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Apoptosis and Parkinson's disease

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Abstract

Parkinson's disease (PD) is a severe and progressive neurodegenerative disease. It is the second most common neurodegenerative disease, after Alzheimer's disease. It is caused by the selective loss of the dopaminergic neurons in the substantia nigra (SN) pars compacta. Although subject to intensive research, the etiology of PD is still enigmatic and treatment is basically symptomatic. Many factors are thought to operate in the mechanism of cell death of the nigrostriatal dopaminergic neurons in PD. In recent years, evidence for the role of apoptotic cell death in PD arises from morphological, as well as molecular, studies in cell cultures, animal models for PD, as well as human studies on postmortem brains from PD patients. These studies indicate that apoptosis takes place in PD and that there is a proapoptotic environment in the nigrostriatal region of parkinsonian patients. It is of utmost importance to conclusively determine the mode of cell death in PD because new "antiapoptotic" compounds may offer a means of protecting neurons from cell death and of slowing the rate of neurodegeneration and disease progression.

Keywords: Apoptosis; Parkinson's disease

1. Introduction

Parkinson's disease (PD) is a severe and progressive neurodegenerative disease characterized by resting tremor, cogwheel rigidity, bradykinesia, and loss of postural reflexes (Parkinson, 1817). PD is caused by a deficiency of the neurotransmitter dopamine at the nerve terminals of the nigrostriatal dopaminergic neurons in the striatum, due to selective loss of the dopaminergic neurons in the substantia nigra (SN) pars compacta. Most of PD are sporadic and age-related, and only approximately 5% is a familial disease.

Although subject to intensive research, the etiology of PD is still enigmatic and the treatment is basically symptomatic. Many factors are speculated to operate in the mechanism of cell death of the nigrostriatal dopaminergic neurons in PD, including oxidative stress and cytotoxicity of reactive oxygen spices (ROS), disturbances of intracellular calcium homeostasis, exogenous and endogenous toxins, and mitochondrial dysfunction.

Neurodegenerative processes are generally characterized by a long-lasting course of neuronal death. Two main forms of cell death are known: necrosis and apoptosis. Necrosis is the result of cellular "accidents," such as those occurring in tissues subject to chemical trauma. The necrotizing cells swell, rupture, and provoke an inflammatory response. Apoptosis is a programmed cell death. It is characterized by morphological changes including cell shrinkage, nuclear condensation, and DNA degradation. The apoptotic process is caused by a cascade of events in which a family of cysteine proteases known as caspases leads to the cleavage of multiple cellular substrates. The apoptotic death is characterized by the expression of genes, mostly oncogenes, that enhance the apoptotic process (i.e., *bax*, *bcl-x*) and others that inhibit the death process (e.g., *bcl-2*, *bcl-xL*). Moreover, a large range of induced transcription factors (ITFs) (e.g., c-Fos, Fos B, Fos-related antigen, c-*jun*, jun B, jun D, Krox 20, Krox 24) are related to the apoptotic process (Offen et al., 2000).

Abbreviations: BDNF, brain-derived neurotrophic factor; DTT, dithiothreitol; 6-OHDA, 6-hydroxydopamine; ITF, induced transcription factor; JNK, c-*jun* N-terminal kinase; MKK4, JNK kinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MAP, mitogen-activated protein; NAC, *N*-acetyl-L-cysteine; NGF, nerve growth factor; PD, Parkinson's disease; PARP, poly(ADP-ribose) polymerase; ROS, reactive oxygen spices; SN, substantia nigra; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; TNF-α, tumor necrosis factor-alpha; TNFR1, TNF-α receptor R1.

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Evidence of apoptotic cell death in various neuronal and non-neuronal cells was seen through DNA fragmentation and typical morphological changes in PD brains. Although the concept of programmed cell death (apoptosis) in PD is still controversial, data from postmortem brains of PD patients, animal models, and in vitro culture studies indicate the presence of apoptotic cell death as well as a proapoptotic environment in the nigrostriatal region in PD. It is of utmost importance to conclusively determine the mode of cell death in PD because new "antiapoptotic" compounds may offer a means of protecting neurons from cell death and of slowing the rate of neurodegeneration and disease progression.

2. Evidence from cell culture models

In vitro experimental models of PD have yielded clues about the possible pathways involved in the disease. Oxidative stress generated by toxic metabolites of dopamine could be one of the factors underlying the selective vulnerability of nigral dopaminergic neurons in PD. Indeed, exposure to dopamine, 6-hydroxydopamine (6-OHDA), and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces characteristic biochemical, histochemical, and morphological changes typical of apoptosis in various neuronal (and also non-neuronal) cell cultures.

Dopamine was shown to induce apoptosis in several cell cultures, including chick sympathetic neurons (Ziv et al., 1994; Zilkha-Falb et al., 1997; Massenaro et al., 1996), human neuroblastoma NMB cell line (Gabbay et al., 1996), human neuroblastoma cell line SH-SY5Y (Junn and Mouradian, 2001), and non-neural cells (Offen et al., 1995). Dopamine-induced apoptosis can be partially suppressed by the overexpression of the antiapoptotic factor, bcl-2 (Offen et al., 1997a; Ziv et al., 1997). Barzilai et al. (2000) found that dopamine toxicity is mediated through its oxidative metabolites and that there is up-regulation of collapsin-1 (an axonal guidance molecule) and of TCP-1-delta (a member of the heat shock family) in sympathetic neurons that undergo dopamine-induced apoptosis. Antibodies directed against collapsin-1 as well as the inhibition of TCP-1-delta expression (using antisense technology) significantly reduced dopamine-induced neuronal apoptosis. Junn and Mouradian (2001) showed that dopamine-induced apoptosis in SH-SY5Y neuroblastoma cells, as demonstrated by the activation of caspase-9 and caspase-3, the cleavage of poly(ADP-ribose) polymerase (PARP), and nuclear condensation, is mediated by the activation of p38 mitogen-activated protein (MAP) kinase and mitochondrial cytochrome *c* release. Another MAP kinase involved in many apoptotic signaling events is c-*jun* N-terminal kinase (JNK). Dopamine also appears to increase the levels of JNK activity, the JNK phosphorylation of c-*jun*, and c-*jun* levels in striatal cultures; both inhibition of JNK phosphorylation and c-*jun* inhibition prevent apoptosis in these cultures (Luo et al., 1998). Daily et al. (1999) also showed that a marked threefold increase in p53 phosphorylation was associated with dopamine-induced apoptosis. Using a temperature-sensitive p53 activation system in leukemia LTR6 cells, they found that p53 inactivation dramatically attenuated dopamine toxicity. Therefore, p53 activation may also have a role in mediating dopamine-induced apoptosis.

Oxidative stress is a leading hypothesis as a potential mechanism of dopaminergic neuronal degeneration in PD. One of the suggested causes for oxidative stress in the PD brain is ROS generated during normal dopamine metabolism, either by autooxidation or by the action of monoamine oxidase. Dopamine-induced apoptosis was blocked in several culture studies by the addition of antioxidants such as *N*-acetyl-L-cysteine (NAC) or dithiothreitol (DTT) (Massenaro et al., 1996; Luo et al., 1998; Barzilai et al., 2000; Junn and Mouradian, 2001; Ziv et al., 1994; Offen et al., 1997b). This protection correlated with the inhibition of caspase-9 and caspase-3 activation, indicating that dopamine triggers apoptosis via a signaling pathway that is initiated by the generation of ROS (Junn and Mouradian, 2001; Barzilai et al., 2000).

Offen et al. (1997c) found that dopamine–melanin may induce typical apoptotic death in PC12 cells. Antioxidants offered a protective effect against the toxicity of dopamine and dopamine–melanin. These findings support a possible role of neuromelanin in the vulnerability of the dopaminergic neural degeneration in PD.

6-OHDA is a dopaminergic neurotoxin used in experimental PD models. It was shown to induce apoptosis in dopaminergic PC12 cells via the activation of caspases (Takai et al., 1998; Andersen, 2001) as well as the induction of the proapoptotic factor, bax (Blum et al., 1997). 6-OHDA-induced apoptosis was also shown in cerebellar granule cells, primary mesencephalic dopaminergic cells, the mesencephalic-derived dopaminergic MN9D cell line, neuroblastoma NB41 cells, the murine embryonic carcinoma P19 cell line, cultured microglial cells, and thymocytes (Blum et al., 2001). 6-OHDA induces an early increase in p53 cellular content in PC12 cultures (Blum et al., 1997). Activation of caspase-3 and caspase-9 was also demonstrated recently in 6-OHDA-induced apoptosis of SH-SY5Y cells (Coelln et al., 2001). Pretreatment with caspase inhibitors, acetyl–Try–Val–Ala–Asp–aldehyde and acetyl–Asp–Glu–Val–Asp–aldehyde, prevented the 6-OHDA-induced apoptosis (Takai et al., 1998). Overexpression of the antiapoptotic protein bcl-2 prevented 6-OHDA-mediated cell death (Takai et al., 1998; Offen et al., 1997a, 1998; Andersen, 2001).

Significant insights into the pathogenesis of PD have been achieved by the use of the neurotoxin MPTP. In several mammalian species, MPTP reproduces most of the biochemical and pathological hallmarks of PD, including the dramatic degeneration of dopaminergic neurons (Przedborski and Jackson-Lewis, 1998). The effects of MPTP on animals depend on several parameters, such as the mode of administration, dosage, and animal age (Blum et al., 2001).

In young mice, MPTP damages the dopaminergic terminals in the stratum (Ricaurte et al., 1986), while a chronic administration of MPTP causes a loss of dopaminergic neurons in the SN (Petroske et al., 2001). MPTP is a mitochondrial toxin that elicits its destructive action by conversion to MPP^+ through monoamine oxidase B. MPP^+ is then selectively taken up by nigral dopaminergic neurons through dopamine transporters. MPP^+ kills these cells by specifically inhibiting mitochondrial complex I activity, leading to the subsequent initiation of apoptosis (Fall and Bennett, 1999). MPP^+ has been found to cause the apoptotic cell death of both dopaminergic PC12 cells and primary midbrain (mesencephalic) cell cultures via the activation of caspase 3 (Hartmann et al., 2000; Andersen, 2001). It also causes apoptosis of dopaminergic SH-SY5Y cells (Fall and Bennett, 1999). Using flow cytometry, Fall and Bennett (1999) found that MPP^+-induced apoptosis was mediated by the induction of ROS and lactate production consistent with inhibition of the mitochondrial electron transport chain. Rho(0) cells, lacking the functional electron transport chain, showed no ROS or lactate production, or apoptosis after exposure to MPP^+.

Several works suggest that MPTP-induced apoptosis may be under the control of p53 protein, *bcl-2* family genes, and caspase activity (Blum et al., 2001; Kitamura et al., 1998). Kitamura et al. (1998) showed that treatment of human neuroblastoma SH-SY5Y cells with MPP^+ induced ROS production, p53 expression, cleavages of caspase-3, and PARP, and apoptotic cell death with DNA fragmentation and characteristic morphological changes. Bcl-2 overexpression protected these cells against MPP^+ toxicity, whereas decreased bcl-2 levels enhanced MPP^+-induced cell death (Kitamura et al., 1998). There is also accumulating evidence that the JNK pathway is involved in MPTP toxicity. Human SH-SY5Y neuroblastoma cells that are submitted to MPP^+ treatment exhibit JNK activation (Cassarino et al., 2000). MPTP/MPP^+ led to the sequential phosphorylation and activation of JNK kinase (MKK4), JNK, and c-*jun*; the activation of caspases; and apoptosis in human SH-SY5Y neuroblastoma cells (Xia et al., 2001).

Cell death associated with PD appears to involve mitochondrial damage, especially via inhibition of the activity of mitochondrial complex I. Betarbet et al. (2000) reported that chronic, systemic inhibition of complex I by rotenone causes highly selective nigrostriatal dopaminergic degeneration that is associated behaviorally with hypokinesia and rigidity. Nigral neurons in rotenone-treated rats accumulate fibrillar cytoplasmic inclusions that contain ubiquitin and alpha-synuclein. These results indicate that chronic exposure to rotenone can reproduce the anatomical, neurochemical, behavioral, and neuropathological features of PD. Sherer et al. (2002) showed that chronic low-grade complex I inhibition, caused by rotenone exposure, induces accumulation and aggregation of alpha-synuclein and ubiquitin, progressive oxidative damage, and caspase-dependent apoptotic death in human neuroblastoma cells. Inhibition of complex I by rotenone and MPTP caused apoptotic cell death also in dopaminergic PC12 cells (Hartley et al., 1994). Hartley et al. (1994) found that both rotenone and MPTP (at low concentrations) cause apoptosis but necrosis at high concentrations.

3. Evidence from animal models of PD

In vivo animal models of PD have strongly suggested a role for apoptosis in the pathophysiology of the disease. There are several animal models of PD: PD mice produced by repeated injections of MPTP; hemiparkinsonian rats produced by injecting 6-OHDA into one side of the ventrotegmental bundle; and inhibition of complex I via rotenone and other inhibitors that can result in decreased ATP production and increased ROS generated in the mitochondria. Transgenic animals also serve as an important tool to verify the role of different genes in the pathogenesis of PD.

He et al. (2000) showed that intracerebral injection of 6-OHDA causes apoptotic cell death of dopaminergic neurons in the SN. 6-OHDA injection into the medial forebrain bundle of adult rats resulted in an increase in the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining (Zuch et al., 2000). There was a loss of tyrosine hydroxylase immunoreactivity in the SN. Morphological examination of these neurons supported the conclusion of death via apoptosis (Zuch et al., 2000). Yamada et al. (1999) demonstrated that the injection of a bcl-2-producing vector into the SN 1 week prior to 6-OHDA injection increased neuronal survival in the SN after the lesion.

Selective degeneration of dopaminergic neurons of the SN, as well as DNA fragmentation, were also shown in an MPTP-induced PD mouse model (Tatton and Kish, 1997). MPTP-mediated cell death was prevented by PARP inhibitor (Cosi et al., 1996). MPTP administration increased nigrostriatal expression of c-*jun* (Nishi, 1997) and activation of JNK and JNK kinase (also known as MKK4) (Saporito et al., 2000). JNK-specific inhibitor administration attenuated dopaminergic cell loss in the SN of MPTP-treated mice (Saporito et al., 1999). In the latter, adenoviral gene transfer of the JNK binding domain of JNK-interacting protein-1 (an inhibitor of JNK) inhibited JNK, c-*jun*, and caspase activation as well as the death of dopaminergic neurons and the loss of catecholamines in the striatum (Xia et al., 2001). There is a dramatic up-regulation of bax mRNA and protein in the SN pars compacta in MPTP-treated mice (Vila et al., 2001; Hassouna et al., 1996). Mutant mice lacking *bax* were shown to be resistant to MPTP toxicity as compared to their wild-type littermates (Vila et al., 2001). In contrast with bax up-regulation, there is a decrease in bcl-2 (an antiapoptotic protein) in the ventral midbrain of these mice, at the same time period (Vila et al., 2001). These data suggest that after MPTP injection, a cascade of events take place with up-regulation of bax and down-regulation of bcl-2 leading to

apoptotic cell death. Consistent with this scenario are the observed protective effects of bax ablation (Vila et al., 2001) as well as overexpression of bcl-2 (Offen et al., 1998; Yang et al., 1998) against MPTP toxicity. Vila et al. (2001) found that in adult mice, there is an up-regulation of bax in the SN after MPTP administration and a decrease in bcl-2. These changes parallel MPTP-induced dopaminergic neurodegeneration. They also showed that mutant mice lacking bax are significantly more resistant to MPTP than their wild-type littermates. Yang et al. (1998) showed that bcl-2 overexpression blocked MPP^+-induced activation of caspases. Offen et al. (1998) showed, using transgenic mice expressing human bcl-2 in their neurons, that while striatal dopamine level after MPTP injections was reduced by 32% in the wild type, the concentration remained unchanged in the transgenic mice.

4. Evidence from human PD patients

Determining whether dopaminergic nigral neurons die via apoptosis or necrosis in postmortem PD brains involves serious technical and theoretical problems. PD is a slowly progressive neurodegenerative disease and the rate of neuronal death in PD is very low (no more than a few cells per day). Several studies failed to identify apoptotic neurons using in situ end-labeling (TUNEL) methods or morphological signs of apoptosis in PD patients' brains (Kosel et al., 1997; Banati et al., 1998; Jellinger, 2000). However, utilizing the common biochemical and histological methods, it is difficult to detect those few cells undergoing apoptosis within the whole tissues; therefore, very sensitive methods are required. Mochizuki et al. (1996) reported that by utilizing in situ nick end-labeling method, they found apoptotic neurons in the SN of PD patients. Tatton et al. (1998) demonstrated apoptotic dopaminergic neurons in the SN pars compacta in PD utilizing fluorescent in situ double labeling method combined with a cyanine dye that binds to DNA and confocal laser microscopy. An electron microscopy study also identified apoptotic nuclei with the typical ultrastructural changes in SN of PD patients (Anglade et al., 1997).

All the abovementioned studies relied on the presence of morphological markers of apoptosis. A different approach is to identify molecular markers of apoptoic cell death in autopsied SN tissue isolated from PD patients. Examination of genes involved in the apoptotic process in postmortem PD tissues as compared to controls demonstrated up-regulation and higher expression of p53 and CD95 (de la Monte et al., 1998). Hartmann et al. (2000) reported that using an antibody raised against activated caspase-3, the percentage of active caspase-3-positive neurons among dopaminergic neurons was significantly higher in PD patients than in controls. Electron microscopy analysis in the human brain and in vitro data suggest that caspase-3 activation precedes and is not a consequence of apoptotic cell death in PD (Hartmann et al., 2000). In another human PD brain study, Hartmann et al. (2001) observed a significantly higher percentage of dopaminergic SN neurons that displayed caspase-8 activation in PD patients compared with controls. Activated forms of both caspase-8 and caspase-9 were reported to be present in dopaminergic SN neurons from PD patients (Andersen, 2001). The activities of both caspase-1 and caspase-3 were significantly higher in the SN from PD patients as compared to normal controls (Mogi et al., 2000). Several studies failed to find differences in the expression of the apoptotic regulators bcl-2, bax, and bcl-x in PD SN compared to controls (Jellinger, 2000; Vyas et al., 1997). However, two other studies found an increased expression of the antiapoptotic factor bcl-2 in the SN of PD patients compared to the cerebral cortex and age-matched controls, probably as a compensatory mechanism (Mogi et al., 1996; Marshall et al., 1997). Hartmann et al. (2002) found increased expression of the antiapoptotic factor bcl-xL in mesencephalic sections from PD patients.

Deprivation of neurotrophins and the presence of proinflammatory cytokine TNF-α can be a potent apoptotic signal (Nagatsu, 2002). The level of TNF-α receptor R1 (TNFR1, p55) was elevated in the SN in PD compared with control (Mogi et al., 2000). TNF-α-immunoreactive glial cells were also found in the SN in PD patients (Boka et al., 1994). TNF-α was found to cause apoptosis in NGF-deprived neurons (Nagatsu, 2002). Many of these neurons were rescued by blocking antibodies against TNF-α or TNFR1 (Barker et al., 2001). A similar situation may exist in the SN of PD patients. Depletion of neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in the nigrostriatal dopaminergic region of PD patients compared to controls, as described by Mogi and Nagatsu (1999) and Mogi et al. (1999), may trigger the process of apoptosis in PD. The presence of a proapoptotic environment in the nigrostriatal region of PD brain suggests vulnerability of neurons and glial cells towards a variety of toxic factors, such as oxidative stress.

5. Conclusions

There is accumulating evidence from in vitro, in vivo, and human studies that implicates apoptotic cell death in the etiology of PD. Elucidation of the cascade of events that lead to programmed cell death in PD and especially identification of the triggers of this process into action may serve as a potent tool for halting or slowing the disease process as well as identifying subjects prone to PD at early and preclinical stages. Establishing a better understanding of the apoptotic process in PD is vital in terms of developing treatment for PD that may alter the course of the disease and not only supply symptomatic relief. The goal of such therapies would be to deliver the required agents to the dopaminergic neurons in the SN and protect them from apoptotic death without interfering with the activity of other

cells, preventing imbalance in the immune system or oncogenesis.

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