An Overview of Endogenous Catechol-Isoquinolines and Their Related Enzymes: Possible Biomarkers for Parkinson's Disease

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Abstract For the most common neurodegenerative disorders, there is no simple, obvious, and effective biomarker for disease identification. Plasma biomarkers of neurodegenerative disorders should play an important role in early detection and diagnosis of diseases. The catechol isoquinoline compounds are considered as possible neurotoxins involved in the pathogenesis of Parkinson's disease (PD). These catechol isoquinolines and enzymes involved in their biosynthesis and metabolism may be endogenous factors in the pathogenesis of PD. As we know, there exists a salsolinol N-methyltransferase (SNMT) in human brain and lymphocyte. In normal postmortem human brain regions, frontal cortex, caudate, putamen, and thalamus, the SNMT activity was significantly higher than the control, and was well relevant to PD. In parkinsonian lymphocytes, the SNMT activity was much higher than in control patients. In addition, there was significant positive correlation between the SNMT activity in the lymphocyte and the level of N-methylsalsolinol in cerebrospinal fluid from the same untreated PD patients. Mass spectrometry-based biomarker discovery, undoubtedly, is still in its early stage of infancy. However, in light of the potential of this technology to provide deep coverage of the body fluid proteomes, it will certainly consolidate its role in development molecular medicine into clinical practice.

Keywords Parkinson's disease · Biomarker · Salsolinol · N-methylsalsolinol · NMSal · Salsolinol N-methyltransferase · SNMT

Introduction

Parkinson's disease (PD) is an age-related neurodegenerative disease that is caused by selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). A classic macroscopically pathological phenomenon appears when up to 50 % nigral dopaminergic neurons loss; that is, hypopigmented substantia nigra. The neurotransmitter dopamine (DA) also reduces, so that it hard to maintain the normal function of regulating the nervous system, which consequently result in appearance of PD symptoms.

However, before the clinical symptoms appear, 50–70 % dopaminergic neurons have been missing, which often makes the patients miss out on early diagnosis, treatment, and preventive intervention. Biomarkers that can be correlated with the risk or presence of PD, or change over time in a way that can be linked to disease progression, are urgent needed. Neuromelanin (NM) is produced in specific populations of catecholaminergic neurons in the brain, including dopaminergic neurons. When neurons die in PD brains, NM is released and removed from the brain by the cells of the immune system [1–3]. A novel NM enzyme-linked immunosorbent assay demonstrated an increased antibody response in the sera of PD patients when compared with age-matched control patients [4]. However, there was a negative correlation with disease duration. DJ-1 and α -synuclein in the cerebrospinal fluid (CSF) are potential index for PD diagnosis [5], but not for PD severity. Shi et al. [6•] found that total tau, phosphorylated tau, amyloid beta peptide 1-42 (A β [1-42]), Flt3 ligand, and fractalkine levels in CSF alone or in combination with DJ-1 and α -synuclein could aid in PD diagnosis, which could differentiate PD patients from those with Alzheimer's disease (AD) or multiple system atrophy (MSA).

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Fig. 1 Possible mechanism of the formation of catechol isoquinolines in brain. Aldehyde, one product of lipid peroxidation, can condense with dopamine to form alkyl-substituted catechol tetrahydroisoquinolines (CTIQs). These CTIQs can be metabolized via *N*-methylation to *N*-methyl-TIQs and oxidation to cytotoxic MPP⁺-like neurotoxins. MPP methyl-4-phenylpyridinium

The death of neurons in PD patients is likely due to combinations of aging, genetic factors, and environmental factors. However, long-term studies have shown that people who are exposed to certain environments show a higher risk for PD [7]. It suggests that environmental factors are an important and major risk factor to be considered in the pathogenesis of PD. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) received extensive attention when it was found that drug addicts who took MPTP accidentally developed a syndrome that clinically and pathologically resembled PD [8]. So far, as frequent endogenous toxins, tetrahydroisoquinoline compounds and β -carboline derivatives are widespread in plants and food, and pass through the blood–brain barrier (BBB) easily. It has been proved that heterogeneous isoquinoline derivatives and β -carboline derivatives exist in the CSF of PD patients but not in normal ones [9, 10].

MPTP-induced symptoms and pathological changes are very similar to pathogenesis of PD, and it implicates that a long-term accumulation of certain toxic substances that have similar structure to MPTP but generate naturally in brain will lead to PD. Catecholamine isoquinoline compounds (CIQs) are members of isoquinoline derivatives, and their nitrogen methylation and oxidation products are the structural analogues of MPTP and methyl-4-phenylpyridinium (MPP⁺), which caused widespread concern. Among the CIQs, salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline

Fig. 2 Structures of MPTP, MPP⁺, and CIQs. CIQs are synthetic or metabolic products of DA with active aldehyde. MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP⁺ 1-methyl-4-phenylpyridinium; CIQs catechol isoquinolines; NorSal norsalsolinol or 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline; NMNorSal N-methylnorsalsolinol or 2-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline; MeDHIQ⁺ 2-methyl-6,7-dihydroxyisoquinolinium ion; Sal salsolinol or 1-methyl-6,7-

dihydroxy-1,2,3,4-tetrahydroisoquinoline; NMSal N-methylsalsolinol or 1(R),2-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline; DiMeDHIQ⁺ 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion; ADTIQ 1-acetyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline; NMADTIQ N-methyl ADTIQ or 1-acetyl-2-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline; MeAcDHIQ⁺ 1-acetyl-2-methyl-6,7-dihydroxyisoquinolinium ion

[Sal]) derivatives were first found in the urine and brain of PD patients who were treated by L-3,4-dihydroxyphenylalanine (L-DOPA) [11, 12] and have the most significant toxicity on cell and animal models [13–15]. With the confirmation of synthesis and metabolism of CIQs, more attention were paid to the role of Sal derivatives in the pathogenesis of PD as promising candidates of critical endogenous neurotoxin.

Hypothesis on the Formation of Catechol Isoquinolines in Brain

Oxidative stress had been considered as a key factor contributed to dopaminergic neurons degeneration in PD [16]. Oxidative stress can produce reactive oxygen species (ROS), and stimulation of lipid peroxidation in mitochondria can lead to generate abundant aldehydes [17]. These aldehydic lipid peroxidation products condense with dopamine to form alkyl-substituted catechol tetrahydroisoquinolines (CTIQs). These CTIQs can be metabolized via *N*-methylation and oxidation to cytotoxic MPP⁺-like neurotoxins [18–20] that in turn inhibit mitochondria Complex I that makes a vicious cycle which finally leads to neurodegeneration (Fig. 1). These CIQs and

enzymes that involved in their biosynthesis and metabolism may be endogenous factors in the pathogenesis of PD, and furthermore become an early clinical biomarker for PD.

Endogenous Catechol Isoquinolines

Currently, the endogenous neurotoxins are concentrated in tetrahydroisoquinoline (TIQ) compounds. Among TIQs, CIQs, *N*-methyl-TIQ, and oxidation products of TIQs are MPTP and MPP⁺ analogues. Figure 2 shows the chemical structures of these compounds.

6,7-Dihydroxyl-1,2,3,4-tetrahydroisoquinoline (norsalsolinol) is the condensation product of DA and formaldehyde; Sal results from the condensation of DA and acetaldehyde; and 1-acetyl-6,7- dihydroxyl-1,2,3,4-tetrahydroisoquinoline (ADTIQ) is the condensation product of DA and methylglyoxal. The methylated products of these compounds are *N*-methylnorsalsolinol (NMNorSal), *N*-methylsalsolinol (NMSal), and *N*-methyl ADTIQ (NMADTIQ) and the ion oxide products are MeDHIQ⁺, DiMeDHIQ⁺, and MeAcDHIQ⁺, respectively (Fig. 2). The C₁ location of CIQ has a chiral center, and thus has (R) and (S) enantiomers.

Fig. 3 Full-scan mass spectra of salsolinol and *N*-methylsalsolinol in manganese-exposed PC12 cells. The selected ion of *m/z* 180.11 represents salsolinol and *m/z* 194.11 represents *N*-methylsalsolinol. The

chemical structures also were shown in this figure. This figure was adapted from Deng Y. et al. [36] with permission

Table 1 Effect of $MnCl_2$ on the levels of salsolinol and N-methyl-salsolinol in PC12 cells

	Control, ng/mg protein ^a	0.6 mmol/L, ng/mg protein ^a	1.2 mmol/L, ng/mg protein ^a	1.8 mmol/L, ng/mg protein ^a
Sal (ng/mg protein)	0.18±0.18	0.31±0.27*	0.35±0.39*	0.67±0.30*
NMSal (ng/mg protein)	0.02±0.12	0.22±0.31**	0.28±0.47**	0.43±0.60**

$MnCl_2$ manganese chloride; Sal salsolinol; NMSal N-methyl-salsolinol

^a The means of three experimental values are presented as mean ± standard deviation

* $P < 0.05$ compared to the control ** $P < 0.01$ compared to the control

ADTIQ is an endogenous salsolinol-like compound that was found for the first time in caudate nucleus, putamen, substantia nigra, frontal cortex, and cerebellum of PD patients [21]. The formation of ADTIQ is closely related with glucose metabolism because methylglyoxal is a byproduct produced from the glycolysis. Clinical data have shown that diabetic patients have more potential to suffer from PD than the healthy ones [22, 23]. The results implicate that there may be some relationship between diabetes and PD, although the etiology of this relationship remains elusive. Based on our hypothesis and experimental observations, the level of methylglyoxal increased in the brain of diabetic patients under hyperglycemic conditions, which subsequently enhanced the formation of CIQs [24]. It might implicate that ADTIQ acts as a linkage between diabetes and PD.

Synthesis and Metabolism of Salsolinol and Its Derivatives

Research data have shown that Sal and NMSal are neurotoxins in vivo or in vitro [25–28]. Sal can be enzymatically synthesized from DA and acetaldehyde. The Sal synthase was purified and characterized from human and rat brain tissues [20, 29•]. It was also observed that this enzyme can produce (R) enantiomer of Sal by using DA and acetaldehyde as substrates, and the results explain the predominance of (R) enantiomer in brain [20, 30–32].

As essential for the neurotoxicity of MPTP that an N-methyl group is transformed to the pyridine ring, Sal is methylated into NMSal by an N-methyltransferase (SNMT). The enzymatic

reaction was confirmed in vitro [18] and in vivo [19]. A neutral SNMT activity had been found in the striatum, but the level of NMSal in the striatum did not correlate with its activity [33]. In PD rat model with a unilateral 6-hydroxydopamine lesion, the activity of SNMT in the peripheral lymphocytes of injured side significantly increased [34•].

NMSal is not the final product in the metabolism of salsolinol. It will be oxidized to DMDHIQ⁺ consequently by non-enzymatic reaction or cytochrome P450 [33]. DMDHIQ⁺ was found to accumulate in the stratum and substantia nigra, where the level was higher than NMSal [20].

NMSal and DMDHIQ⁺ were considered to accumulate in the striatum, where NMSal is taken up into the nerve terminal by a DA-transport system, and then oxidized into DMDHIQ⁺. DMDHIQ⁺ may accumulate in the mitochondria or by binding to neuromelanin to manifest a higher level than NMSal [20, 32, 35].

Formation of Salsolinol and Its Metabolites in Cells Exposed to Oxidative Stress

The effect of manganese occurs mainly in the respiratory tract and brain. It was found that manganese poisoning can cause symptoms similar to PD. Using rat adrenal pheochromocytoma cell line PC12 cells exposed to manganese, data showed that manganese increased the levels of malondialdehyde (MDA) in a dose-dependent manner [36], and the production of Sal and NMSal was found in PC12 cells. Figure 3 shows the mass spectra of the samples obtained from PC12 cells exposed to manganese, and the

Table 2 Effects of Fe^{2+} on dopamine-induced intracellular dopamine, salsolinol, and NMSal production in SH-SY5Y cells

	Control, ng/mg protein ^a	Dopamine, ng/mg protein ^a	Dopamine+ Fe^{2+} (40 μ mol/L), ng/mg protein ^a	Dopamine+ Fe^{2+} (80 μ mol/L), ng/mg protein ^a
Dopamine	22.14±5.19	46.31±5.76	47.05±6.08	52.40±5.57*
Salsolinol	4.06±1.08	4.20±1.52	6.60±2.09*	5.68±1.72
NMSal	ND	1.95±0.47	2.69±1.14	6.92±2.56

Fe^{2+} iron; NMSal N-methyl-salsolinol; ND not detected

^a The means of three experimental values are presented as mean ± standard deviation

* $P < 0.05$, compared to control

selected ions of Sal and NMSal clearly identified. Table 1 gives the levels of Sal and NMSal in PC12 cells exposed to different concentrations of manganese chloride (MnCl_2).

Similar results also were observed with different cell models. When human SH-SY5Y neuroblastoma cells treated with high doses of DA (150 $\mu\text{mol/L}$) with certain amount iron (Fe^{2+}), the production of Sal and NMSal was confirmed as shown in Table 2, significant increasing of hydroxyl radicals also was observed [37]. More interestingly, MPP^+ enhanced not only the oxidative stress significantly in primary cultured cerebellar, mesencephalic, and striatal neurons but also the level of Sal and NMSal in MPP^+ -treated neurons compared to control groups, as shown in Fig. 4.

Enzymes Involved in the Synthesis and Metabolism of Salsolinol and Its Metabolites

As shown in Fig. 1, there are stepwise reactions for CIQs in brain, and enzymes are involved in their biosynthesis and metabolism. Sal can be generated from DA and acetaldehyde by an (R)-Sal synthesis-mediated condensation, and then *N*-methylated into (R)-NMSal by a neutral (R)-Sal *N*-methyltransferase (SNMT). NMSal can be activated by enzymatic and nonenzymatic oxidation.

A novel enzyme isolated from human brain was found to catalyze the enantioselective synthesis of (R)-Sal from DA and acetaldehyde [20, 29••]. The enzyme was purified and characterized. The K_m and V_{max} are 5.11 ± 0.62 mM and 342.4 ± 40.5 nmol/min mg, respectively, for the substrate DA, and 0.59 ± 0.12 mM and 18.8 ± 3.5 nmol/min mg, respectively, for acetaldehyde. A neutral *N*-methyltransferase

Fig. 4 Comparison of the amount of dopamine (DA), salsolinol (Sal), and *N*-methylsalsolinol (NMSal) in the primary cells of different regions after the MPP^+ treatment. Primary cells from different regions of new born SD rats were treated with 1 mmol/L MPP^+ for 24 h. HPLC-ECD was used to detect the level of DA, Sal, and NMSal. There were no significant changes for the level of DA, but the level of Sal increased after treated with MPP^+ in all the regions. The level of NMSal significantly increased in striatum and midbrain but not in cerebellum. Data present the means of three experimental values obtained from the measurement by an HPLC/MS method ($P < 0.05$, compared to control). *MPP* methyl-4-phenylpyridinium; *SD* Sprague–Dawley; *HPLC-ECD* high-performance liquid chromatography electrochemical detection

Fig. 5 Enzyme activity of SNMT in different brain regions of control (C), sham operation (SH), and PD rats. No significant changes showed between control and shamed operation, but the activity of SNMT was significantly increased in 6-hydroxydopamine-treated PD rat substantia nigra on the damaged side ($n=5$; $P < 0.01$ compared to control). *SNMT* salsolinol *N*-methyltransferase; *PD* Parkinson's disease; *ST/L* striatum on left side; *ST/R* striatum on right side; *SN/L* substantia nigra on left side; *SN/R* substantia nigra on right side

was found to be involved in the *N*-methylation of (R)-Sal into (R)-NMSal. The *N*-methyltransferase was isolated from both human and rat brain tissues [19] and found to be stereospecific to (R)-Sal, but (S)-Sal is not the substrate. The K_m and V_{max} for (R)-Sal are 126 ± 72.7 μM and 29.2 ± 15 pmol/min/mg protein, respectively.

SNMT was considered to play a more important role in PD. In normal postmortem human brain regions, frontal cortex, caudate, putamen, and thalamus, the SNMT activity in the PD patients was significantly higher than the control patients. The SNMT activity was also determined in Wistar rat gray matters that were injected with 6-hydroxydopamine at the area of substantia nigra. Figure 5 presents the SNMT activity in control, sham operation, and 6-hydroxydopamine-treated rats, and the activity in the PD animals was dramatically elevated. More interestingly, SNMT was found in the blood lymphocytes, and the activity in the PD rats is significantly higher than that in both sham operation and control rats Fig. 6.

N-Methyltransferase May Be a Promising Early Biomarker for Parkinson's Disease Diagnosis

It was repeatedly reported that the level of salsolinol and NMSal was found higher levels in the brain and CSF of

Fig. 6 SNMT activity in lymphocytes of PD, sham operation (SH), and control (C) rats. The activity of SNMT was significantly increased in PD model rat ($n=5$; $P < 0.05$ compared to control). *SNMT* salsolinol *N*-methyltransferase; *PD* Parkinson's disease

Table 3 The case information for the patient's blood samples

	Patients, <i>n</i>	Age, <i>y</i> ± <i>SD</i>	Sex	Group	Number	Age, <i>y</i> ± <i>SD</i>
Control	76	52.8±14.8	Male 38; female 38	< 40 y	13	30.85±9.49
				41–49y	15	43.07±2.60
				50–59y	19	53.05±2.48
				60–69y	16	63.94±2.77
				>70 y	11	74.36±3.38
PD	26	64.8±10.7	Male 4; female 12	< 70	15	57.27±7.99
				> 70	11	75.27±3.44

SD standard deviation; *PD* Parkinson's disease

patients with untreated PD cases than in controls [38–47]. Furthermore, the increased activity of *N*-methyltransferase was observed in lymphocytes of PD rats. It is obvious that the SNMT activity in patients' blood is worthy to be investigated. A total of 102 blood samples were obtained from the China-Japan Friendship Hospital of China, and Table 3 gives the brief information of samples. The SNMT activity was measured in the lymphocytes of control patients grouped with different ages. As shown in Table 4, the SNMT activity was increased with age, which shows a correlation between the activity and age. Comparison of PD and control cases, the SNMT activity in the lymphocytes of PD patients was obviously higher (Fig. 7) than in control patients. The elevated level of SNMT activity in the lymphocytes in PD patients is consistent with the results observed from the PD animal model both in brain and blood, and thus, it indicated that SNMT may be a promising biomarker for the diagnosis of PD.

The Application of Proteomics Technology in the PD Biomarker Discovery

With the development and wide applications of proteomics, biomarker discovery has become a hot area of disease proteomics [43, 48–51]. Mass spectrometry and two-dimensional electrophoresis (2-DE) provide a powerful tool for screening of protein biomarkers. A single proteomics assay can

identify hundreds of differentially expressed proteins between disease and control samples. Many identified proteins displayed significant differences in their relative abundance between the disease and normal groups. Human CSF is one of the ideal sources for identifying biomarkers for PD because it is the most proximal to the central nervous system. In CSF samples, Radu found ubiquitin and β -microglobulin were at higher levels, while chromogranin B fragments were expressed in lower levels in atypical parkinsonian disorders (APD) compared with PD and control patients [52]. Using isobaric tags for relative and absolute quantitation (iTRAQ), Abdi et al. [53], identified 73 proteins with differentiated expression in PD patients; some of the unique proteins, including apolipoprotein (apo) H/ β -2-glycoprotein 1 (Apo-H), ceruloplasmin, and chromogranin B (secretogranin I) that are unique to PD, were further confirmed in the initial study using Western blotting. Whereas Pan et al. [54] used SCX in combination with liquid chromatography–mass spectrometry (LC-MS)/MS and addition of stable isotope-labeled peptides to absolute quantify five proteins. Several other markers have been tested in a different and larger set of patients, in which a panel of eight CSF proteins (brain-derived neurotrophic factor, interleukin-8, vitamin D-binding protein, β 2-microglobulin, apo AII, apo E, plus tau and A β 1–42) appears to be able to classify PD patients with 95 % sensitivity and 95 % specificity using Luminex-based multi-analyte profiling technology [38]. Moreover, CSF levels

Table 4 The SNMT activity in the lymphocytes of control patients grouped with different ages

Groups	Age, <i>y</i>	Age range, <i>y</i> ± <i>SD</i>	SNMT activity, <i>pmol</i> /μg/h ± <i>SD</i>
Group I	< 40	30.8±9.4	0.22±0.43
Group II	41–49	43.0±2.6	1.21±0.91
Group III	50–59	53.0±2.4	2.81±3.55
Group IV	60–69	63.9±2.7	4.56±5.64
Group V	> 70	74.3±3.3	11.03±5.20

SNMT salsolinol *N*-methyltransferase; *SD* standard deviation

Fig. 7 The SNMT activity in the lymphocytes of PD (PD) and control patients (C). Blood samples of PD patients and healthy people were obtained. Data show the activity of SNMT of PD patients was significantly increased (PD patients: *n*=26; control: *n*=76 [*P*<0.05]). *SNMT* salsolinol *N*-methyltransferase; *PD* Parkinson's disease

of DJ-1 were found to be significantly increased in sporadic PD and well correlated with disease progression [39]. DJ-1 is located in the cytoplasm, mitochondria, and nucleus in the form of soluble dimers; it is a recognized PD-related protein because its gene mutation leads to the early onset of autosomal recessive PD. It is important to appreciate that the SNpc is composed of different cell types, among which only 5–10 % are DA neurons. In human substantia nigra specimens or mesencephalon specimens, there were different results shown from several research groups. DJ-1 expressed at a constant level between control and patients [40]. Using 2-DE, Basso and colleagues [41] performed the first unbiased profiling work in human SNpc, with 44 proteins identified and nine of them differentially expressed in PD tissues with respect to controls. Moreover, immune mechanisms may contribute to neuronal damage, as suggested by the immune disturbances reported in PD [42]. By means of 2-DE and mass spectrometry protein identification, Silvia and colleagues [55••] compared patients under L-DOPA treatment, patients under subthalamic nucleus deep-brain stimulation and healthy controls, and found cytoskeletal protein dysregulation and oxidative stress in PD patients [55••].

Proteomic studies performed in the field of PD already have generated a large amount of data to support existing pathogenic theories and have produced numbers of original findings regarding PD pathogenesis and biomarkers. However, due to different sample preparation and selected criteria, it is worthy establishing reasonable and detectable standard to find effective biomarkers for diagnosis of PD, especially samples from peripheral tissue.

Conclusions

In conclusion, oxidative stress might promote the formation of CIQs, and then initiate the vicious cycle and lead to cell degeneration. This CIQ family and enzymes that involved in their biosynthesis and metabolism may be endogenous factors in the pathogenesis of PD. The elevated level of SNMT activity was found in the lymphocytes in PD patients, consistent with the results observed from the PD animal model both in brain and blood. These results indicated that SNMT may be a promising biomarker for the diagnosis of PD. Proteomic studies performed in the field of PD have produced numbers of original findings of biomarkers. In light of the potential of this technology to provide deep coverage of the body fluid proteomes, it will certainly consolidate its role in development molecular medicine into clinical practice.

Disclosures No potential conflicts of interest relevant to this article were reported.

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