

Protective effect of curcumin in transgenic *Drosophila melanogaster* model of Parkinson's disease

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Abstract

Studies on model organisms have been found to be invaluable in clarifying the cellular and molecular basis of normal cellular processes and disease pathogenesis. *Drosophila* mutants and transgenes have provided a platform to understand the mechanisms associated with degenerative disease. Studies on the role of polyphenols in protecting against neurodegenerative diseases are limited. In the present study, the effect of curcumin at various doses was studied on the climbing ability of the transgenic *Drosophila melanogaster* that expresses normal human α -synuclein in the neurons. A significant dose-dependent protection against loss of climbing ability was observed. The results suggest that curcumin can strongly improve the climbing ability of Parkinson's disease model flies and also supports the utility of this model in studying the symptoms of Parkinson's disease.

Introduction

Parkinson's disease (PD) is a common neurodegenerative syndrome characterized by the loss of dopaminergic neurons in the substantia nigra, formation of filamentous intraneuronal inclusions (Lewy bodies) and extra pyramidal movement disorders.¹ PD is usually classified as a movement disorder, although it also gives rise to several non-motor types of symptoms, such as sensory deficits, cognitive difficulties or sleep problems.² Two forms of human PD have been identified: the hereditary form, which accounts for less than 10% of PD cases, and the idiopathic form, accounting for the remaining 90%.^{3,4} At least 5% of people are now known to have forms of the disease that occur because of mutation of one of the several specific genes,⁵ such as alpha synuclein (*SNCA*), Ubiquitin Carboxy-terminal hydrolase

L1 (*UCH-L1*), parkin (*PRKN*), Leucine rich repeat kinase 2 (*LRKK2*), PTEN-induced putative kinase 1 (*PINK1*), *DJ-1* and *ATP B2*. The most extensively studied PD-related genes are *SNCA* and *LRKK2*.⁵⁻⁶ There are genetic models of PD based on α -synuclein, primarily the transgenic overexpression of mutant or wild-type forms in mice or flies.⁷⁻¹¹ Overexpression of either wild-type or mutant α -synuclein in *Drosophila* leads to lewy body like synuclein containing inclusions and loss of dopaminergic neurons, as well as a behavioral abnormality that appears to be corrected by levodopa or dopaminergic agonist.^{1,12} Curcumin is the active ingredient of the turmeric plant (*Curcumin longa*).¹³ It has been shown to possess a number of pharmacological properties: antimutagenic,¹⁴ antigenotoxic,^{15,16} antitumor,¹⁴ anti-inflammatory,¹⁷ anti-ischemic,¹⁸ etc. We studied the effect of curcumin on the locomotor ability of Parkinson's disease model flies exhibiting α -synuclein in the neurons.

Materials and Methods

Drosophila stocks

Transgenic fly lines that express wild-type human alpha synuclein under UAS control in neurons (*w*[*]; *P*{*w*+*mC*}=*USA-Hsap/SNCA.F*"}*5B* and *GALA* "*w*[*]; *P*{*w*+*mC*}=*GAL4-elavL*}*3* were obtained from Bloomington *Drosophila* stock center (Indiana University, Bloomington, IN, USA). When the males of UAS-Hsap/SNCA.F strains are crossed with the females of *GALA-elav.L* (and vice-versa) the progeny will express the human α -synuclein in their neurons.¹

Drosophila culture and crosses

The flies were cultured on standard *Drosophila* food containing agar, corn meal, sugar and yeast at 25°C (24±1).¹⁹ Crosses were set up using 6 virgin females of UAS-Hsap/SNCA.F5B who were mated to 3 *GALA-elav* males. The progeny will express the human α -synuclein in the neurons and the flies were referred to as Parkinson's disease (PD) flies. First, the climbing assay was performed for the PD flies and the UAS-Hsap/SNCA.F (control). The PD flies were exposed to different doses of curcumin mixed in the culture medium. Curcumin was added in the medium at final concentrations of 1 μ L/mL, 5 μ L/mL and 10 μ L/mL. The UAS-Hsap/SNCA.F were used as control. Vials of PD flies without curcumin were used as positive control.

Drosophila climbing assay

The climbing assay was performed as described by Pedleton *et al.*¹² Ten flies were

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placed in empty glass vials (10.5 cm×2.5 cm). A horizontal line was drawn 8 cm above the bottom of the vial. After the flies had acclimated for 10 min at room temperature, both controls and treated groups were assayed at random for a total of 10 trials each. The procedure involved gently tapping the flies down to the bottom of the vials. The number of flies above the mark of the vial was counted after 10 sec of climbing and repeated 10 times to get the mean number above the mark of the flies in each vial. These values were then averaged, and a group mean and standard error were obtained. The mean values of various fly groups were statistically compared using an unpaired group of Student's t-test. All behavioral studies were performed at 25°C under standard lighting conditions.

Results and Discussion

A time course evaluation showed that the climbing response of control flies remained essentially unchanged over 21 days (Figure 1). From Day 9, however, the response of the PD flies was significantly lower than that of controls. Based on these results, standard duration of treatment was set at 21 days for the subsequent treatments with various doses of curcumin. The climbing assay was performed after 21 days of treatment. It was seen that 1.0, 5 and 10 μ L/ml of curcumin significantly improved the deteriorating climbing ability of the PD flies (Figure 2).

The results of the present study show that the addition of curcumin to the culture medi-

um significantly improved the climbing ability of the PD flies. A time-dependent loss of dopaminergic neurons in the dorsomedial group and the intracellular aggregates of α -synuclein (Lewy bodies) were reported by Feany and Bender in the transgenic flies. These changes were followed by functional loss in climbing ability.¹

PD is characterized by several abnormalities, including inflammation, mitochondrial dysfunction, iron accumulation, and oxidative stress.¹² Pharmaceutical intervention in the molecular pathways of disease initiation and progression is one of the strategies currently being employed. Most research focuses on providing protection against the loss of dopaminergic neurons in the substantia nigra. The efficacy of the drug levodopa declines as PD progresses. Recently, there have been strategies aimed at preventing progressive cell death in the nigral dopamine neuron.¹¹ Both wild-type and mutant α -synuclein form amyloid fibrils resembling those seen in lewy body, as well as non-fibrillary oligomers termed *protofibrils*. The accumulation of α -synuclein leads to toxicity and oxidative stress.^{20,21} It remains unclear whether misfolded proteins directly cause toxicity or damage cells via the formation of protein aggregates (Lewy body).¹¹ However, in our present study, the treatment of PD flies with curcumin showed a protective effect and reduced the possible loss of climbing ability in PD flies as they got older. Curcumin is an antioxidant and a free radical scavenger. Its protective effect may be due to the reduction in oxidative stress, or due to the inhibition of the expression of the α -synuclein, or by preventing the damage of dopaminergic neurons. It has been hypothesized that oxidative stress is linked to both the initiation and the progression of PD.

The present study was carried out using the *Drosophila* model of PD developed by Feany and Bender in 2000 that expresses human wild-type α -synuclein in the neurons of the fly, with consequent locomotor dysfunction.¹ The proteomic analysis of this panneural expression of human wild-type α -synuclein in the transgenic flies showed a differential expression of proteins indicating a perturbation of molecular pathways involving metabolism and signaling.²² Gene expression changes for genes in these molecular pathways have been shown to be greatest in this model at the pre-symptomatic stage, when the potential for neuroprotection is greatest, thus validating this model for identifying potential targets for neuroprotective strategies.²³ Similar results have been reported in the transgenic mice expressing human α -synuclein in the neocortex, hippocampus, and substantia nigra. The mice showed progressive damage in the dopaminergic neurons and impaired rotorod performance.⁹

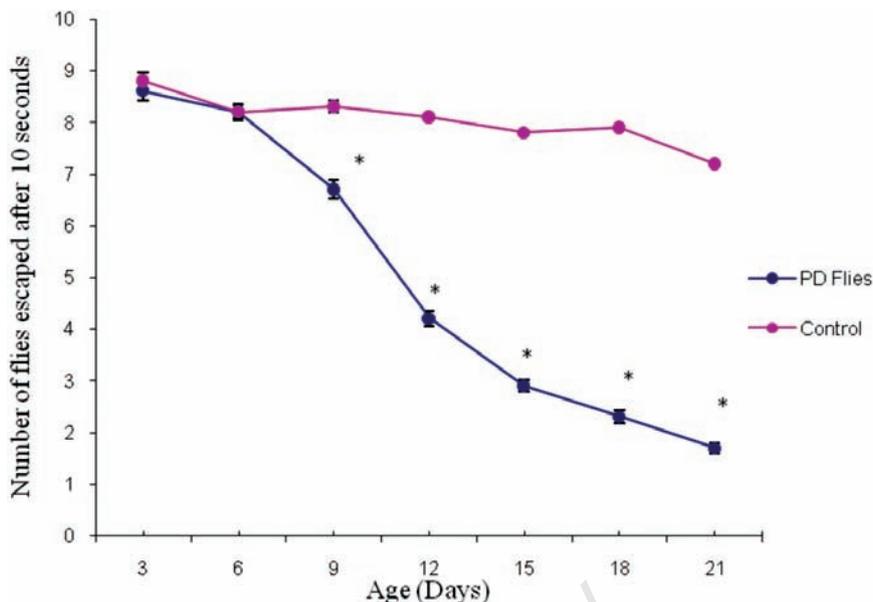


Figure 1. Climbing ability in Parkinson's disease flies and control over a period of 21 days. The values are the mean of 3 assays (*significant with respect to control $P < 0.05$).

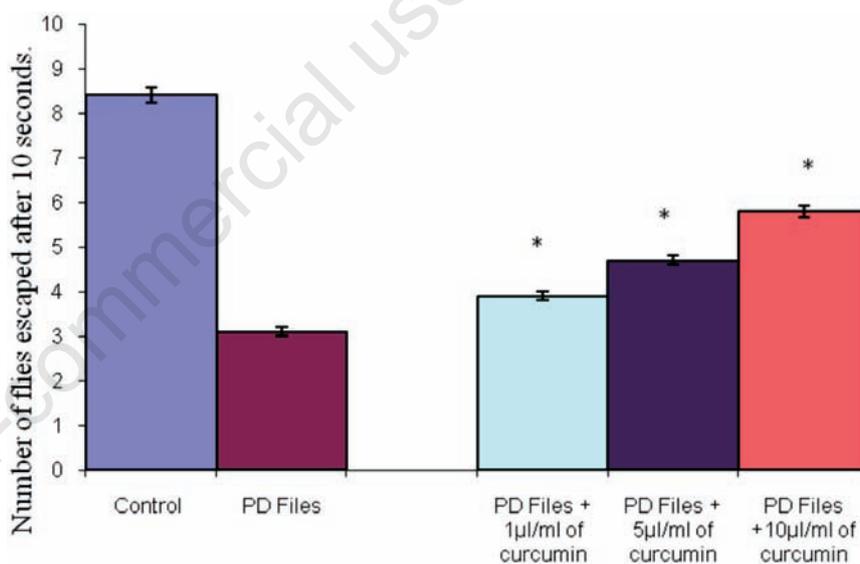


Figure 2. Effects of curcumin on fly climbing ability. The flies were treated with curcumin for 21 days and then assayed for climbing ability. Values are the mean of 3 assays (*significant with respect to PD flies $P < 0.05$).

The results of the present study suggest that the transgenic fly model mimics the motor impairments associated with PD and a climbing assay can be performed to determine whether or not a variety of compounds or drugs mixed in the fly culture medium prevent the progressive loss of climbing ability.¹²

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