Neuroprotective mechanism of modafinil on Parkinson disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

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ABSTRACT

AIM: To observe the neuroprotective mechanism of modafinil on Parkinson disease (PD) models induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). **METHODS:** The model of PD was induced by intraperitoneally injecting MPTP into C57BL/6J mice for 4 d. Modafinil (ip, 50 or 100 mg·kg $^{-1}$·d^{-1}) was administered at 30 min following MPTP for 4 d and for another 10 d continuously. The contents of dopamine (DA), noradrenaline (NA), 5-hydroxytryptamine (5-HT), γ-aminobutyric acid (GABA) , glutamine (Glu) in the striatum, and the contents of GABA, Glu, malondialdehyde (MDA), and glutathione (GSH) in the substantia nigra (SN) of model mice were determined. **RESULTS:** Modafinil (50 and 100 mg/kg) prevented against the decrease of the contents of DA, 5-HT, and NA in the striatum and GSH, GABA in the SN induced by MPTP, but reduced the increase of MDA in the SN and GABA in the striatum induced by MPTP. Modafinil preferentially inhibited striatal GABA release, but it did not change the increase of nigrostriatal Glu release induced by MPTP. **CONCLUSION:** The anti-oxidation and the modulation of nigrostriatal GABA and striatal NA and 5-HT release contributed to the neuroprotective effects of modafinil on PD induced by MPTP.

INTRODUCTION

Parkinson disease (PD) is a common dopaminergic neurodegenerative disorder. At present, chronic use of several approved drugs to alleviate PD symptoms is often associated with aggravating side effects, and none of these drugs seems to prevent the progress of PD. This is probably due to the fact that the etiology of PD is still unknown. The cardinal theories have been proposed to explain the cause of PD including the basal ganglia circuitry theory and the oxidative stress-free radicals theory, and the one on oxidative stress is presently the most persuasive one and gains major interest [1]. Thereby a variety of potential neuroprotective agents are under investigation.

Previous studies have shown that modafinil, a vigilance-enhancing agent[2], was also effective in protecting neurons from a range of toxic insults, including damage to the nigro-striatal pathway[3-8]. We demonstrated that modafinil could improve the behavioral deficits and protect the monoaminergic neurons lesion in serious 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model by neurochemical, neurohistopathological, and behavioral evidences. However, the mechanism is

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unclear. To explore the neuroprotective mechanism of modafinil on PD, we observed the effects of modafinil on neurochemistry of MPTP mouse.

MATERIALS AND METHODS

Materials MPTP (Sigma-Aldrich, USA) was dissolved in saline. Modafinil (Chongqing Pharmaceutical Research Institute, Chongqing, China) was dissolved in 0.4 % sodium carboxymetylcellulose. Dopamine, 5-hydroxytryptamine, and noradrenaline (Fluka, Switzerland) were dissolved in 0.01 mol/L hydrochloride. GABA and Glu were respectively dissolved in Li-S (Sigma-Aldrich, USA) .

Animal model Male old C57BL/6J mice, weighing 20 g±2 g, were provided by the Experimental Animal Center of Sichuan University. The 40 mice were randomly divided into four groups: the control group was ip injected 0.4 % sodium carboxymethylcellulos (15 mL·kg^{-1}·d^{-1}) for 14 d; the model group was ip injected MPTP (30 mg·kg^{-1} ·d^{-1}) for 4 d; the treatment groups were administered ip modafinil (50 and 100 mg·kg^{-1}·d^{-1}) at 30 min after injection of MPTP for 4 d and for another 10 d continuously.

Treatment All mice were killed after the last injection and the brain tissues were quickly freezed at -80 ºC. The contents of dopamine (DA) in the striatum were determined with spectrofluorophotometer (λ_{Ex}=310 nm, λ_{Em} =390 nm, RF-5000, Japan) , 5-HT (λ_{Ex}=355 nm, $\lambda=_{Em}$ 495 nm), and noradrenaline (NA) (λ_{Ex}=400 nm, $\lambda=_{Em}$ 500 nm). The contents of malondialdehyde (MDA) in the SN were measured with the thiobarbituric acid-reaction to indicate the LPO, and contents of glutathione (GSH) in the substantia nigra (SN) were based on the dithionitrobenzonic acid (DTNB) determination. The contents of GABA and Glu in the striatum and SN were shown by high performance amino acid auto-analyser (System-6300 Beckman, USA) . The mobile phase consisted of Li-A, Li-C, and Li-R. The flow rate was 20 mL/h and the temperature of chromatography column were 33.5 ºC and 77 ºC. The wavelength of ultraviolet spectrophotometer was 570 nm. The limit of detection for GABA and Glu were 0.2 μmol/L and 7 μmol/L, respectively.

Statistical analysis The data were expressed as mean±SD, and analyzed by one-way factorial analysis of variance (ANOVA) followed by Newman-Keuls test for multiple comparisons. P<0.05 was considered significant.

RESULTS

Effects of modafinil on the contents of DA, NA, and 5-HT The content of DA, NA, and 5-HT in the striatum was significantly decreased in MPTP group compared with control group (P<0.05, n=10). Modafinil (50 and 100 mg/kg) increased DA, NA, and 5-HT contents compared with model group (P<0.05, n=10) (Tab 1) .

Tab 1. Effects of modafinil on the concentration DA, NA, and 5-HT in the striatum of PD mice induced by MPTP. n=10. Mean±SD. ^{b}P<0.05 *vs* the control group. ^{e}P<0.05 *vs* MPTP group.

Group	DA	NA	5-HT
	/μg·g^{-1} wet tissue		
Control	953±106	638±60	326±18
MPTP	475±118^{b}	409±57^{b}	246±22^{b}
MPTP+modafinil (50 mg/kg)	847±89^{e}	568±52^{e}	312±29^{e}
MPTP+modafinil (100 mg/kg)	867±105^{e}	549±67^{e}	313±28^{e}

Effects of modafinil on the contents of MDA and GSH The level of nigral GSH in model group was markedly decreased (P<0.01, n=10) and the contents of nigral MDA was increased compared with those in control group (P<0.01, n=10). Modafinil (50 and 100 mg/kg) markedly lowered the MDA level while relatively increased the GSH level in PD model (P<0.01, n=10) (Fig 1) .

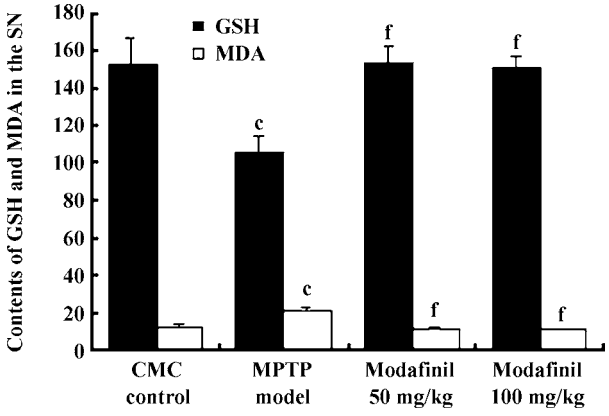

Fig 1. Effects of modafinil on the concentration of GSH (g·g^{-1} protein), MDA (μmol·g^{-1} protein) in the substantia nigra of PD mice induced by MPTP. n=10. Mean±SD. ^{c}P<0.01 *vs* control group. ^{f}P<0.01 *vs* MPTP group.

Effect of modafinil on the contents of GABA and Glu MPTP markedly increased the striatal GABA level (P<0.01, n=10) while decreased those in the SN (P<0.05, n=10) compared with control group, which were prevented by modafinil 50 and 100 mg/kg. Moreover, the change of striatal GABA contents was higher than those of the SN in treatment groups. However, modafinil did not change the increase of nigrostriatal Glu release induced by MPTP (Tab 2) .

DISCUSSION

MPTP can selectively damage dopaminergic neurons, which leads to impaired dopaminergic neurotransmission. MPTP is oxidized by the monoamine oxidase-B to its toxic metabolite MPP^+, which is selectively taken up by dopaminergic neurons. MPP^+ could produce oxidative stress and inhibit NADH dehydrogenase in the mitochondria leading to ATP loss[9]. At present, the basal ganglia circuitry and the oxidative stress are implicated in the mechanism of MPTP-induced neurotoxicity in animals.

Older C57BL/6J mice injected with MPTP are used in our study. The MPTP mice model, for studying the PD mechanisms and evaluating the antiparkinsonian drugs, is superior to the MPTP primates model[10,11]. Our present results raise the possibility that modafinil might intervene in multi-intermediate links of pathogenesis to prevent the progress of PD. Therefore three anti-parkinsonian mechanisms of modafinil are discussing.

Some of the toxic effects of MPTP are mediated by both superoxide radicals and hydroperoxides, and they occur prior to dopaminergic neurodegeneration in the SNc[12]. The level of GSH in the SN was greatly reduced only in PD. Glutathione system could scavenge the superoxide radicals and hydroxyl radicals, which is the very important non-enzyme protective mechanism. Following the decrease of GSH in neurons of PD, the reaction of lipid peroxidation in SN was enhanced and the content of MDA was increased. At the same time, the enhanced DA autoxidation and oxidative stress caused damage of the SN neurons. These suggest that the altered GSH/GSSG ratio in the SN in PD is consistent with the concept of oxidative stress, a major component in the pathogenesis of nigral cell death in PD[13-15]. Our results provided direct evidence for involvement of anti-oxidative effect in the action of modafinil on MPTP mouse.

Post-mortem studies provided evidence for a decrease in the content of NA and 5-HT in the putamen and globus pallidus of PD. To date, it is not known whether the decrease of NA and 5-HT is a secondary or primary change[16]. Modafinil have an effect on the transformation of 5-HT in central nervous system of conscious rats[17,18]. Our results showed that the contents of striatal NA and 5-HT in the MPTP mice were markedly lower than those of the normal mice, and modafinil increased striatal DA, NA, and 5-HT levels. It might indirectly improve the progress of PD.

GABA neurons are abundant in the SN as the dopaminergic population, and may be related with the neurotoxicity of MPTP. In normal mice, the striatal neurons' activity is controlled by the inhibition of nigra dopaminergic neurons and the excitation of pallium Glu neurons. Once dopaminergic neurons degenerated, the balance in basal ganglia-thalamo-cortical circuitry is broken, and too much inhibition from the thalamus and cerebral cortex circuitry causes expression of Parkinsonian syndrome at last[19-21]. Modafinil reduced the GABA release in the several normal brain regions, and the decrease of GABA is related with the NA and 5-HT neurotransmission[22-26]. Our results showed that modafinil preferentially inhibited striatal GABA release without influencing local Glu release in PD model, which

Tab 2. Effects of modafinil on the concentration of GABA (μmol·g^{-1} wet tissue) and Glu in the substantia nigra and striatum of PD mouse induced by MPTP. n=10. Mean±SD. $^c P$<0.01 *vs* control group. $^d P$>0.05, $^e P$<0.05 , $^f P$<0.01 *vs* MPTP group.

Group	Substantia nigra		Striatum	
	GABA	Glu	GABA	Glu
Control	4.9±0.4	27.3±2.3	4.4±1.6	23.9±2.3
MPTP	2.1±0.5^c	32.6±2.9^c	8.6±1.5^c	34±5^c
MPTP+modafinil (50 mg/kg)	2.9±0.3^e	31±4^d	4.7±1.3^f	33±4^d
MPTP+modafinil (100 mg/kg)	2.8±0.4^e	30±4^d	4.9±1.7^f	34±4^d

is the first demonstration that modafinil restored normal GABAergic transmission and ameliorated the basal ganglia circuitry in PD. So we presumed that modafinil first increased NA level, then indirectly activated 5-HT, at last restored the balance of GABA release between in the striatum and SN to inhibit the over-activity of glutamate pathway from the subthalamic nucleus to the internal segment of the globus pallidus and SN. In addition, our results further confirmed that the content of striatal GABA in MPTP mouse was significantly increased, which was the same with other reports.

In conclusion, modafinil prevented against the neurotoxicity of MPTP by anti-oxidation and modulation of the striatal NA and 5-HT and nigrostriate GABAergic activity. The neurotoxicity of MPTP is related with the free radicals and the unbalance of neurotransmitters between the striatum and SN. Thereby modafinil may be a valuable neuroprotective agent for the treatment of PD.

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