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Review

Metallothionein-mediated neuroprotection in genetically engineered mouse models of Parkinson's disease

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Abstract

Parkinson's disease is characterized by a progressive loss of dopaminergic neurons in the substantia nigra zona compacta, and in other sub-cortical nuclei associated with a widespread occurrence of Lewy bodies. The cause of cell death in Parkinson's disease is still poorly understood, but a defect in mitochondrial oxidative phosphorylation and enhanced oxidative and nitrative stresses have been proposed. We have studied control$_{wt}$ (C57B1/6), metallothionein transgenic (MT$_{trans}$), metallothionein double gene knock (MT$_{dko}$), α-synuclein knock out (α-syn$_{ko}$), α-synuclein–metallothionein triple knock out (α-syn–MT$_{tko}$), weaver mutant (wv/wv) mice, and Ames dwarf mice to examine the role of peroxynitrite in the etiopathogenesis of Parkinson's disease and aging. Although MT$_{dko}$ mice were genetically susceptible to 1, methyl, 4-phenyl, 1,2,3,6-tetrahydropyridine (MPTP) Parkinsonism, they did not exhibit any overt clinical symptoms of neurodegeneration and gross neuropathological changes as observed in wv/wv mice. Progressive neurodegenerative changes were associated with typical Parkinsonism in wv/wv mice. Neurodegenerative changes in wv/wv mice were observed primarily in the striatum, hippocampus and cerebellum. Various hallmarks of apoptosis including caspase-3, TNFα, NFκB, metallothioneins (MT-1, 2) and complex-1 nitration were increased; whereas glutathione, complex-1, ATP, and Ser(40)-phosphorylation of tyrosine hydroxylase, and striatal ^{18}F-DOPA uptake were reduced in wv/wv mice as compared to other experimental genotypes. Striatal neurons of wv/wv mice exhibited age-dependent increase in dense cored intra-neuronal inclusions, cellular aggregation, proto-oncogenes (c-*fos*, *c-jun*, caspase-3, and GAPDH) induction, inter-nucleosomal DNA fragmentation, and neuro-apoptosis. MT$_{trans}$ and α-Syn$_{ko}$ mice were genetically resistant to MPTP-Parkinsonism and Ames dwarf mice possessed significantly higher concentrations of striatal coenzyme Q$_{10}$ and metallothioneins (MT 1, 2) and lived almost 2.5 times longer as compared to control$_{wt}$ mice. A potent peroxynitrite ion generator, 3-morpholinosydnonimine (SIN-1)-induced apoptosis was significantly attenuated in MT$_{trans}$ fetal stem cells. These data are interpreted to suggest that peroxynitrite ions are involved in the etiopathogenesis of Parkinson's disease, and metallothionein-mediated coenzyme Q$_{10}$ synthesis may provide neuroprotection.

Theme: Neurodegenerative disorders
Topic: Parkinson's disease

Keywords: Metallothionein double gene knockout mice; Metallothionein transgenic mice; a-synuclein knockout mice; Homozygous weaver mutant mice (WMhomo); Ames dwarf mice; Fetal stem cell transplantation; 18F-DOPA; MicroPET imaging; Parkinson's disease

Abbreviations: AD, Alzheimer's disease; DA, dopamine; DA-ergic neurons, dopaminergic neurons; ^{18}F-DOPA, 6-^{18}fluoro-L-dihydroxy phenylalanine; GIRK channel, G-protein-activated inward-rectifying K$^+$ channel; wv/$^+$, heterozygous weaver mutant mice; wv/wv, homozygous weaver mutant mice; MT$_{dko}$ mice, metallothionein double gene knockout mice; MT$_{trans}$ mice, metallothionein transgenic mice; PD, Parkinson's disease; PCR, polymerase chain reaction; SNpc, substantia nigra pars compacta; α-Syn, α-synuclein; α-Syn$_{ko}$ mice, α-synuclein knockout mice; α-Syn$_{ko}$–MT$_{tko}$ mice, α-synuclein–metallothionein triple knockout mice; TH, tyrosine hydroxylase

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Contents

1. Introduction

Zinc-containing neurons in the brain are a subclass of glutamatergic neurons, which are found predominantly in the telencephalon. These neurons store zinc in their presynaptic terminals and release it by a calcium-dependent mechanism. These "vesicular" pools of zinc are viewed as endogenous modulators of ligand-gated and voltage-gated ion channels.

The term *metallothionein* (MTs) refers to a low-molecular-weight metal-binding protein (M_r=6000–7000) that has unusual biochemical characteristics, such as a high content of cysteine (25–30%), large proportions of serine and lysine (12–18%), and complete absence of histidine and aromatic amino acids such as tyrosine and phenylalanine. The precise functions of metallothioneins, which may vary in different tissues and organisms, have not been established. However, evidence indicates that the hepatic and renal metallothioneins are involved primarily in metal detoxification [15], whereas the brain metallothionein isoforms I–III participate in the homeostasis of essential trace metals, such as zinc, and in scavenging free radicals caused by oxidative stress. In addition to scavenging free radicals, metallothionein isoforms exert their neuroprotective effects, in part, by enhancing the concentration of ubiquinol from ubiquinone [11,13–15].

A mutation, by definition, is a change in gene sequence and may be associated with a specific human disease. Genetically engineered mice are produced by induced mutations, including mice with transgenes, mice with targeted mutations ("knockouts"), and mice with retroviral or chemically induced mutations. Transgenic mice possess a segment of foreign DNA that is inserted in the host cell genome via pronuclear microinjection (non-homologous recombination), via infection with a retroviral vector, or in some situations via homologous recombination. Gene knockout mice are prepared by first introducing gene disruptions, replacements, or duplications into embryonic stem (ES) cells by homologous recombination. Genetically modified ES cells are microinjected in the host embryos at the eight-cell blastocyst stage. The embryos are then introduced in the pseudo-pregnant females that bear chimeric progeny. The chimeric progeny that carry the knockout gene in their germ line are subsequently bred to establish the gene knockout mice. Chemically induced mutations are produced by mutagens, such as ethyl–nitrosourea (ENU), which induces point mutations. ENU mutation is induced by treating male mice with ENU, and then breeding is performed with untreated females. The progeny are screened for phenotypes of interest carrying point mutations by polymerase chain reaction (PCR) analysis of tail DNA. Detailed information and research applications for different genetically engineered mice are now available on the JAX® Mice Database.

Various experimental models have been proposed to explore basic molecular mechanisms of neurodegeneration in Parkinson's disease (PD) [2,8,18]; however, an appropriate animal model of PD remains unavailable. Like any other experimental model, genetically engineered animals have limitations. On occasions, they do not breed well, or their growth potential may be impaired due to a compromised immune system. Irrespective of these limitations, genetically engineered animals have provided unique opportunities to understand the disease process and novel therapeutic strategies for neurodegenerative disorders. Genetically manipulated mice have been and are being used to investigate amyotrophic lateral sclerosis [1,6], Alzheimer's disease [7], motor neuron disease [5,19], Prion diseases [20,24,25], catecholaminergic regulatory systems [3], and Huntington's disease [22,26,27].