Comparative Biochemistry and Physiology, Part C 211 (2018) 15–24

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Insights on the age dependent neurodegeneration induced by Monocrotophos, (an organophosphorous insecticide) in *Caenorhabditis elegans* fed high glucose: Evidence in wild and transgenic strains

Chinnu Salim[a,b], Nidheesh Thadathil[a,c], Muralidhara M.[d], P.S. Rajini[a,b,*]

[a] Academy of Scientific and Innovative Research (AcSIR), Anusandhan Bhawan, New Delhi, India
[b] Department of Food Protectants and Infestation Control, CSIR-Central Food Technological Research Institute, Mysore 570 020, India
[c] Meat and Marine Science Department, CSIR-Central Food Technological Research Institute, Mysore 570 020, India
[d] Department of Biochemistry, CSIR-Central Food Technological Research Institute, Mysore 570 020, India

ARTICLE INFO

Keywords:
Monocrotophos
High glucose
C. elegans
Dopaminergic neurodegeneration
α-Synuclein

ABSTRACT

The higher susceptibility of high glucose fed *C. elegans* to Monocrotophos (MCP, an organophosphorus insecticide) - induced dopaminergic (DA) neuronal degeneration was recently demonstrated. Employing this acute exposure model, the impact of MCP on DA degeneration among worms of two age groups (8 and 13 d old) fed control (CO) and high glucose (GF) diet with specific focus on phenotypic alterations, oxidative impairments and associated molecular perturbations employing both wild (N2) and transgenic strains(BZ555 and NL5901) was investigated. In general, 13 d worms exhibited higher susceptibility to MCP intoxication compared to 8 d old worms. Further, MCP-exposure caused an enhanced degree of DA degeneration among glucose fed (GF) worms as evidenced by lower chemotaxis index, reduced long-term memory and increased nonanone repulsion. Biochemical analysis of 13 d GF worms also revealed a significant increase in ROS, protein carbonyls and reduced ADP/ATP ratio. Interestingly, marked increase in degeneration of dopaminergic neurons and increased in α-synuclein content was evident among 13 d GF worms exposed to MCP. Significant alterations in the mRNA expression levels of *daf-2*, *age-1*, *sir 2.1* and *aak-2* among 13 d GF worms was evident. Collectively these findings suggest that high intake of glucose diet aggravates MCP associated dopaminergic neuronal degeneration and the impact of increasing age under such a condition. Moreover it provides an experimental paradigm to explore the molecular targets and mechanism/s underlying the possible relationship between insecticide exposure-associated dopaminergic degeneration in humans under hyperglycemic conditions.

1. Introduction

Owing to the increase in life expectancy, the prevalence of neurodegenerative diseases (NDD) is bound to increase in the coming years (Brown et al., 2006). Epidemiological evidence suggests pesticide exposure to be a risk factor in the development of various age-related NDD such as Parkinson's disease (PD) (Franco et al., 2010).Oxidative stress and mitochondrial dysfunction appear to be a crucial link between environmental factors, such as exposure to pesticides, a major risk factor in the pathogenic mechanism of PD (Lukaszewicz-Hussain, 2010). Long-term adverse effects of organophosphorus Insecticide (OPI) such as delayed polyneuropathy (Lewis et al., 2009), and adverse developmental neurobehavioral effects (Costa et al., 2008; Lewis et al., 2009) have been well documented. Besides PD, numerous studies have clearly highlighted that occupational pesticide exposure and its direct link in developing Alzheimer's disease (AD), dementia, amyotrophic lateral sclerosis (ALS) and discrepancies in cognitive function (Cannon and Greenamyre, 2011; Kamel et al., 2012). Although association between neurodegenerative effect and OPI is consistent regarding acute poisoning in humans, data are scarce on the adverse effects of chronic low doses of OPI (Salim and Rajini, 2017).

Excess intake of sugars can potentially alter the pharmacological and toxicological implications of several xenobiotics. Earlier studies have unequivocally shown that OPI besides inducing neurotoxicity also possess the propensity to disrupt glucose homeostasis in experimental models through several mechanisms (Joshi et al., 2012). Recent research has also shown that the metabolic pathways downstream of raised glucose have a damaging effect on neurons whose functional consequences are yet to be comprehensively understood (Tomlinson and Gardiner, 2008).Studies have ascribed a multidimensional role of

* Corresponding author at: Food Protectants and Infestation Control Department, CSIR-Central Food Technological Research Institute, Mysore 57002, India.
E-mail address: rajini29@yahoo.com (P.S. Rajini).

https://doi.org/10.1016/j.cbpc.2018.05.002
Received 18 January 2018; Received in revised form 25 April 2018; Accepted 8 May 2018
Available online 12 May 2018
1532-0456/

glucose transport in the maintenance of brain structure and function and a damaging interaction with AD pathology(Winkler et al., 2015).Interestingly, reports suggest a strong link between type 2 diabetes (T2D) and PD (Pablo-Fernandez et al., 2017).

C. elegans is an excellent candidate for aging research and ecotoxicological evaluations (Leung et al., 2008). They have been successfully employed in the evaluation of the toxicants at environmentally relevant concentrations (Rajini et al., 2008). Short lifespan and availability of specific mutant strains make them ideal models to obtain insights on various NDD, metabolic diseases as well as aging research (Choi, 2011, Copes, 2015,Fitzenberger et al., 2013, Schlotterer et al., 2009).

C.elegans has been shown to be a model to understand the relationship between neurodegeneration in a scenario of high glucose diet (Salim and Rajini, 2017). In the present study, 8 and 13-day old worms were exposed to OPI (which will be approximately equivalent to human exposure when they are in their late 40s) to understand the relevance to human health under occupational exposure in a scenario of high glucose diet. The most extensively studied pathway that regulates *C. elegans* lifespan is insulin/IGF-1 signaling pathway (Mondoux et al., 2011). In this study, much importance has been given to genes related to aging and insulin signaling pathway in *C. elegans*. Recent reports are available which indicate the independent action of insulin/IGF1 signaling machinery to control dopaminergic neurodegeneration and lifespan in *C. elegans* (Apfeld and Fontana, 2017).

Monocrotophos (MCP) is one of the most commonly used OPI worldwide (Pohanish, 2008). Spices like chillies and tea have also been reported to receive a higher number of applications with MCP (Dewan and Rajendran, 2009). MCP residues have been reported in fresh fruit samples of both grapes and pomegranate and long-term exposure is possible due to contaminated vegetable consumption (Arora and Singh, 2004). MCP has been found to induce neuropathy in affected farmers (Singh et al., 2004) and infertility in offspring (Rao and Kaliwal, 2002). Several reports have demonstrated the potential of MCP to induce Parkinson's like features in both mice models and *C. elegans* (Ali and Rajini, 2012, 2016). Previously it has been demonstrated that high glucose diet augments MCP induced neurotoxicity and dopaminergic degeneration in *C. elegans* (Salim and Rajini, 2014, 2017). This prompted the present study to understand the underlying mechanism/s in this model and the complexities associated with high glucose diet and MCP exposure.

Accordingly, the primary focus of this investigation was to understand the impact of acute MCP exposure on DA degeneration among worms of two age groups fed control (CO) and high glucose (GF) diet with a specific focus on phenotypic alterations (chemotaxis studies, nonanone repulsion assay), and oxidative impairments. Further analyzed various molecular markers genes related to the mechanism of aging (*sir 2.1, age-1, daf-2,aak-2* mRNA expressions) and dopaminergic degeneration employing both wild (N2) and transgenic strains(BZ555 and NL5901 with the GFP/YFP tag for dopaminergic as well as α-synuclein deposit). We found that as the worms ages (from 8 d to13 d old) they will be more vulnerable to MCP -induced dopaminergic degeneration when fed high glucose diet compared to their normal counterparts.

2. Material and methods

2.1. Chemicals

Monocrotophos (MCP) (99% pure) was procured from Sigma Chemical Co. (St. Louis, MO, USA). ApoSENSOR™ ADP/ATP Ratio Bioluminescent Assay Kit was purchased from BiovisionCatalogue #K255–200); Verso cDNA Synthesis Kit (Cat.No: AB1453A) from Thermo Fisher Scientific Pvt. Ltd.; Sso Fast EvaGreenSupermix (cat.no:1725201) from Bio-radLaboratories (USA); RNeasy Mini Kit (cat.no:74104) from Qiagen GmbH, Germany. All other chemicals used for the experiment were of analytical grade.

2.2. C. elegans strain preparation

Three strains of *Caenorhabditis elegans* Viz., wild-type (N2), NL5901(*pkIs*2386), BZ555 (egls1-dat-1p::GFP) along with *E. coli* (OP50, an uracil auxotroph) were obtained from the Caenorhabditis Genetics Center (CGC, Minneapolis, MN, USA), which is funded by the National Center for Research Resources (NCRR). All strains of worms were cultivated on nematode growth medium (NGM) plates with OP50 and maintained at 20 °C (Williams and Dusenbery, 1988; Brenner, 1974). Worms were synchronized by separating the eggs from gravid adults by sodium hypochlorite treatment (Fabian and Johnson, 1994).

2.3. Experimental procedure

Synchronous eggs were allowed to develop on plates with NGM incorporated with 2% glucose (111 mM) in a molten state and were seeded with heat-killed OP50 (Schlotterer et al., 2009). The worms were allowed to develop in the glucose-rich NGM by changing the plate frequently to avoid F1 generation. Worms maintained on NGM (Control worms/CO) or in 2% glucose NGM medium (Glucose-fed worms/GF) till gravid stage (after egg laying stage) on days 8 and 13 for the experiments. For each time point, worms were exposed to sublethal concentration of MCP (8d and 13d- 50 μM) in NGM plates with heat-killed *E. coli* and incubated for 24 h at 20 °C. Control (CO) worms were maintained on NGM plates without MCP. Transgenic strains- BZ555 (8 dopaminergic neurons tagged with GFP) and NL5901 (α-synuclein tagged with YFP) were used for quantification of dopaminergic neurodegeneration.

Following the exposure period, the worms were collected, washed three times with K-medium and subjected to behavioral phenotype assessment, biochemical measurements and to quantify mRNA expression levels. Glucose content in the worms was assessed before starting the individual experiment to confirm that they had consumed the glucose provided. The concentration of MCP employed was selected based on our previous study (Ali and Rajini, 2012). For biochemical determinations, approximately 800 to 1000 worms were taken for each group, while ~1000 worms from each group were used for mRNA extraction. For microscopic studies, 15 worms were taken for each group. All Experiments were repeated three times in triplicate.

2.4. Assessment of behavioral phenotype

Chemotaxis assay was performed to determine the learning behavior of worms as described previously (Kauffman et al., 2011; Saeki et al., 2001). Worms (n = 300 worms per replicate; three replicates per group) were washed thrice with M9 buffer and then transferred to NGM plates for training. Worms were starved for different time intervals before the long and short-term memory assays with butanone. After the training phase, worms were transferred to the starting point of test plates with butanone and alcohol for a different time interval. After 1 h, the number of nematodes in the section with butanone and with ethanol were counted manually using sodium azide anesthesia. The chemotaxis index (number within butanone gradient– number within ethanol)/ total number of worms on the plate) was calculated for each treatment.

Nonanone repulsion assay was carried out to measure indirectly the dopamine content in the worms (Bargmann et al., 1993). Pelletized worms washed thrice with K-medium were placed in NGM plate without food. A minute drop of nonanone was placed in front of a forward-moving worm in the plate. The time taken by the worm to reverse the path responding towards nonanone was noted. 15 worms were studied from each group. The assay was carried out three times in triplicates and the response time was measured in seconds.

2.5. Determination of biochemical endpoints

After exposure, the worms were washed with K-medium, pelletized