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SCIENCE DIRECT

Brain Research 1037 (2005) 1–6

BRAIN RESEARCH

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Research report

# Simvastatin prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced striatal dopamine depletion and protein tyrosine nitration in mice

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Accepted 21 February 2004

**Abstract**

Parkinson's disease is a neurological disorder involving the progressive loss of dopaminergic neurons in the substantia nigra pars compacta. There is increasing evidence that inflammation plays a role in the propagation of neurodegenerative processes in Parkinson's disease. We investigated the neuroprotective effects of simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A inhibitor with anti-inflammatory properties, in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Oral administration of simvastatin attenuated the depletion of dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid in the striatum caused by MPTP in a dose-dependent manner. Simvastatin also inhibited the formation of 3-nitrotyrosine in striatal proteins in MPTP-treated mice. Simvastatin had no effect on cholesterol concentrations in the plasma or in the striatum. Simvastatin inhibited the production of tumor necrosis factor (TNF)-α, nitric oxide, and superoxide in cultured rat microglia stimulated by lipopolysaccharide. The results suggest that simvastatin inhibits the formation of TNF-α and peroxynitrite in activated microglia thereby protecting dopaminergic neurons from inflammatory damage. Simvastatin may be a potential new treatment to slow the progression of Parkinson's disease.


*Theme:* Disorders of the nervous system
*Topic:* Degenerative disease: Parkinson's

*Keywords:* Simvastatin; Parkinson's disease; 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Dopamine; 3-Nitrotyrosine

## 1. Introduction

Parkinson's disease is a neurological disorder caused by the death of dopaminergic neurons in the substantia nigra resulting in progressive bradykinesia, rigidity, and tremor [17]. The current dopaminergic treatments improve the motor symptoms and quality of life for patients during the early stages of Parkinson's disease but do not prevent the progression of the disease and are associated with disabling side effects [29]. There is an urgent need for more effective treatments that address the underlying neurodegenerative processes in Parkinson's disease.

There is growing recognition that inflammation plays an important role in the pathology of Parkinson's disease (for review, see Hirsch et al. [13]). There is an increase in HLA-DR (MHC class II)-positive microglial cells in the substantia nigra of patients with Parkinson's disease compared to control brains [21]. There is a marked increase in cytokine concentrations such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-2, IL-4, IL-6, transforming growth factor (TGF)-α, TGF-β1, and TGF-β2 in the striatum, and cerebrospinal fluid in patients with Parkinson's disease compared with control subjects [23]. There is evidence that cytokine production may be confined to lesion sites because the density of glial cells expressing TNF-α, IL-1β, and IFN-γ is increased in the substantia nigra of Parkinson's patients compared to control subjects [12].

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doi:10.1016/j.brainres.2004.02.083

There is an increase in the concentration of nitrite in the cerebrospinal fluid in patients with Parkinson's disease [28]. The density of glial cells expressing inducible nitric oxide synthase (iNOS) is increased in the substantia nigra of patients with Parkinson's disease compared to control subjects [11]. The increased density of cytokine and iNOS-producing glial cells in the substantia nigra suggests that cytokines induce the expression of iNOS in glial cells [11,12]. Nitric oxide exerts its toxic effect by reacting with superoxide to produce peroxynitrite, which is a powerful oxidant [3]. It has been shown that there is an increase in 3-nitrotyrosine immunostaining in nigral dopaminergic neurons in Parkinson's patients [10].

The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mouse has been widely used as a tool to investigate the pathogenesis of Parkinson's disease [26]. Inflammatory processes have been described in the brains of animals treated with MPTP including the infiltration of CD4+ and CD8+ T-cells in the substantia nigra [15], the up-regulation of MHC class I and class II antigens on microglia [15], and increased IL-1β production in the striatum [22]. There is a marked increase in 3-nitrosotyrosine concentrations in rat striatum after MPTP administration [25]. The up-regulation of microglial iNOS plays an important role in the neurotoxicity of MPTP [19] and mice lacking the iNOS gene are more resistant against MPTP toxicity [6,19]. The nitration of striatal tyrosine hydroxylase after MPTP administration is prevented in transgenic mice overexpressing superoxide dismutase [1].

The statin class of drugs inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase thereby lowering circulating cholesterol concentrations and reducing the incidence of coronary events [14]. Recent data suggest that statins may also have immunomodulatory effects relevant to the treatment of neuroinflammatory conditions [7,16,24,33]. Statins inhibit the induction of MHC-II expression by IFN-γ on antigen-presenting cells [16]. Statins inhibit leukocyte function antigen-1 and inhibit binding of lymphocytes to intracellular adhesion antigen-1 [33]. Lovastatin inhibits the production of iNOS, TNF-α, IL-1β, and IL-6 in rat primary astrocytes, microglia, and macrophages [24]. Statins reduce superoxide production by NADPH oxidase in THP-1-derived monocytes [7]. The suppression of acute experimental autoimmune encephalomyelitis (EAE) in rats by oral administration of lovastatin is accompanied by a decrease in iNOS and TNF-α production [31]. Oral atorvastatin prevents or reverses chronic and relapsing EAE in the mouse, inhibiting Th1 cytokines (IL-2, IL-12, IFN-γ, TNF-α) and inducing Th2 cytokines (IL-4, IL-5, and IL-10) [35]. The anti-inflammatory actions of statins are related to their neuroprotective properties in cerebral ischemia and stroke [32]. The decreased prevalence of Alzheimer's disease in patients receiving statins may also be related to their anti-inflammatory effects [5].

We speculated that statins may be a potential new therapy for the treatment of Parkinson's disease. Simvastatin and lovastatin penetrate the blood–brain barrier while pravastatin atorvastatin, fluvastatin, and cervistatin do not [14]. Therefore, we examined the effects of simvastatin on MPTP toxicity in the mouse. We also studied the effect of simvastatin on the production of TNF-α, nitric oxide, and superoxide by cultured microglia.

## 2. Methods

### 2.1. Treatment protocol

Studies with animals were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male C57BL mice (8 weeks old; 22–25 g; $n$ = 10/group) were treated with 0, 10, 20, or 40 mg/kg/day of simvastatin by gavage for 10 days. On day 5, the mice received four ip injections of MPTP hydrochloride (15 mg/kg free base) dissolved in 0.1 ml of phosphate-buffered saline at 2-h intervals. A control group was treated with 0.1 ml of normal saline. On day 11, the mice were sacrificed by decapitation and the striata rapidly dissected and stored at −80 °C.

### 2.2. Determination of striatal dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid concentrations

The striata from five mice in each group were homogenized in 500 μl of 0.1 M of $HClO_4$ and centrifuged at 10,000 × $g$ for 10 min at 4 °C to precipitate proteins. The concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the supernatants were determined by high performance liquid chromatography (HPLC) with electrochemical detection [27].

### 2.3. Determination of striatal protein-bound 3-nitrotyrosine

The concentration of protein-bound 3-nitrotyrosine in striatal pellets was determined by GC/MS following alkaline hydrolysis and derivatization to the heptaflurobutylamide *tert*-butyldimethylsilyl ester using [$^{13}C_9$]nitrotyrosine as the internal standard [9].

### 2.4. Determination of striatal TNF-α concentrations

The striata from five mice in each group were homogenized on ice in 0.1 M phosphate buffer containing protease inhibitors at pH 7.4 [4]. The homogenates were centrifuged for 10,000 × $g$ for 10 min at 4 °C and the concentration of TNF-α in the supernatants determined using a commercial ELISA kit (BioSource International, Camarillo, CA).