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Research Report

PKU is a reversible neurodegenerative process within the nigrostriatum that begins as early as 4 weeks of age in *Pah*^{enu2} mice

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ABSTRACT

Phenylketonuria (PKU) is a common genetic disorder in humans that arises from deficient activity of phenylalanine hydroxylase (PAH), which catalyzes the conversion of phenylalanine to tyrosine. There is a resultant hyperphenylalanemia with subsequent impairment in cognitive abilities, executive functions and motor coordination. The neuropathogenesis of the disease has not been completely elucidated, however, oxidative stress is considered to be a key feature of the disease process. Hyperphenylalanemia also adversely affects monoaminergic metabolism in the brain. For this reason we chose to evaluate the nigrostriatum of *Pah*^{enu2} mice, to determine if alterations of monoamine metabolism resulted in morphologic nigrostriatal pathology. Furthermore, we believe that recent developments in adeno-associated virus (AAV)-based vectors have greatly increased the potential for long-term gene therapy and may be a viable alternative to dietary treatment for this metabolic disorder. In this study we identified neurodegenerative changes with regenerative responses in the nigrostriatum of *Pah*^{enu2} mice that are consistent with oxidative injury and occurred as early as 4 weeks of age. These neuropathologic changes were reversed following portal vein delivery of a recombinant adeno-associated virus-mouse phenylalanine hydroxylase-woodchuck hepatitis virus post-transcriptional response element (rAAV-mPAH-WPRE) vector to *Pah*^{enu2} mice and corresponded to rapid reduction of serum Phe levels.

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Abbreviations:
AAV, adeno-associated virus
BDNF, brain-derived neurotrophic factor
CX, cortex
DA, dopamine
DAB, 3,3′-diaminobenzidine tetrachloride
DOPAC, 3,4-dihydroxy-phenylacetic acid
GFAP, glial fibrillary acidic protein
H_20_2, hydrogen peroxidase
5-HIAA, 5-hydroxyindoleacetic acid
5-HT, 5-hydroxytryptamine (serotonin)
HPA, hyperphenylalanemia
HVA, homovanillic acid
INOS, inducible nitric oxide synthase
MPTP, 1-methyl-4 phenyl-1,2,3,6-tetrahydropyridine
PAH, phenylalanine hydroxylase
PD, Parkinson's disease
PKU, phenylketonuria
PHE, phenylalanine
rAAV-mPAH-WPRE, recombinant adeno-associated virus-mouse phenylalanine hydroxylase-woodchuck hepatitis virus post transcriptional response element
RS, reactive species
SN, substantia nigra
SNPc, substantia nigra pars compacta
STR, striatal
TH, tyrosine hydroxylase
TYR, tyrosine
VTA, ventral tegmental area

1. Introduction

Phenylketonuria (PKU) is a disorder of amino acid metabolism caused by a deficiency of hepatic phenylalanine hydroxylase (PAH) activity. Under these conditions, phenylalanine (Phe) is unable to be converted to tyrosine (Tyr), which results in millimolar concentrations of serum Phe. The increased Phe is subsequently metabolized to the organic acid ketones phenylpyruvate, phenylacetate, and phenylactate. The disease manifests itself primarily as a neurologic disorder, and if untreated, can result in microcephaly, mental retardation, epilepsy, and progression of motor disorders in the second or third decade. The treatment is difficult to implement and consists of lifetime dietary restriction of phenylalanine (Krause et al., 1985; Scriver, 1995; Smith and Wolff, 1974).

The etiology of the neurologic disease process in PKU patients has not been fully elucidated but is thought to occur secondarily to increased concentrations of phenylalanine in the blood. One factor that is considered to play a role in the neuropathogenesis of the disease is oxidative stress. The role of oxidative damage in the neuropathology of organic acidurias is presented in a review by Wajner et al. (2004) who discusses considerable evidence in which organic acid accumulation associated with various metabolic disorders will induce free radical and reactive species (RS) generation and will also decrease antioxidant defenses in tissues. These reactive species can inhibit various complexes of the respiratory chain and critical enzymes necessary for energy production resulting in metabolic failure, cell injury and cell death (Castro et al., 1994, 1998; Radi et al., 2002; Wyse et al., 2001). Free radicals also provoke cell injury and death directly by breaking cell lipid membranes, destroying proteins, and oxidizing DNA (Halliwell, 2001; Halliwell and Gutteridge, 1989a). In some of these disorders, the toxic metabolites were thought to be organic acids such as the phenylketones in PKU (Huttenlocher, 2000).

Hyperphenylalanemia (HPA) of PKU also results in the disruption of amino acid transport and metabolic pathways