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Research Report

Neuroprotective effects of anthocyanin- and proanthocyanidin-rich extracts in cellular models of Parkinson's disease

Katherine E. Strathearn[a], *Gad G. Yousef*[b], *Mary H. Grace*[b], *Susan L. Roy*[a,1], *Mitali A. Tambe*[a], *Mario G. Ferruzzi*[c], *Qing-Li Wu*[d], *James E. Simon*[d], *Mary Ann Lila*[b], *Jean-Christophe Rochet*[a,*]

[a]*Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, 575 Stadium Mall Drive, West Lafayette, IN 47907-2091, United States*
[b]*Plants for Human Health Institute, Department of Food Bioprocessing and Nutrition Sciences, North Carolina State University, Kannapolis, NC 28081, United States*
[c]*Departments of Food Science and Foods and Nutrition, Purdue University, West Lafayette, IN 47907, United States*
[d]*Department of Plant Biology & Pathology, Rutgers University, New Brunswick, NJ 08901, United States*

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ABSTRACT

Neuropathological evidence indicates that dopaminergic cell death in Parkinson's disease (PD) involves impairment of mitochondrial complex I, oxidative stress, microglial activation, and the formation of Lewy bodies. Epidemiological findings suggest that the consumption of berries rich in anthocyanins and proanthocyanidins may reduce PD risk. In this study, we investigated whether extracts rich in anthocyanins, proanthocyanidins, or other polyphenols suppress the neurotoxic effects of rotenone in a primary cell culture model of PD. Dopaminergic cell death elicited by rotenone was suppressed by extracts prepared from blueberries, grape seed, hibiscus, blackcurrant, and Chinese mulberry. Extracts rich in anthocyanins and proanthocyanidins exhibited greater neuroprotective activity than extracts rich in other polyphenols, and a number of individual anthocyanins interfered with rotenone neurotoxicity. The blueberry and grape seed extracts rescued rotenone-induced defects in mitochondrial respiration in a dopaminergic cell line, and a purple basal extract attenuated

Abbreviations: Aβ, amyloid-β; ANC, anthocyanin; BB, blueberry; BC, blackcurrant; BCA, bicinchoninic acid; C3G, cyanidin-3-O-glucoside; C3Sa, cyanidin-3-O-sambubioside; C3So, cyanidin-3-O-sophoroside; D3G, delphinidin-3-O-glucoside; D3Sa, delphinidin-3-O-sambubioside; DAPI, 4′,6-diamidino-2-phenylindole; DMEM, Dulbecco's minimal essential media; DMSO, dimethyl sulfoxide; EU, endotoxin units; FBS, fetal bovine serum; GS, grape seed; LC–MS, liquid chromatography–mass spectrometry; LPS, lipopolysaccharide; M3G, malvidin-3-O-glucoside; MAP2, microtubule-associated protein 2; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; PA, phenolic acid; PAC, proanthocyanidin; PB, purple basil; PBS, phosphate buffered saline; PD, Parkinson's disease; PTFE, polytetrafluoroethylene; RIPA, radioimmunoprecipitation assay; ROS, reactive oxygen species; SNpc, *substantia nigra pars compacta*; SPE, solid-phase extraction; TFA, trifluoroacetic acid; TH, tyrosine hydroxylase

*Corresponding author. Fax: +1 765 494 1414.
E-mail address: jrochet@purdue.edu (J.-C. Rochet).
[1]Current address: National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, United States.

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nitrite release from microglial cells stimulated by lipopolysaccharide. These findings suggest that anthocyanin- and proanthocyanidin-rich botanical extracts may alleviate neurodegeneration in PD via enhancement of mitochondrial function.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder that involves a loss of dopaminergic neurons in a region of the midbrain referred to as the *substantia nigra pars compacta* (SNpc). A neuropathological hallmark of PD is the presence in some surviving neurons of Lewy bodies, cytosolic inclusions rich in fibrillar forms of the presynaptic protein α-synuclein (Spillantini et al., 1997). The postmortem brains of PD patients are also characterized by reduced activity of complex I, an enzyme of the mitochondrial electron transport chain (Betarbet et al., 2000). This complex I defect causes a 'leakage' of electrons from the transport chain, leading to the accumulation of reactive oxygen species (ROS) that promote the formation of aSyn aggregates (Betarbet et al., 2000; Rochet et al., 2012). Dopaminergic neurons of the SNpc contain relatively high basal levels of ROS resulting from the metabolism and auto-oxidation of dopamine (Betarbet et al., 2000; Graham, 1978). Therefore, these neurons are thought to be particularly susceptible to pathogenic mechanisms that upregulate ROS in PD. Moreover, the SNpc has a relatively high density of microglia compared to other brain regions, and microglial activation likely contributes to neurodegeneration in PD by triggering neuroinflammation (Block et al., 2007). Current PD therapies act by controlling the disease symptoms but do not slow the underlying neurodegeneration in the brains of PD patients.

Epidemiological evidence suggests that PD risk increases as a result of chronic exposure to environmental pollutants, including rotenone, a complex I inhibitor used as an insecticide and as a pesticide to control fish populations (Tanner et al., 2011). Rats or primates subjected to prolonged, low-dose rotenone exposure develop a PD-like phenotype characterized by motor dysfunction, a loss of dopaminergic neurons, the formation of Lewy-like inclusions, and microglial activation (Betarbet et al., 2000; Sherer et al., 2003a). In addition, rotenone triggers preferential dopaminergic cell death and aSyn aggregation in primary midbrain cultures (Liu et al., 2008a, 2008b). Rotenone is thought to elicit neurotoxicity by disrupting mitochondrial electron transport, thereby causing a buildup of ROS (Sherer et al., 2003b). In turn, this increase in ROS levels promotes the conversion of aSyn to oxidatively modified species with a high propensity to form potentially neurotoxic oligomers (Conway et al., 2001; Mirzaei et al., 2006; Rochet et al., 2012).

Multiple lines of evidence suggest that diets rich in polyphenols may have neuroprotective effects that result in a lower risk of neurodegenerative disorders including PD (Albarracin et al., 2012; Chao et al., 2012; Lau et al., 2007b). A number of phytochemicals have exhibited neuroprotective effects in cellular and animal models of PD (Chao et al., 2012; Song et al., 2012), including curcumin (Zbarsky et al., 2005), green tea flavan-3-ols (Choi et al., 2002; Guo et al., 2007; Levites et al., 2001; Mercer et al., 2005), and stilbenes including resveratrol and oxyresveratrol (Blanchet et al., 2008; Chao et al., 2008; Khan et al., 2010). Although polyphenolic compounds are well known for their ROS scavenging ability, the fact that their peak concentrations in the brain are lower than endogenous glutathione levels has led to the suggestion that they may alleviate neurodegeneration via additional protective mechanisms (Del Rio et al., 2013; Milbury and Kalt, 2010; Williams et al., 2004). Consistent with this idea, polyphenols have been found to exhibit an array of neuroprotective activities independent of ROS scavenging (reviewed in Chao et al., 2012; Ramassamy, 2006; Song et al., 2012), including suppression of oxidative stress via effects on mitochondrial respiratory chain function (Morin et al., 2003; Zini et al., 2002) and alleviation of inflammatory responses associated with glial activation (Guo et al., 2007; Kao et al., 2009; Lau et al., 2007a).

Recent epidemiological findings suggest that the consumption of berries (e.g. blueberries, strawberries) rich in two classes of polyphenols, anthocyanins (ANC) and proanthocyanidins (PAC), may reduce the risk of PD (Gao et al., 2012). Although a number of polyphenolic extracts or individual polyphenols have been tested for neuroprotective activity in PD models as outlined above, much less is known about the effects of botanical extracts rich in ANC and/or PAC on PD-related neurodegeneration, or how the neuroprotective activities of these extracts compare to those of extracts rich in other classes of polyphenols. In this study, we characterized ANC- and PAC-rich extracts and a number of individual ANC in terms of their ability to alleviate neurotoxicity in primary midbrain cultures exposed to rotenone, and we examined potential underlying mechanisms. Our findings suggest that extracts rich in ANC and PAC protect against rotenone neurotoxicity by alleviating mitochondrial dysfunction.

2. Results

2.1. *Study design*

The underlying hypothesis of this study was that botanical extracts rich in ANC and/or PAC have neuroprotective activity against PD stresses (Gao et al., 2012). To address this hypothesis we characterized a series of extracts with high levels of ANC and/or PAC, in addition to significant amounts of phenolic acids (PA) and stilbenes (Table 1) (Del Rio et al., 2013; Ramassamy, 2006), in terms of their ability to alleviate neuronal cell death elicited by the PD-related neurotoxin, rotenone. We also examined resveratrol and a Chinese