PERSPECTIVE

# A new mechanism for protection of dopaminergic neurons mediated by astrocytes

For a long time, the question about the mechanism involved in the degenerative process of the nigrostriatal system in Parkinson's disease (PD), resulting in the loss of dopaminergic neurons containing neuromelanin, has remained open. The discovery of genes associated with familial forms of PD, such as α-synuclein (SNCA), parkin, DJ-1, PINK-1, LRRK-2, ATP13A2, PINK-1 and others resulted in important input into the basic research in this field with the aim of understanding the role of these proteins in sporadic PD. In the scientific community, there is a general agreement that the loss of dopaminergic neurons containing neuromelanin in the nigrostriatal system involves mitochondria dysfunction, protein degradation dysfunction, and SNCA aggregation in neurotoxic oligomers, oxidative stress, neuroinflammation and endoplasmic reticulum stress (Segura-Aguilar et al., 2014). The question concerns the identity of the neurotoxin that triggers these mechanisms and the existence of a possible synergism between these mechanisms that has been proposed. It seems to be plausible that the neurotoxin involved in the degenerative process of the dopaminergic nigrostriatal system must be of endogenous origin, since the progression of the neurodegenerative process in PD is very slow and takes years for the development of motor symptoms, when up to 60–70% of dopaminergic neurons containing neuromelanin are lost. The slow progression of the degenerative process in PD contrasts with the extremely rapid degeneration observed in humans who are injected with drugs containing 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), who after only 3 days develop severe motor symptoms. The possibility that *o*-quinones derived from dopamine oxidation are responsible for the loss of dopaminergic neurons containing neuromelanin in PD is supported by the fact that these neurons require dopamine oxidation to *o*-quinones to generate neuromelanin. Interestingly, *o*-quinones generated during dopamine oxidation have been reported to induce mitochondria dysfunction, SNCA aggregation in neurotoxic oligomers, protein degradation dysfunction of both proteasomal and lysosomal systems, oxidative and endoplasmic reticulum stress (Segura-Aguilar et al., 2014).

Dopamine oxidation to *o*-quinones seems to be a natural process resulting in the formation of neuromelanin, since this pigment is found in dopaminergic neurons localized in the substantia nigra of the brain of healthy individuals. Dopamine oxidation takes place in several steps: (i) Dopamine-*o*-quinone formation which immediately undergoes molecular cyclization to form aminochrome at a constant rate of 0.15/s at physiological pH; (ii) Aminochrome is the most stable *o*-quinone at physiological pH but after 40 minutes aminochrome initiates its rearrangement to 5,6-indolequinone at a rate of 0.06/minute; and (iii) finally, 5,6-indolequinone polymerizes to neuromelanin. However, under certain conditions, the *o*-quinones formed during dopamine oxidation participate in neurotoxic reactions, such as the formation of adduct with proteins and one-electron reduction, to generate an *o*-semiquinone radical. Dopamine oxidation to *o*-quinones has been found to inactivate chaperone-mediated autophagy. Dopamine-*o*-quinone has been found (i) to inactivate the proteasome system by forming adducts with parkin; (ii) to inactivate mitochondrial complexes I, III and V, which results in mitochondrial dysfunction; and (iii) to inactivate other proteins such as dopamine transporter, tyrosine hydroxylase, UCHL-1, and DJ-1. Aminochrome has been reported to induce the formation of neurotoxic oligomers (Muñoz et al., 2015). Aminochrome induces mitochondria dysfunction by forming adducts with mitochondrial complex I. One-electron reduction of aminochrome to leukoaminochrome *o*-semiquinone radicals induces oxidative stress. Aminochrome induce endoplasmic reticulum stress (Xiong et al., 2014). In addition, aminochrome induces protein degradation dysfunction by (i) impairing the proteasomal system; (ii) inducing lysosome dysfunction (Huenchuguala et al., 2014); and (iii) inhibiting autophagy (Muñoz et al., 2012; Huenchuguala et al., 2014). 5,6-Indolequinone has been reported to form adducts with SNCA (Segura-Aguilar et al., 2014; **Figure 1**).

Glutathione transferase M2-2 (GSTM2) is a human form of glutathione transferase that is expressed in astrocytes and it has been proposed to play an important protective role in the neurodegenerative process in PD by preventing aminochrome and dopamine *o*-quinone toxicity. GSTM2 is the most active form of glutathione transferases that catalyzes glutathione (GSH) conjugation of aminochrome to 4-*S*-glutathionyl-5,6-dihydroxyindoline. Interestingly, 4-*S*-glutathionyl-5,6-dihydroxyindoline is stable in the presence of biological oxidizing agents such as oxygen, hydrogen peroxide and superoxide radicals, suggesting a protective role of this reaction (Baez et al., 1997; Segura-Aguilar et al., 1997). GSTM2 also conjugates with GSH the precursor of aminochrome dopamine *o*-quinone to 5-*S*-glutathionyl dopamine (Dagnino-Subiabre et al., 2000), which is finally degraded to 5-*S*-cysteinyl dopamine. Interestingly, 5-*S*-cysteinyl dopamine has been detected in neuromelanin, substantia nigra, putamen, caudate nucleus, globus pallidus, and the cerebrospinal fluid of PD patients, suggesting that this conjugate is an end-product that will be eliminated from dopaminergic neurons (Rosengren et al., 1985; Carstam et al., 1991; Cheng et al., 1996).

The presynaptic dopaminergic neurons release dopamine into the synaptic cleft for synaptic neurotransmission. The removal of dopamine from the synaptic cleft is essential in order to continue this synaptic neurotransmission, since the permanence of dopamine in the synaptic cleft will prevent new neurotransmission signals as a consequence of receptor desensitization. The reuptake of dopamine into dopaminergic neurons is mediated by the dopamine transporter localized on the plasma membrane. However, astrocytes surrounding the synaptic cleft of dopaminergic neurons are also able to remove dopamine due to their ability to take up dopamine. The uptake of dopamine into astrocytes will result in the formation of *o*-quinones as a consequence of dopamine oxidation, but astrocytes have constitutive expression of GSTM2 to prevent aminochrome toxicity (Huenchuguala et al., 2014). Recently, it has been demonstrated that the GSH conjugation of aminochrome with GSTM2 was essential to protect astrocytes from aminochrome-induced cell death, since the silencing of GSTM2 with constitutive

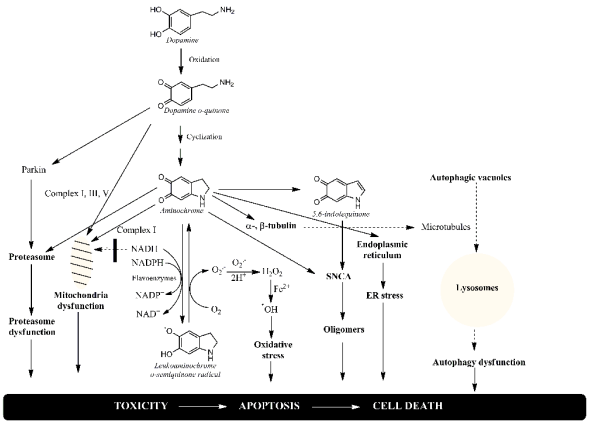


**Figure 1 Toxic reactions of *o*-quinones generated during dopamine oxidation.**
Dopamine oxidizes to form dopamine *o*-quinone, which at physiological pH undergoes intramolecular cyclization in two steps to generate aminochrome. Dopamine *o*-quinone inactivates parkin and mitochondrial complexes I, III, and V complexes inducing both mitochondria and proteasomal system dysfunction. Aminochrome (i) inactivates mitochondrial complex I, inducing mitochondrial dysfunction; (ii) induces protein degradation dysfunction by impairing the proteasomal and lysosomal protein degradation system; (iii) induces oxidative stress with hydroxyl radical formation; and (iv) induces and stabilizes the formation of neurotoxic oligomers of α-synuclein (SNCA); and (V) induces endoplasmic reticulum strss 5,6-Indolequinone also induces neurotoxic oligomers of SNCA.

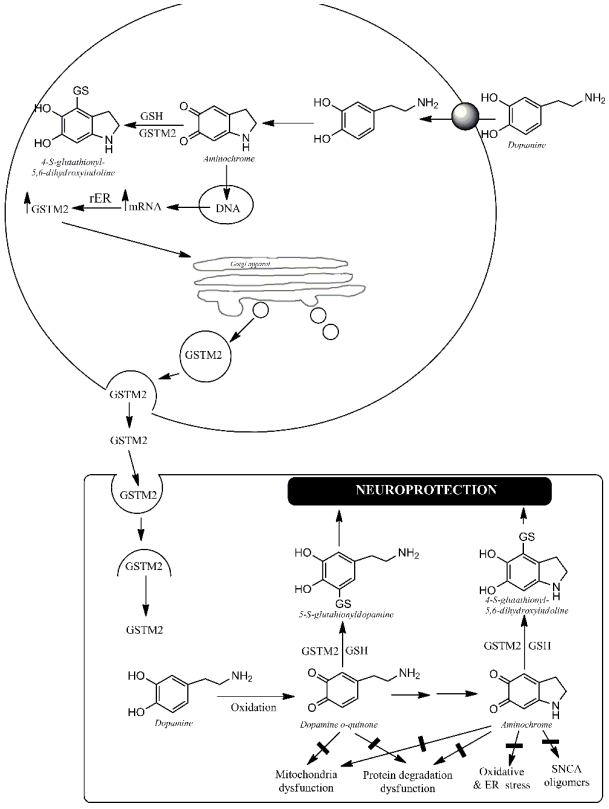


**Figure 2 Possible mechanisms for astrocyte protection of dopaminergic neurons against dopamine oxidation to *o*-quinones.**
Astrocytes take up dopamine, which oxidizes to aminochrome. Aminochrome induces an increase in the expression of glutathione transferase M2-2 (GSTM2), which conjugates aminochrome with glutathione (GSH). Astrocytes also secrete GSTM2 into the synaptic cleft, where dopaminergic neurons internalize the enzyme into the cytosol. The mechanisms for excretion of GSTM2 from the astrocytes into the conditioned medium and for GSTM2 uptake into dopaminergic neurons are unknown. However, it seems to be plausible that the synthesis of GSTM2 protein in the rough endoplasmic reticulum (rER) is followed by its transport to the plasma membrane mediated by the Golgi, where a vesicle containing GSTM2 is released and fused with plasma membrane to secrete the enzyme to extracellular space. The internalization of GSTM2 into dopaminergic cytosol is probably dependent on a process of endocytosis where GSTM2 is released into the cytosol where the enzyme can protect these neurons against aminochrome toxicity. In the dopaminergic neurons, GSTM2 catalyzes the conjugation of both dopamine *o*-quinone and aminochrome, preventing their neurotoxic action by inducing mitochondria dysfunction, protein degradation dysfunction, oxidative stress and the formation of neurotoxic oligomers of α-synuclein (SNCA). GSTM2 protects both astrocytes and dopaminergic neurons against *o*-quinones generated during dopamine oxidation.

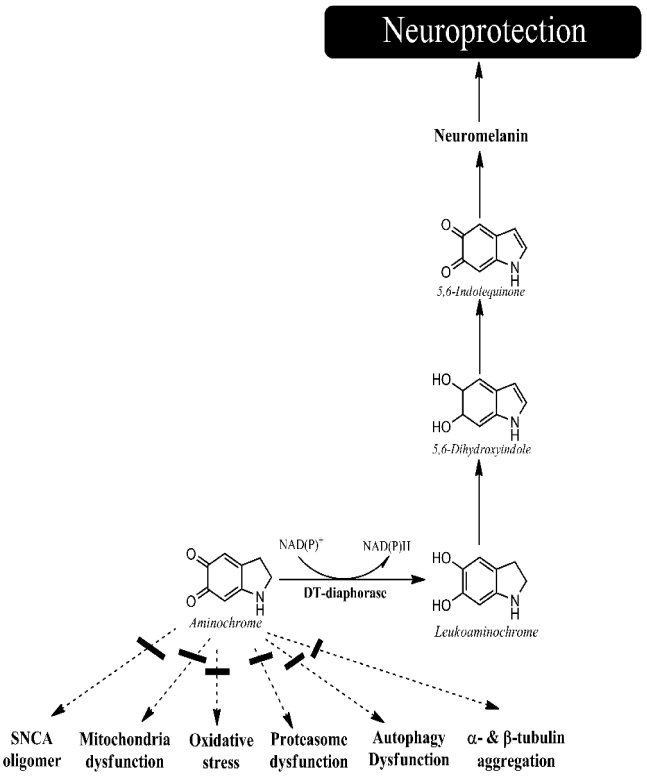


**Figure 3 DT-diaphorase protects against aminochrome neurotoxicity.**
DT-diaphorase catalyzes the two-electron reduction of aminochrome to leukoaminochrome, preventing the neurotoxic actions of aminochrome, such as (i) formation of adducts with α-synuclein (SNCA); (ii) formation of adducts with complex I of mitochondria and subsequent mitochondrial dysfunction; (iii) one-electron reduction to leukoaminochrome *o*-semiquinone radical and subsequent oxidative stress; (iv) inhibition of degradation of proteins as a consequence of proteasome inactivation; (v) inhibition of degradation of proteins and organelles with autophagy; and (vi) formation of adducts with α- and β-tubulin and subsequent aggregation. Leukoaminochrome can tautomerize to 5,6-dihydroxyindole, which can oxidize to 5,6-indolequinone which is the precursor of neuromelanin.

expression of an siRNA against this enzyme inhibits autophagy and induces lysosome dysfunction (Huenchuguala et al., 2014). These results support the idea that GSTM2 plays an important role in the prevention of aminochrome toxicity in astrocytes.

Astrocytes seem to play an important role in the protection of neighboring neurons by releasing energy substrates. The intracellular level of GSH in neurons plays a protective role against oxidative stress, and the decrease of intracellular GSH will lead to apoptosis. Surrounding astrocytes supply all amino acids to neurons required for GSH synthesis (L-glutamic acid, L-cysteine, and glycine). Recently, we reported a new mechanism for the protection of dopaminergic neurons against *o*-quinone toxicity formed during dopamine oxidation. We reported that an astrocyte model cell line secreted GSTM2 into the conditioned medium where a model cell line for dopaminergic neurons internalizes GSTM2 into neurons to protect these neurons against aminochrome neurotoxicity (Cuevas et al., 2015). The presence of aminochrome in the astrocytes increases the expression of GSTM2, which is secreted into the conditioned medium. However, the mechanism by which astrocytes secrete and dopaminergic neurons can internalize the enzyme in order to increase their neuroprotective defense against *o*-quinones formed during dopamine oxidation remains unclear (Cuevas et al., 2015; **Figure 2**).

Dopaminergic neurons are the most vulnerable cells exposed to neurotoxic *o*-quinones generated during dopamine oxidation. Dopaminergic neurons have both dopamine synthesis from tyrosine and dopamine reuptake mediated by dopamine transporters from the synaptic cleft. Dopamine is stored in monoaminergic vesicles to be used for neurotransmission, and dopamine excess is degraded by monoamino oxidase, which catalyzes the oxidative deamination of the dopamine amino group. However, under certain unknown conditions, free dopamine in the cytosol is oxidized to *o*-quinones that normally result in the formation of neuromelanin. The presence of neuromelanin in substantia nigra in the brain of healthy individuals support the idea that dopamine oxidation occurs *in vivo* despite the presence of vesicular monoamine transporter-2 and monoamine oxidase. The constitutive expression of DT-diaphorase prevents *o*-quinones that formed during dopamine oxidation from participating in neurotoxic reactions, such as the formation of adducts with proteins and one-electron reduction to o-semiquinone radicals. DT-diaphorase catalyzes the two-electron reduction of aminochrome to leukoaminochrome and this is expressed both in neurons and astrocytres. DT-diaphorase protects dopaminergic neurons from aminochrome-induced (i) cell death; (ii) formation of neurotoxic α-synuclein oligomers (Muñoz et al., 2015); (iii) mitochondria dysfunction; (iv) inhibition of the proteasomal system; (v) inhibition of autophagy (Muñoz et al., 2012); (vi) inhibition of α- and β-tubulin aggregation and cell shrinkage; and (vii) inhibition of oxidative stress (**Figure 3**). GSTM2 is not expressed in dopaminergic neurons, and therefore, the importance of this new mechanism of dopaminergic neuron protection, mediated by the internalization of GSTM2 secreted from astrocytes, is to reinforce the neuroprotection provided by DT-diaphorase against aminochrome generated during dopamine oxidation. GSTM2 catalyzes the conjugation of both aminochrome and dopamine *o*-quinone, which provides a wider protection in dopaminergic neurons. Therefore, astrocytes seem to play a very important role in the prevention of neurodegeneration of dopaminergic neurons in PD.

**Juan Segura-Aguilar***

**Molecular and Clinical Pharmacology ICBM, Faculty of Medicine, University of Chile, Santiago, Chile**

***Correspondence to:** Juan Segura-Aguilar, Ph.D., jsegura@med.uchile.cl.*
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