Vol.2, No.3, 70-74 (2013)
http://dx.doi.org/10.4236/apd.2013.23014

Potential of a planarian model to study certain aspects of anti-Parkinsonism drugs*

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Received 15 February 2013; revised 30 March 2013; accepted 22 April 2013

ABSTRACT

We previously created and investigated a planarian model for the study of drug action, abuse, physical dependence, receptor affinity, the toxicity of heavy metals in wastewater, and seizures. For the present pilot study, we investigated the possibility that this model might be useful for studying certain aspects of drugs used in treatment of Parkinson disease. For the first step, we were interested in finding an *in vivo* metric for the inhibition of L-DOPA by an inhibitor of DOPA decarboxylase. The direct clinical relevance of the endpoint was of secondary concern during this preliminary phase of model development. Two metrics were explored: L-DOPA-induced inhibition of motility (locomotor velocity) and dopamine-mediated toxicity, which was quantified using a Kaplan-Meier survival curve. L-DOPA produced both dose- and time-related toxicity. The water-soluble DOPA decarboxylase inhibitor benserazide dose-dependently inhibited the effect of L-DOPA, as manifested by a leftward shift in the Kaplan-Meier curve. Additional work was initiated using the more sensitive and a graded metric of spontaneous locomotor velocity. The encouraging results of this pilot study suggest that: 1) planarians contain DOPA decarboxylase or an equivalent enzyme, and 2) the planarian model might be useful for the study of certain aspects of anti-Parkinsonism pharmacotherapy.

Keywords: L-DOPA; Benserazide; Planarians

1. INTRODUCTION

Parkinson disease (PD) is manifested as generalized abnormalities in locomotor movements (hypokinesias) (*paralysis agitans*) such as a stooped posture, difficulty in initiating and stopping walking, a short and shuffling gait, and a tendency to fall over [1-6]. The mean age of PD onset is about 60 years [7]; its prevalence is approximately 1% of those older than 65 years [8]. The disorder occurs throughout the world [9].

Post-mortem brains of PD patients have a decreased level of dopamine [10] that correlates with the loss of dopaminergic neurons within the substantia nigra *pars compacta* (SNpc) and degeneration of nerve terminals in the corpus striatum (basal ganglia) (**Figure 1**) [1]. Because the basal ganglia participate in regulation of neuronal transmission from the cerebral cortex to motor neurons of the spinal cord, decreased levels of dopamine disrupt the balance between dopamine and other neurotransmitters that are involved in a coordinated locomotor activity, including acetylcholine, GABA (γ-aminobutyric acid), glutamate, 5-HT (5-hydroxytryptamine or serotonin), norepinephrine, and neuropeptides in striatal interneurons [11]. The SNpc target neurons express both major 7-TM-GPCR (seven-transmembrane G protein-coupled receptor) dopamine receptor (DAR) types: excitatory DAR D_1 *DRD*1 (transduced by stimulated formation of cAMP and phosphatidyl inositol hydrolysis) and inhibitory DAR D_2 *DRD*2 (transduced by decreased cAMP and modulation of K^+ and Ca^{2+} currents). In experimentally-lesioned animals, damage to the nigrostriatal pathway results in a hyperactivity of the remaining dopaminergic neurons, increased transmitter turnover rate, and state of denervation *super* sensitivity [12].

Current pharmacologic management of PD involves a restoration of the balance between dopamine and other neurotransmitters, most commonly by replenishment of dopamine [13]. Since direct administration of dopamine

*All authors contributed significantly to this study and there are no conflicts of interest.

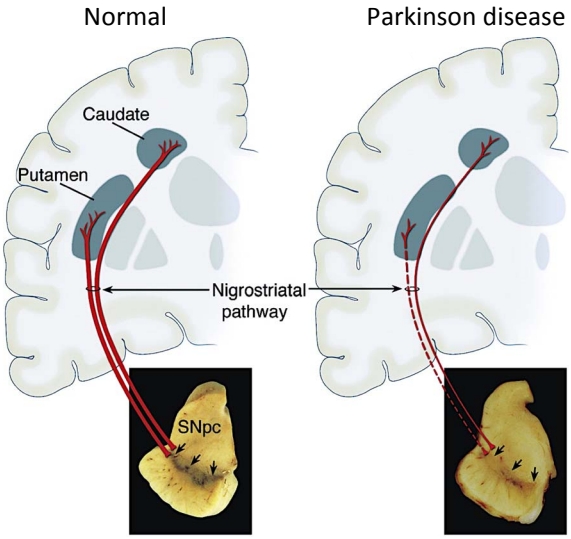

Figure 1. Schematic representation of the normal nigrostriatal pathway and marked degeneration in Parkinson disease. SNpc (substantia nigra *pars compacta*). Reprinted from Dauer *et al*. [1] with permission.

is ineffective or inefficient, because it does not readily cross the blood-brain barrier, indirect approaches are used. The most common is administration of the amino acid levodopa (L-DOPA) [13,14]. L-DOPA enters the brain by active amino acid transport systems [15] and is then converted to dopamine by the enzyme L-aromatic amino acid decarboxylase (DOPA decarboxylase) [16].

There are several animal models of PD [1]. Most of the models use mammals and are generally both time- and resource-intensive. We here report a convenient and facile non-mammalian model for *in vivo* demonstration and quantitative assessment of DOPA decarboxylase or an equivalent enzyme activity in a species with primitive nervous system and mammalian-analogous neurotransmitter and 2nd messenger systems [17-26]. The model is useful for the study of drug action and for physiological aspects of drug abuse [27-43].

2. MATERIALS AND METHODS

2.1. Animals and Chemicals

The planarians (*Dugesia dorotocephala*) were obtained from Carolina Biological Supply Co. (Burlington, NC). They were acclimated to laboratory conditions and were tested within 48 h of receipt. L-DOPA and benserazide (a competitive inhibitor of DOPA decarboxylase) were obtained from commercial sources and were prepared at the desired concentration in tap water. N = 12 per group.

2.2. Testing

Phase 1. In order to construct a survival Kaplan-Meier curve, planarians were placed individually into observation (petri) dishes that contained either water (as control) or L-DOPA at a concentration that had previously been established in pilot studies. Each of the groups was tested with or without benserazide. The number of the planarians remaining alive at specified times during the observation period was recorded.

Phase 2. In order to measure the *p*LMV, as has been previously described [31], each planarian was placed individually into a clear plastic petri dish that was located over graph paper having gridlines that were spaced 0.5 cm apart in a square pattern. The dish contained either water as the control or L-DOPA at a concentration that was established in pilot studies. *p*LMV was quantified by counting the number of gridlines that each planarian crossed or re-crossed during each minute over the observation period. The results are graphed as the group mean number of (re)crossings for each minute during the observation period.

3. RESULTS

Phase 1. Exposure of planarians to L-DOPA resulted in a dose- and time-dependent toxicity that was manifested by a decrease in number over the 2-h observation period. At each of the doses of L-DOPA, the effect of L-DOPA was attenuated by the DOPA decarboxylase inhibitor benserazide (see **Figure 2**).

Phase 2. Consistent with previous results [31], untreated planarians displayed a relatively constant and a near linear spontaneous locomotor activity (*p*LMV) of about 10 - 15 gridlines per minute over the entire obser-

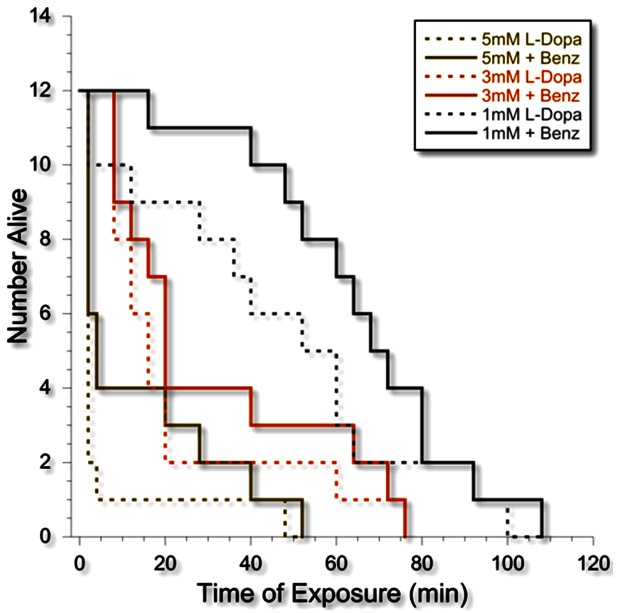

Figure 2. Kaplan-Meier survival curve of planarians exposed to L-DOPA alone (dotted lines) or L-DOPA plus benserazide (solid lines).

vation period of 10 minutes. L-DOPA decreased the *p*LMV. Importantly, the *p*LMV in the presence of L-DOPA was linear (see **Figure 3**).

4. DISCUSSION

The present work investigated the use of a simple *in vivo* planarian model in order to study the *in vivo* effect of L-DOPA, specifically, the metabolic conversion of L-DOPA by DOPA decarboxylase or the planarian equivalent. Planarians have a simple nervous system with mammalian-like neurotransmitter systems (e.g., [17,24, 26]), including dopaminergic. Therefore, they are the lowest form of extant animal that would display relevant dopaminergic-related neurotoxicity. They have been shown to respond with quantifiable dose-related behavioral changes to drug exposure or to withdrawal (e.g., [17,19,21,22,25-27,30,33-34,37]). We and others have used planarian models for investigation of drug action and related physiological processes (for a review, see the monograph [31]).

In the present study, the dose-related L-DOPA-induced (neuro)toxicity was attenuated by benserazide, which is an inhibitor of DOPA decarboxylase. It is used clinically together with L-DOPA in order to minimize the conversion of L-DOPA to dopamine in the periphery–thereby increasing the percent of the administered dose that is converted to dopamine in the CNS (**Figure 4**).

These results provide *in vivo* evidence for the presence and functional activity of DOPA decarboxylase or for an equivalent enzyme in planarians. A *D. japonica* aromatic amino acid decarboxylase-like activity (*DjAADCA*) gene,

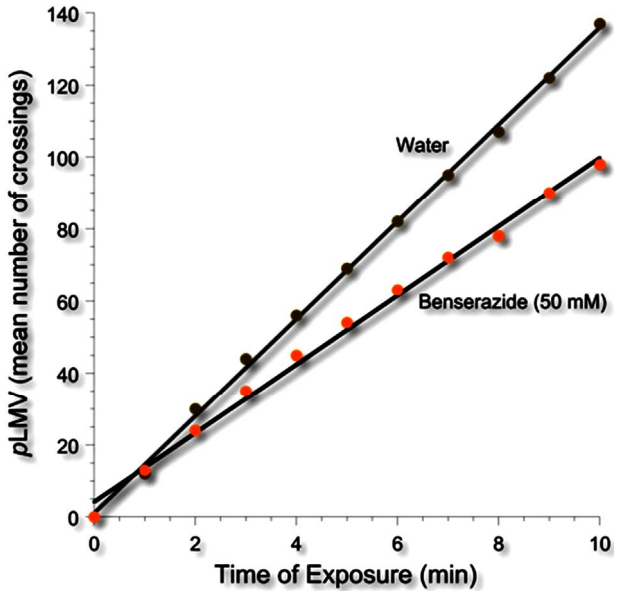

Figure 3. Spontaneous locomotor activity of planarians (*p*LMV) in water or the DOPA decarboxylase inhibitor benserazide (50 mM).

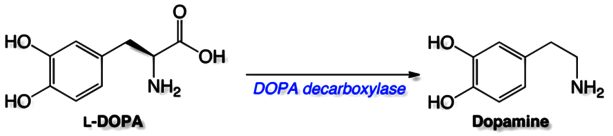

Figure 4. Conversion of L-DOPA to dopamine catalyzed by DOPA decarboxylase.

and mRNA and protein expression have been identified in *D. japonica* by Nishimura and colleagues [44]. It thus seems that *D. dorotocephala* shares the same or similar enzyme.

In summary, L-DOPA produced dose- and time-related lethality in planarians that was attenuated by a reversible inhibitor of DOPA decarboxylase (benserazide). These results are consistent with a previous demonstration of such an enzyme in planarians [44] and they support the use of a facile planarian model to study certain biochemical aspects of anti-Parkinson drugs.

5. ACKNOWLEDGEMENTS

The authors thank Timothy Shickley, Ph.D., for the suggestion of *Planaria* as a model. This work was supported by NIH grants DA15378 (RBR) and DA022694 (SMR).

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