State and perspectives on flavonoid neuroprotection against aminochrome-induced neurotoxicity

Victor Silva, Juan Segura-Aguilar*

Parkinson's disease (PD) motor symptoms are induced by the loss of dopaminergic neurons containing neuromelanin in the nigrostriatal system. The exact mechanism that triggers the degeneration of the nigrostriatal neurons is still unknown but there is general consensus in the scientific community that mitochondrial dysfunction, alpha-synuclein aggregation to neurotoxic oligomers, protein degradation dysfunction of both lysosomal and proteasomal systems, endoplasmic reticulum stress, neuroinflammation and oxidative stress are involved in the degeneration of dopaminergic neurons containing neuromelanin. The pigmentation of dopaminergic neurons in the substantia nigra results from dopamine oxidation to neuromelanin. The hydroxyl groups of the dopamine catechol structure oxidize to carbonyl groups generating quinones. Dopamine oxidizes to dopamine ortho-quinone that is completely unstable at physiological pH and cyclizes spontaneously to aminochrome. Aminochrome is the most stable quinone formed during dopamine oxidation to neuromelanin; it can be one- or two-electron reduced by flavoenzymes or form adducts with proteins such as alpha synuclein, parkin, dopamine transporter, and other proteins. Interestingly, aminochrome has been reported to induce mitochondrial dysfunction, alpha-synuclein aggregation to neurotoxic oligomers, protein degradation dysfunction, endoplasmic reticulum stress, neuroinflammation and oxidative stress (**Figure 1**; for a review, see Segura-Aguilar, 2019). Therefore, we have proposed that aminochrome is the endogenous neurotoxin formed in neurons containing neuromelanin that triggers the degeneration of the nigrostriatal neurons in PD.

The usual treatment for PD is based on drugs that alleviate the symptoms, but they cannot halt the progression of the disease. One of the most effective drugs is levodopa, which have a spectacular effect causing the patients to recover motor deficiencies induced by the disease; however, after 4–6 years of chronic levodopa treatment, severe side effects are developed, such as dyskinesia, disabilities in balance, posture, speech, gait, and "wearing off" or "on-off" fluctuations. In this sense, the attention in neuroprotective compounds has increased in the scientific community. Flavonoids are a group of compounds that have shown a promising effect on neuroprotection in preclinical studies and present potential to inhibit the cellular and molecular alterations caused by aminochrome. Therefore, this perspective article is aimed at presenting an overview of multipotential effects of flavonoids as a neuroprotective drug in PD and how studies regarding aminochrome can be used to provide new insights into its therapeutic application.

Flavonoids as a multipotential drug for PD. Flavonoids are plant-derived compounds chemically characterized by a fifteen-carbon skeleton consisting of two benzene rings (A and B rings) linked by a heterocyclic pyrane ring (C ring; **Figure 1**) (Rice-Evans et al., 1996; Kumar and Pandey, 2013). They are widely present in the human diet and have been investigated as functional food or potential drug due to their ability to interact and change biological systems. The most explored pharmacological activity of flavonoids is their antioxidant properties, especially the radical scavenging activity that has the effectiveness depending on differential chemical structures. For example, it is known that aglycone quercetin, which presents a 3-hydroxyl group attached to the 2,3-double bond and adjacent to the 4-carbonyl in the C ring, has a higher antioxidant potential than the flavonoid rutin (glycosylated form) and the flavonoid apigenin, which does not have 3-hydroxy (Rice-Evans et al., 1996).

The oxidative stress is an important element in neurodegenerative disease pathogenesis. However, it is a part of multiple elements involved in the loss of the neuron. In this sense, flavonoids with low radical scavenging effectiveness are also an important drug in perspectives to pharmacology. They have shown a potential application for neurodegenerative diseases, such as PD. The cause of dopaminergic neuronal loss in sporadic form of PD is not totally clear, but it is known that in addition to oxidative stress, protein aggregation, impairment of protein degradation, mitochondrial dysfunction, endoplasmic reticulum stress, loss of neurotrophic factors and neuroinflammation contribute to neuronal death (Antony et al., 2013)

The discovery of flavonoids with multiple neuroprotective actions has moved the field forward with studies in several models. There is an emergent PD study model induced by the neurotoxin aminochrome, capable of reproducing the seven alterations related to the PD pathogenesis and the dopaminergic neuronal loss. This is a model in perspective for the investigation on the flavonoid mechanism of action and it was recently used to investigate the potential of agathisflavone to inhibit lysosomal dysfunction (Santos et al., 2020).

Aminochrome must be considered for research on flavonoids neuroprotection. The outstanding findings in PD pathogenesis and in the use of analogue experimental models impede the drug discovery for neuroprotection. In this sense, the aminochrome-induced PD study model emerges as a novel tool with a potential use for researches of neuroprotective compounds with a variety of mechanisms of action. In an *in vivo* model, aminochrome induced contralateral rotation when the animals are stimulated with apomorphine, cell shrinkage, reduction of dopamine release, increase in GABA release, decrease in the number of monoaminergic presynaptic vesicles, increase in dopamine concentration inside of monoaminergic vesicles, mitochondrial damage and dysfunction (Herrera et al., 2016; for a review, see Segura-Aguilar, 2019).

Research with classic study models of PD, such as those induced by 6-hydroxydopamine, rotenone and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have shown several neuroprotective effects of flavonoids. Among them are antioxidant, antiapoptotic, anti-inflammatory properties and the inhibition of the formation of toxic alpha-synuclein oligomers, highlighting these compounds as a potential drugs for PD (for a review, see Costa et al., 2016). However, clinical studies based on preclinical models with exogenous neurotoxins failed to translate positive results into clinical treatments of the disease (Athauda and Foltynie, 2015). Exogenous neurotoxins are not able to reproduce what happens in PD where the degeneration prior motor symptoms and the disease progression take years while MPTP induces a severe parkinsonism in humans exposed to this compound in just three days (Williams, 1986). Therefore, aminochrome as a preclinical model is more physiological because it is formed inside the dopaminergic neurons containing neuromelanin loss in PD substantia nigra. In addition, the degeneration of a single neuron induced by aminochrome does not induce degeneration of surrounding neurons, suggesting that aminochrome-induced generation should be a very slow process similar to what is observed in PD. In this context, neuroprotective studies in aminochrome-induced PD model provide a promising strategy to amplify the prospection of new compounds and better characterize the mechanism of action.

The flavonoids are classified into six main subclasses (flavones, flavonols, flavanones, flavanols, isoflavones and anthocyanins). The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the C ring (for a review, see Jung and Kim, 2018). There is report of neuroprotective action for PD of different flavonoids belonging to the six subclasses (Jung and Kim, 2018). Recently, we used SHSY-5Y cells exposed to aminochrome to investigate the effect of agathisflavone, a member of the flavone subclass that has been never studied in PD models. A protective action against lysosomal dysfunction and protection against aminochrome-induced cytotoxicity was shown (Santos et al., 2020). Agathisflavone is a biflavonoid (bis-apigenin), isolated from the Brazilian plant *Poincianella pyramidalis*, which acts via estrogen receptors to induce neurogenesis and neuronal differentiation (Costa et al., 2016). The effect of agathisflavone on the lysosomal dysfunction may be also associated to its action in estrogen receptors (Santos et al., 2020). It is important to note that mechanisms associated to lysosomal dysfunction have been little explored in studies regarding neuroprotection of flavonoids for PD and the model induced by aminochrome is an excellent tool for these investigations.

DT-diaphorase should be considered in studies with flavonoids with catechol moiety. The dopamine oxidation to aminochrome is accompanied by the formation of superoxide radicals that generate hydroperoxide, mitochondrial damage and cell death via apoptosis in the substantia nigra of rat RCSN-3 cell line (Arriagada et al., 2004). The amount of these neurotoxic effects is inhibited by DT-diaphorase, an enzyme responsible

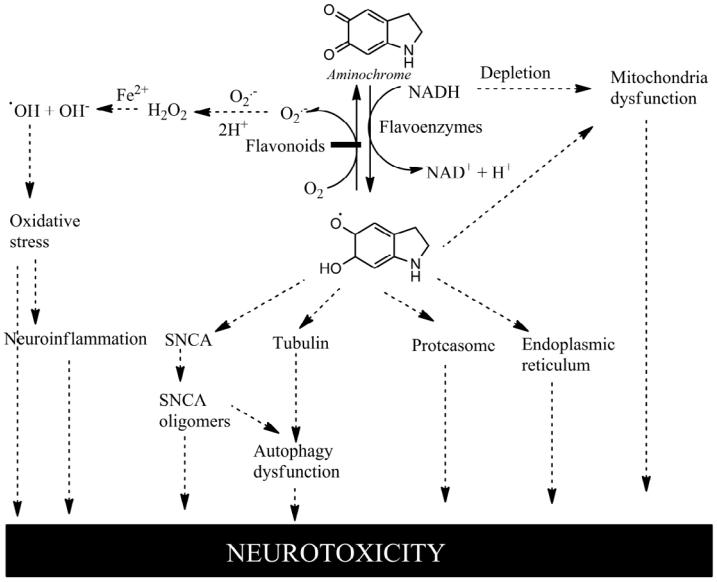

Figure 1 | **Potential effects of flavonoids on the protection against aminochrome-induced neurotoxicity.** Aminochrome induces neuronal death via oxidative stress, alpha-synuclein aggregation, impairment of protein degradation, mitochondrial dysfunction, endoplasmic reticulum stress, and neuroinflammation. Several studies have demonstrated the effects of flavonoids on the inhibition of these molecular alterations. We proposed the use of aminochrome to study the antioxidant effects of flavonoids and others mechanisms of neuroprotection for Parkinson's disease therapy.

for preventing the leukoaminochrome o-semiquinone radical generation during one-electron reduction of aminochrome (Arriagada et al., 2004). The DT-diaphorase also prevents aminochrome-induced alpha-synuclein oligomer formation in RCSN-3 cells, lysosome dysfunction in human neuroblastoma SH-SY5Y cells and loss of dopaminergic neurons in animals intracerebrally injected with aminochrome (for a review, see Segura-Aguilar, 2019).

Flavonoids with high effectiveness for radical scavenging, such as quercetin from the subclass flavonol, are predicted as protective against the oxidative damage induced by aminochrome. However, based on the structural properties of quercetin, one ought to consider that its catechol moiety can be oxidized to toxic o-quinone followed by DT-diaphorase metabolization. Surprisingly, the product of two-electron reduction of quercetin catalyzed by DT-diaphorase is pro-oxidant and cytotoxic in CHO cell line (derived from the Chinese hamster ovary; Metodiewa et al., 1999). It has been demonstrated that quercetin has both a pro-oxidant or antioxidant action and the pro-oxidant role depends on the quercetin concentration and the presence of transition metals (Gao et al., 1997). In this sense, advances in research on the effects of flavonoids with catechol moiety from different subclasses, for example rutin (flavonols subclass), luteolin (flavones subclass) and toxifolin (flavanonols subclass) in DT-diaphorase catalysis and biological action of its product must be considered for PD drug development.

Briefly, we consider the prospect of using aminochrome to study different mechanisms of action of flavonoids as a potential breakthrough for the application of these compounds in the PD therapy.

The perspective on the use of flavonoids in the therapy of PD has been evidenced. However, the preclinical model used to demonstrate its therapeutic action in the disease may be of crucial importance. Clinical studies of antioxidants in the treatment of PD based on preclinical models that use neurotoxins have failed (Parkinson Study Group QE3 Investigators et al., 2014), because preclinical models based on exogenous neurotoxins do not represent what happens in the disease. For example, neurodegeneration in PD is very slow both before and after motor symptoms, while the mode of action of exogenous neurotoxins is extremely fast. For example, MPTP induces Parkinsonism in humans in just 3 days. On the other hand, flavonoids have several mechanisms of action in addition to the antioxidant potential that must be investigated in a study model capable to reproduce different cellular and molecular alterations seen in PD. For this reason, aminochrome is a more physiological preclinical model to evaluate the protective effect of flavonoids because (i) it is formed in neurons that are lost in disease; (ii) it induces all mechanisms related to the disease, such as mitochondrial dysfunction, addition of alpha-synuclein to neurotoxic oligomers, dysfunction of both lysosomal and proteasomal protein degradation systems, endoplasmic reticulum stress, neuro-inflammation and oxidative stress; (iii) it induces slow progressive neuronal dysfunction that ultimately leads to neurodegeneration; (iv) its neurotoxicity is induced within the dopaminergic neuron, which implies that only one neuron dies and therefore is not expansive, which implies that the degenerative process will be slow as what occurs in the disease.

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