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A cell-permeable peptide inhibitor TAT-JBD reduces the MPP+-induced caspase-9 activation but does not prevent the dopaminergic degeneration in *substantia nigra* of rats

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Abstract

Many studies showed that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which was widely used to produce Parkinson's disease (PD)-like models in animals can elicit apoptosis with increase of caspase activity via its neurotoxic metabolite 1-methyl-4-phenylpyridinium ion (MPP^+). Another pathway shown in MPTP-mediated nigrostriatal dopaminergic cell death involved the c-Jun-N-terminal kinases (JNKs) which are stress-activated protein kinases (SAPKs). Activation of the JNKs leads to the activation of transcription factors such as c-Jun that regulates its own expression. However, it is not known whether the activation of c-Jun is crucial in the stimulation of caspases leading to apoptosis observed in PD-like models. The aim of this study was to investigate the cellular expression and phosphorylation of c-Jun and the caspase-9 activity in rat injured with an intranigral injection of MPP^+. Furthermore, we determined the effects of a cell-permeable peptide TAT-JBD, inhibiting selectively JNKs, on apoptosis markers and on the expression of tyrosine hydroxylase (TH). Our results showed that MPP^+ induced not only an activation of c-Jun but also an early and robust stimulation of caspase-9 in midbrain of rats. Furthermore, a preliminary intravenous injection of TAT-JBD reduced the caspase-9 activation specifically induced by MPP^+ suggesting a control of the JNKs pathway on the intrinsic way of apoptosis in MPP^+-toxicity. However, the inhibition of the JNK pathway did not prevent TH inhibition, DNA fragmentation and Bad expression in MPP^+-lesioned *substantia nigra* of rats. Therefore, the possibility of intervention on the JNK pathway as a therapeutic strategy in Parkinson's disease is questionable.

Keywords: MPP^+; Caspase-9; c-Jun; Rat; Neuropeptide; *Substantia nigra*

Abbreviations: Akt/PKB, protein kinase B; Apaf-1, apoptotic protease activating factor-1; JNK, c-Jun N-terminal kinase; LEHD, Leu-Glu-His-Asp acid; MAPK, mitogen-activated protein kinase; MPP^+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PC12 cells, rat pheochromocytoma cell line; PD, Parkinson's disease; *p*-NA, *p*-nitroaniline; RS6K, ribosomal S6 kinase; SAPK, stress-activated protein kinase; SH-SY5Y cells, human neuroblastoma cells; SN, substantia nigra; TH, tyrosine hydroxylase; TUNEL, terminal deoxynucleotidyl transferase TdT-mediated BrdUTP nick-end labelling.

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1. Introduction

Parkinson's disease (PD) is a degenerative disorder of the extrapyramidal motor system. Specifically, failure of the nigrostriatal system due to the dopaminergic cell loss in the *substantia nigra*, results in the classic symptoms of resting tremor, rigidity, bradykinesia and postural instability. Although the exact cause of this neuronal loss is still unknown, human postmortem studies showed the involvement of apoptosis in this disease (Mochizuki et al., 1996; Tatton, 2000; Hartmann et al., 2000, 2001). Therefore, it is important to define cellular actors of this cell death presumably critical for the nigral degeneration. To date, the determination of the sequence of events leading to this apoptotic cell death is easier in animal models using neurotoxic compounds that mimic dopaminergic degeneration seen in PD.

Interestingly, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which was widely used to produce a PD-like model in both primates (Burns et al., 1983; Langston et al., 1984) and rodents (Chiueh et al., 1984; Heikkila et al., 1984) leads to apoptosis via its neurotoxic metabolite 1-methyl-4-phenylpyridinium ion (MPP^+) (Dipasquale et al., 1991; Mochizuki et al., 1994; Kitamura et al., 1998). On the one hand, MPP^+ induces nuclear changes, considered as hallmarks of apoptosis and consisted of nuclear fragmentation from chromatin condensation and internucleosomal DNA breakdown leading to the appearance of a DNA "ladder", as it was observed in PC12 cells (Chalmers-Redman et al., 1999), mesencephalic-striatal cocultures (Mochizuki et al., 1994), SH-SY5Y human neuroblastoma cells (Gómez et al., 2001) and in MPTP-mice (Tatton and Kish, 1997). On the other hand, several molecular factors of programmed cell death have been implicated in MPTP/MPP^+-induced neurotoxicity. In mice treated with MPTP, Bax mRNA (an apoptotic factor) was overexpressed (Hassouna et al., 1996). Moreover, mice overexpressing Bcl-2 (an anti-apoptotic protein) or deficient in p53 (a proapoptotic protein) were resistant to MPTP-induced neurotoxicity (Trimmer et al., 1996; Yang et al., 1998). *In vivo* and *in vitro* studies (Du et al., 1997; Gómez et al., 2001; Viswanath et al., 2001) indicated that aspartate-specific cysteine proteases (caspases), particularly caspases-3, -8 and -9 were activated after MPTP/MPP^+ treatment as it was observed in postmortem parkinsonian brain (Hartmann et al., 2000, 2001). Caspase-3 is a final effector in apoptotic death, it may be activated by caspase-9, activated itself by the apoptosome complex containing cytochrome *c*, apoptotic protease-activating factor-1 (Apaf-1) and procaspase-9. The caspase-9 represents the central initiator caspase for the intrinsic cell death pathway (Li et al., 1997; Lauber et al., 2001). Interestingly, MPP^+ selectively and potently inhibits the complex I of the electron transport chain (Nicklas et al., 1985; Vyas et al., 1986), opens the mitochondrial permeability transition pore and releases cytochrome *c* (Cassarino et al., 1999; Kakimura et al., 2001). Furthermore, the inhibition of caspase-3 and apoptosis in a caspase-9 knockout mice model (Kuida et al., 1998) and in a mutant caspase-9 (Seol and Billiar, 1999) supported the hypothesis that caspase-9 may be a central regulator of caspase-3 in cytochrome *c*-dependent signalling pathway involving Apaf-1.

Another pathway implicated in MPTP-mediated nigrostriatal dopaminergic cell death was the c-Jun NH_2-terminal kinase (JNK) way (Saporito et al., 1999, 2000), a component of a stress-activated protein kinases (SAPKs) which play an important role in triggering apoptosis in response to free radicals (Derijard et al., 1994; Xia et al., 1995; Eilers et al., 2001; Kim et al., 2007). Thus, it was not surprising that JNKs were activated in MPTP model in which the generation of free radicals was clearly demonstrated (for review see Tipton and Singer, 1993). Activation of the JNK pathway leads to the phosphorylation-induced activation of the transcription factor c-Jun that function as heterodimer in association with c-Fos or as homodimer leading to increase the AP-1 transcription activity (Dragunow and Preston, 1995). However, it is not known whether the activation of c-Jun is crucial for stimulation of caspases in the programmed cell death observed in PD-like models.

The current study was designed to extend these findings by investigating the time-course of the expression and phosphorylation of c-Jun protein and the activity of caspase-9 in midbrain of rats injured with an intranigral injection of MPP^+. Moreover, we investigated the effects of a neuroprotective cell-permeable peptide, (L)-HIV-$TAT_{48–57}$-PP-JBD_{20} which blocks selectively the access of JNK to c-Jun by a competitive mechanism (Bonny et al., 2001; Barr et al., 2002; Borsello and Forloni, 2007) on MPP^+-induced caspase-9 activation, apoptosis and tyrosine hydroxylase inhibition.

2. Materials and methods

2.1. Materials

The chemicals were purchased from the following sources: isoflurane (Forene®) from Abbott (Paris, France); the peptide substrate for caspase-9 (Ac-LEHD-*p*-NA) and the cell-permeable peptide (L)-HIV-$TAT_{48–57}$-PP-JBD_{20} (JNK Inhibitor I) from VWR International (Fontenay-sous-Bois, France); desipramine, 1-methyl-4-phenylpyridinium (MPP^+),