Effect of Capsaicin on the Climbing Ability in Drosophila Model of Parkinson’s Disease


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ABSTRACT

Parkinson’s Disease (PD) is characterized by the progressive loss of dopaminergic neurons. Drosophila mutants and transgenes have provided a platform to understand the mechanistic insight associated with the degenerative disease. In the present study, the effect of capsaicin was studied on the climbing ability of the PD model Drosophila expressing normal human alpha synuclein (h-αS) in the neurons. These flies exhibit locomotor dysfunction as the age progresses. Capsaicin at final concentration of 0.1, 0.5, 0.75 and 1.0 μL mL⁻¹ was supplemented with the diet and the flies were allowed to feed for 21 days. Capsaicin showed a dose dependent significant (p<0.05) delay in the loss of climbing ability of PD model flies as compared to the untreated PD flies. The results suggest that the capsaicin is potent in delaying the climbing disability of PD model flies and also supports the utility of this model in studying PD symptoms.

Key words: Capsaicin, Parkinson’s disease, climbing ability, Drosophila

INTRODUCTION

Capsaicin is the active component of chili peppers (Genus: Capsicum). It is the main capsaicinoid in chili pepper (Fujiwake et al., 1980). Besides the use of it in various creams and ointments (Tsui et al., 2007), it has also been reported to influence the carbohydrate metabolism (Brown and Vale, 2011), reduce prostrate cancer (Mori et al., 2006) and the pain in rheumatoid arthritis (Fraenkel et al., 2004).

There are genetic models of PD based on alpha synuclein, primarily the transgenic over expression of mutant or wild forms in mice or flies (Masliah et al., 2000; Van der Putten et al., 2000; Dauer and Przedborski, 2003). The over expression of either wild type or mutant alpha synuclein in Drosophila leads to the formation of Lewy body like synuclein containing inclusions resulting in the loss of dopaminergic neurons as well as behavioural abnormality (Feaney and Bende, 2000; Pendleton et al., 2002). Besides having the therapeutic approaches of increasing the dopaminergic neurons activity or inhibiting the cholinergic effects to the striatum, now-a-days the attention has also been given to the use of flavonoids/natural antioxidants to reduce the oxidative stress (Naoi and Maruyama, 2001; Lu et al., 2010; Ono and Yamada, 2006; Pal et al., 2011). A number of polyphenolic compounds have been identified to have anti-fibrillogenic
properties (Caruana et al., 2011). In this context, the effect of capsaicin was studied on the locomotor ability of the PD model flies exhibiting human alpha synuclein in the neurons.

**MATERIALS AND METHODS**

**Drosophila stocks:** Transgenic fly lines that expresses wild type human alpha synuclein under UAS control in neurons "w[+]; P[w[+mC]=UAS-Hsap/SNCA,F]5B and GAL4 "w[+]; P[w[+mC]=GAL4-elav]3" were obtained from Bloomington Drosophila stock centre (Indiana University, Bloomington, IN). When the males of UAS (Upstream Activation Sequence)-Hsap/SNCA,F strains are crossed with the females of GAL4-elav,L (vice-versa) the progeny will express the human α-synuclein in the neurons (Feany and Bende, 2000).

**Drosophila culture and crosses:** The flies were cultured on standard Drosophila food containing agar, corn meal, sugar and yeast at 25°C (24±1) (Siddique et al., 2011). Crosses were set up using six virgin females of UAS-Hsap/SNCA,F5B mated to three males of GAL4-elav. The progeny will express the human α-synuclein in the neurons and the flies were referred as Parkinson disease (PD) flies. First, the climbing assay was performed for the PD flies and UAS-Hsap/SNCA,F (control). The PD flies were exposed to different doses of capsaicin (CAS: 404-86-4; Hi-Media) mixed in the culture medium. Capsaicin was added in the medium at final concentrations of 0.1, 0.5, 0.75 and 1.0 µL mL⁻¹. The UAS-Hsap/SNCA,F act as a control. The vials of PD flies without capsaicin act as a positive control.

**Drosophila climbing assay:** The climbing assay was performed as described by Pendleton et al. (2002). Ten flies were placed in an empty glass vials (10.5×2.5 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, to a total of 10 trials for 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 for 10 times to get the mean number above the mark of flies in this 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 the student t-test. All behavioral studies were performed at 25°C under standard lighting conditions.

**RESULTS**

The climbing response of control flies remained essentially unchanged over 21 days in a time course evaluation (Fig. 1). From the day 12 on however, the response of the PD flies were significant lower than those of the control. Based on these results, 21 days as standard duration of treatment was selected for the subsequent treatments with various doses of capsaicin. The climbing assay was performed after 21 days of the exposure to various doses of capsaicin. The exposure of PD flies to 0.1 µL mL⁻¹ of capsaicin showed a significant delay in the loss of climbing ability (Fig. 2). Similarly, the exposures of PD flies to 0.5, 0.75 and 1.0 µL mL⁻¹ of capsaicin in the culture medium significantly delayed the climbing disability in the PD flies (Fig. 2).
Fig. 1: Climbing ability in Parkinson disease (PD) flies and control for a period of 21 days. The values are the mean of 5 assays.

Fig. 2: Effects of capsaicin on the climbing ability. The flies were allowed to feed on the diet having capsaicin for 21 days and then assayed for climbing ability. The values are the mean of 5 assays and bar indicate the SD.

DISCUSSION

The treatment of PD flies with capsaicin showed the protective effect and results in the delay of the loss of climbing ability in PD flies. A time dependent loss of dopaminergic neurons, the formation of intercellular aggregates of alpha synuclein (Lewy bodies) leading to the loss in climbing ability was reported in transgenic flies (Feany and Bender, 2000). The accumulation of alpha synuclein leads to the toxicity and oxidative stress (Conway et al., 1988; Giasson et al., 1999). However, it remains unclear whether misfolded proteins directly cause toxicity or damage cells via the formation of protein aggregates (Dauer and Przedborski, 2003). One hypothesis is that the metabolism of the neurons that are being lost in the PD produces endogenous toxins such as hydrogen peroxide and free radicals that leads to the loss of the neurons over time (Fahn, 1989). Capsaicin binds to a vanilloid receptor subtype 1 (VR1) and permits the cations to pass through
the cell membrane. The resulting depolarization of the neuron stimulates it to signal the brain (Story and Orense, 2007). The aggregation of alpha synuclein has also been linked with the pathogenesis of the disease. Hence, the attention has been directed towards the identification of the inhibitors of alpha synuclein aggregation for the prevention and treatment of PD (Amer et al., 2006).

The lethal dose (LD₅₀) of capsaicin in mice is 47.2 mg kg⁻¹ (Johnson, 2007). The selected doses of capsaicin in our present study i.e., 0.1, 0.5, 0.75 and 1.0 μL mL⁻¹ successfully delayed the climbing disability. The present study was on the Drosophila model of PD that expresses the human wild type alpha synuclein in the neurons of the fly with consequent locomotor dysfunction. The European Centre for the Validation of Alternative Methods (EVCAM) has recommended the use of Drosophila as an alternative model for scientific studies (Festing et al., 1998; Benford et al., 2000). Drosophila has lot of similarities with human genome and is easy to handle, culture and ethical problems are less with this model. Drosophila as a model in pharmaceutical research is time efficient and cost effective in comparison to rodents (Avanesian et al., 2009). The results in 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 (Pendleton et al., 2002). In the light of results obtained in the present studies it is concluded that the capsaicin has the potential of delaying the symptoms of PD and could be a possible potential agent in treating the PD symptoms.

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REFERENCES


