Folinic acid is neuroprotective in a fly model of Parkinson’s disease associated with pink1 mutations

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Abstract
Mutations in PTEN-induced kinase 1 (PINK1) cause autosomal recessive and early-onset Parkinson’s disease (PD). PINK1, a kinase involved in a mitochondrial quality control mechanism, acts by promoting the autophagic degradation of damaged mitochondria. Mutations in PINK1 lead to the accumulation of impaired mitochondria and the death of dopaminergic neurons. Foliates act as single carbon donors in metabolic reactions such as nucleotide synthesis from purines. Oral folates are available in two forms, folic and folinic acid (FA and FiA, respectively). In Drosophila pink1 mutants, enhancing nucleotide biosynthesis via dietary supplementation with FA during development rescues mitochondrial function and leads to neuroprotection in adults. Orally available FiA bypasses the deconjugation and reduction steps required with FA and is more metabolically active. Here, we investigated the neuroprotective potential of dietary supplementation with FiA in adult pink1 mutant flies. We show that an FiA-enriched diet begun at early to middle stages of adulthood prevents the degeneration of dopaminergic neurons observed in pink1 mutants. An FiA-enriched diet might therefore delay or prevent the neuronal loss in patients with PINK1 mutations and may ameliorate other diseases linked to mitochondrial defects.

Introduction
It is accepted that mitochondrial defects play an important role in the neuronal demise associated with Parkinson’s disease (PD, reviewed in [1]). Mutations in PINK1, a mitochondrial kinase, lead to an early onset form of autosomal recessive Parkinsonism. PINK1 is involved in mitochondrial quality control (reviewed in [2]). Loss of PINK1 activity results in mitochondrial dysfunction, increased production of toxic reactive oxygen species, and neuronal death (reviewed in [3] [4]). The fruit fly Drosophila melanogaster is a powerful model for exploring the mechanisms of PD-associated neurodegeneration. This invertebrate is also an excellent in vivo platform for testing chemicals with therapeutic potential (reviewed in [5]). Drosophila pink1 mutants show an age dependent loss of dopaminergic neurons [6] that can be blocked via an FA-supplemented diet beginning at early embryogenesis [7]. In humans, FA is generally well absorbed, but the conversion of FA to its metabolically active coenzyme forms is complex (reviewed in [8]). On the other hand, FiA is an immediate precursor of 5,10-methylene-tetrahydrofolate, and oral administration of FiA bypasses the chemical reactions required for the coenzyme conversion of FA. Additionally, unlike FA, dietary FiA might be readily available to the brain (reviewed in [8]).

Objective
To determine whether a dietary supplement containing FiA in adult pink1 flies is neuroprotective.
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**Figure Legend**

**Figure 1.** A FiA-enhanced diet blocks neurodegeneration in *pink1* mutants.

(A) Representative images of normal and defective thorax in control and *pink1* mutant flies. The thoracic defect is indicated with a white arrow.

(B) Dietary supplementation with FA (4 mM) or FiA (4 mM) beginning at the embryonic stage rescues the thoracic defects in *pink1* mutants (asterisks, two-tailed chi-square, 95% confidence intervals, \(\chi^2[Fa](2) = 38.57, \chi^2[FA](2) = 33.92\)). NF indicates normal food. The scoring of thoracic defects was performed 3 days after eclosion.

(C) Schematic diagram of a *Drosophila* brain (sagittal orientation) with the PPL1 cluster of dopaminergic neurons shown in blue.

(D) Anti-TH staining showing cell bodies of PPL1 neurons. Representative images are shown; arrows indicate the individual PPL1 cluster in a control animal.

(E) Aged (30 days) *pink1* mutant flies show a loss of PPL1 neurons (mean ± SD; asterisks, two-tailed unpaired t-test, \(t(36) = 9.18\)).

(F) Dietary supplementation with FA (4 mM) or FiA (4 mM) from embryonic stage is neuroprotective in *pink1* mutants (mean ± SD; asterisks, one-way ANOVA with Dunnett’s multiple comparison test, \(F(2,23) = 11.21\)). A diagram of the dietary intervention protocol is shown on the right.

(G) Time course analysis shows an age dependent loss of PPL1 cluster neurons in *pink1* mutant flies (mean ± SD; asterisks, one-way ANOVA with Dunnett’s multiple comparison test, \(F(3,32) = 39.37\)).

(H) Dietary supplementation with FiA (4 mM) from the adult stage for 30 days or 20 days is neuroprotective in *pink1* mutants (mean ± SD; asterisks, two-tailed unpaired t-test, \(t(35) = 6.27\)).
Results & Discussion

*Drosophila pink1* mutants have impaired mitochondria, resulting in pathologies in tissues with high energy requirements, such as skeletal muscles and brain. One of the most easily measurable features of *pink1* mutant flies is the degeneration of their indirect flight muscles, which results in a defective (crushed) thorax phenotype [6] [9] in young (3 days) adults (Fig. 1A). The incidence of this crushed thorax in *pink1* mutants flies was reported to be significantly decreased by maintaining these flies in a FA-supplemented diet beginning at early embryogenesis [7]. We first evaluated whether FiA can also suppress the crushed thorax phenotype in *pink1* mutants. We confirmed that dietary FA led to a reduction in the appearance of crushed thorax phenotype (from 83% to 63%; Fig. 1B) in *pink1* mutants. Maintaining *pink1* mutants from early embryonic stages on food containing an identical concentration of FiA (4 mM) also led to a significant reduction in the crushed thorax phenotype (from 83% to 61%; Fig. 1B). Taken together, these data show that diets supplemented with either FA or FiA can compensate for muscle degeneration in *pink1* mutant flies.

*Drosophila pink1* mutants exhibit an age-dependent loss of dopaminergic neurons in the protocerebral posterior lateral 1 (PPL1) cluster, and this loss is first detected in 30 day old adults [6] (Fig. 1C-E). We next investigated whether both FA and FiA could suppress this neurodegeneration. *pink1* mutant flies fed on FA since embryonic development are protected from the loss of PPL1 cluster neurons [7]. We therefore tested whether an FiA-supplemented diet was also neuroprotective. A diet supplemented with either FA or FiA through embryonic development to adulthood led to significant neuroprotection (Fig. 1F). As the neuronal loss observed in PD is age-dependent, and first reported in 30 day old *pink1* mutant flies [6], we tested how early the degeneration of the PPL1 cluster of neurons occurs. We first detected a significant loss of PPL1 cluster neurons in *pink1* mutant flies in 20 day old adults (Fig. 1G). Next, we tested how early during adulthood the dietary supplementation with FiA could achieve neuroprotection in *pink1* mutant flies. We began a diet of FiA-supplemented food either on day 1 or day 10 after eclosion of *pink1* adults and quantified the loss of PPL1 neurons in 30 day old animals. These results showed that an FiA-supplemented diet when given as late as 10 days post-eclosion is sufficient to prevent neurodegeneration in *pink1* mutants (Fig. 1H). Altogether, these findings reveal the neuroprotective potential of an FiA-supplemented diet when fed to *Drosophila* in a model of PD linked to mitochondrial dysfunction caused by *pink1* mutations.

Conclusions

This study shows that FiA has therapeutic potential for neuroprotection in adult *pink1* mutant flies.

Limitations

We have used an insect model, *Drosophila melanogaster*, to gain initial insights into whether FiA supplementation has an in vivo neuroprotective potential in a PD model. In contrast to FA, dietary FiA has been proposed to readily cross the blood-brain barrier [8] and might therefore be more capable of counteracting mitochondrial defects in neurons. The blood-brain barrier in *Drosophila*, an invertebrate, is exclusively formed by glial cells, similar to that present in lower vertebrates (reviewed in [10]). To determine whether dietary FiA is capable of counteracting mitochondrial dysfunction in humans, it would be desirable to determine to what degree FiA can cross the blood-brain barrier in a mammalian model system such as a rodent (mouse or rat) before assessing the neuroprotective potential of FiA for neurons in the human central nervous system. PD is a disabling disorder for which no cure is yet available; further, after dopaminergic neurons are lost, only a few palliative treatment options for PD symptoms are available.
Therefore, treatments that either prevent or delay the onset of the disease at an early stage are needed. There is one active clinical trial of FA in PD (World Health Organization ID NCT01238926). FA is already approved and used for applications in the clinic as an adjuvant during chemotherapy [11] and can be administered orally, as a dietary supplement, or intravenously. Thus, the drug safety risk is low, and drug development for repurposing FA as a treatment for PD would be faster than for a novel drug. With this in mind, it seems worthwhile to further test the supplementation of FA in clinical trials as a potential preventative or palliative therapeutic for PD and to expand the repertoire of treatment options.

**Additional Information**

**Methods and Supplementary Material**

Please see https://sciencematters.io/articles/201702000009.

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**Ethics Statement**

Not applicable.

**Citations**


