.

CONCLUSIONS

AD may differ significantly in individuals who have the genetic trait for acute hepatic porphyria because there is the potential to develop more severe neuronal heme deficiency and possibly accumulate ALA and other heme precursors. Epidemiological data confirming a link between AD and porphyria would be an important test of the hypothesis. AD progression (from disease-free state, to mild cognitive impairment, to AD) in individuals with a genetic trait for acute hepatic porphyria could be compared with AD progression in an unaffected cohort. Testing for the presence of a genetic trait for acute porphyria in individuals diagnosed with mild cognitive impairment or early AD might identify a unique subset of AD patients. Management decisions may need to be adjusted in such individuals to avoid potential sensitivity to common medications and novel therapeutic agents which, if porphyrinogenic, could exacerbate porphyria and possibly AD symptoms. Approaches such as these could yield significant new information on AD pathogenesis and treatment.

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