Acetaminophen attenuates dopamine neuron degeneration in animal models of Parkinson's disease

Cody J. Locke, Stacey A. Fox, Guy A. Caldwell, Kim A. Caldwell

Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487, United States

Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disorder with ~2% of people over age 65 suffering from this disease. Risk factors for PD involve interplay between still poorly defined genetic and non-genetic contributors, but appear to converge upon cellular pathways that mediate protein misfolding and oxidative stress that lead to dopaminergic neuron loss. The identification of either new or repurposed drugs that exhibit benefit in slowing the age-dependent neuronal damage that occurs in PD is a significant goal of much ongoing research. We have exploited the nematode Caenorhabditis elegans as a model system by which the neuroprotective capacity of acetaminophen could be rapidly evaluated for efficacy in attenuating dopamine (DA) neurodegeneration. Using three independent and established neurodegenerative models in C. elegans, we assayed for acetaminophen-dependent rescue in response to: (1) over-expression of the PD-associated protein, α-synuclein; (2) acute exposure to 6-hydroxydopamine (6-OHDA); (3) excess intracellular DA production due to over-expression of the DA biosynthetic enzyme, tyrosine hydroxylase (TH). These data suggest that acetaminophen significantly protected C. elegans DA neurons from stressors related to oxidative damage, but not protein misfolding. Taken together, these studies imply an activity for acetaminophen in the attenuation of DA neuron loss that, following essential corroborative analyses in mammalian systems, may represent a potential benefit for PD.

The most logical route toward therapeutic intervention for neurodegenerative diseases involves the identification of small molecules that have the ability to provide neuroprotection. Limited animal model studies have shown that acetaminophen might protect neurons from degeneration. For example, acetaminophen can protect primary rat embryonic DA neurons from glutamate toxicity [6]. This is an important finding for the treatment of PD as DA neurons, which selectively degenerate in this disease, are particularly vulnerable to glutamate toxicity in vitro [15]. Additionally, acetaminophen provided partial neuroprotection in rats treated with 1-methyl-4-phenyl pyridinium (MPP+), a neurotoxin that induces DA neurodegeneration [17]. These results suggest that acetaminophen could be a prophylactic, as well as adjuvant, therapy for neurodegenerative diseases such as PD. However, the mechanism behind acetaminophen-induced neuroprotection is still not known.

DA neuron loss is a hallmark of PD. Several distinct mechanisms have been associated with DA neuron decline. Some of these involve genetic mutations and others are a result of environmental exposures. Our laboratory has been examining the relationship between cellular stress and its functional consequences for PD using the nematode animal model system, C. elegans [5]. Despite its evolutionary distance from humans, C. elegans neurons retain many aspects of mammalian neuronal function including ion channels, neurotransmitters (dopamine, serotonin, acetylcholine, GABA, etc.), vesicular transporters, receptors, and synaptic components [2,7]. In this regard, we have developed several DA neurodegeneration models whereby there is a decline in DA neuron survival over the aging process; these models display degenerative phenotypes from a dominant genetic cause of PD, a toxin, or excess intracellular DA production.

The greatest strength of C. elegans as a model system is its ability to be used for screening purposes—be those genetic, genomic or chemical. There is a growing body of literature showing the utility of using C. elegans for pharmacological research. A variety of compounds ranging from drugs associated with modulating neurotransmitter activity [1,18] to epilepsy modifiers [10,25] have been successfully employed in this nematode. Here, we exploited the advantages of our DA neuron stress assays to determine which cellular malfunctions can be assuaged by acetaminophen exposure.

© 2008 Elsevier Ireland Ltd. All rights reserved.
DA neurons are present. DA neurons when they are 7-day-old adults; only 1 of 4 CEP (arrow) and 1 of 2 ADE (C) Most worms expressing This worm exhibits only 2 of 4 CEP neurons and 1 of 2 ADE neurons (arrowhead); the CEP neuron processes and arrowheads indicate the ADE cell bodies in an adult worm. DA neurons without toxic insult (genetic or environmental); arrows depict the four C. elegans Fig. 1.

9.2 mM. Occasionally 10 higher concentrations of compounds are tested: 0.1, 1, 2, and of compounds [20].

Nematodes were maintained using standard procedures [4]. Acetaminophen (Sigma A7085) was dissolved in molten nematode growth medium (NGM) before pouring in Petri dishes. The following final concentrations of acetaminophen were tested: 0.1, 1, 2, and 9.2 mM. Occasionally 10x higher concentrations of compounds are needed in worm assays as the worm cuticle can impede the uptake of compounds [20].

Compared with the ~100 billion neurons of the human brain, C. elegans hermaphrodites have exactly 302 neurons, precisely eight of which produce DA. Six of the DA neurons are located within the anterior-most region of the animal, consisting of two pairs of CEP (cephalic) and one pair of ADE (anterior deirid) neurons (Fig. 1A).

For the ease of analyses, these six neurons were monitored in our assays; in all cases transgenic worms expressed GFP exclusively within the DA neurons (Pdat-1::GFP) [19]. To visualize these neurons, worms were mounted onto 2% agarose pads and immobilized with 3 mM levamisole before examination with a Nikon Eclipse E800 epifluorescence microscope equipped with an Endow GFP HYQ filter cube (Chroma). A neuron was scored as “wild-type” when its cell body and process (the prominent dendrites in CEP neurons or axons in ADE neurons) were intact. A neuron was scored as degenerating when at least one degenerative change such as “dendrite blebbing” (Fig. 1B), “cell body rounding” (Fig. 1B), or “cell body and/or process loss” (Fig. 1C) was observed. All six anterior DA neurons were scored in each animal. An unequivocal advantage of C. elegans is that detailed quantitative analyses of individual cells are achievable.

Images were captured with a Cool Snap HQ CCD camera (Photometrics) driven by MetaMorph Software (Universal Imaging). Statistics were performed by direct comparison between each treatment/control pair using the Fisher Exact Test (http://www.langsrud.com/fisher.htm).

Recent genetic discoveries have implicated specific proteins, such as α-synuclein, in the pathogenesis of PD. Genomic multiplication of the wild-type α-synuclein gene results in PD, indicating that over-expression of this protein alone can lead to the disease [11,21]. We have established that over-expression of wild-type human α-synuclein under control of Pdat-1 promoter results in age- and dose-dependent neurodegeneration in C. elegans [5,13]. Worm neurons do not display high levels of degeneration until they are adults. Since DA neurodegeneration is slow in these animals (occurs over the course of a week), the complete loss of one or more DA neurons is the most commonly observed phenotype (Fig. 1C). These isogenic worms express Pdat-1::GFP and Pdat-1::α-synuclein (strain UA18 (baEx18)) [5]; herein they will be referred to simply as α-synuclein-expressing worms. Prior confirmation of human α-synuclein expression within the DA neurons of this transgenic strain was performed using both semi-quantitative RT-PCR [13] and immunolocalization [5]. Previously these same animals have been used to validate the neuroprotective capacity of specific gene products, such as torsinA, a protein with chaperone activity, and several Rab GTPases, which are involved in ER to Golgi transport [5,8,12].

To examine the effect of acetaminophen on α-synuclein-induced neurodegeneration, approximately 20 gravid adults (P0 generation) were placed on NGM plates with either acetaminophen or solvent (water). When the F1 larvae reached the L4 stage, ~500–1000 worms were then transferred onto 5–10 NGM plates, prepared with or without acetaminophen and 5-fluoro-2-deoxyuridine (FUDR; Fisher Scientific) at a concentration of 0.04 mg/mL media to prevent the development of offspring. When the F1 animals aged to day 4 of adulthood, between 50 and 100 F1 worms from each acetaminophen concentration were assayed for degenerating DA neurons using the criteria described above. Since the six anterior DA neurons were examined from each worm, and the analyses were repeated between two and four times per condition, with 25 worms/replicate, this corresponded to 300–600 neurons analyzed per concentration.

Acetaminophen was not significantly neuroprotective against α-synuclein-induced neurodegeneration at any concentration tested (Fig. 2). When solvent (water) was added to the media, 33.4% of the neurons from 4-day adult worms displayed degenerative changes while 35.5%, 31.7%, 29.7%, and 34.7% of the neurons were degenerating when worms were treated with 0.1 mM, 1 mM, 2 mM, and 9.2 mM acetaminophen, respectively.

Exposure to 6-OHDA causes formation of reactive oxygen species (ROS) and subsequently leads to DA neuron death via an undefined apoptotic mechanism. Worm DA neurons undergo a
Fig. 2. Acetaminophen does not protect C. elegans DA neurons from degeneration resulting from over-expression of α-synuclein at any concentration tested when compared to animals exposed to the solvent (water) only. Approximately 300 neurons from 100 worms were examined at each concentration.

Fig. 3. Low concentrations of acetaminophen protect C. elegans DA neurons from short-term 6-OHDA exposure. The lowest concentration of acetaminophen tested, 0.1 mM, significantly protected DA neurons at all times assayed while 1.0 mM acetaminophen protected DA neurons at the 6 and 24 h time points (P < 0.05). Approximately 300 neurons from 50 worms were scored at each concentration and at each time point.
ever, several of the genes linked to PD are predicted to function in cellular pathways involving the management of protein degra-
dation and oxidative stress [9]. Thus, maintaining a cytological
threshold against harmful environmental influences is central to
disease etiology. Sporadic PD is traditionally thought to be a late-
onset disorder associated with toxic exposures on the background of
generically susceptible individuals. Moreover, the cumulative
effects of aging clearly are an unequivocal risk factor for PD and
may be linked to the eventual failure of naturally protective cellu-
lar systems that erode over time [23]. Further investigation of
the neuroprotective activity of acetaminophen in mammalian systems
is warranted, given the prospect for this common medication to be
used for prophylactic, as well as adjuvant, therapy for neurodegen-
erative diseases resulting from oxidative damage.

Acknowledgements

We wish to acknowledge the cooperative spirit of all Caldwell
lab members. This work was supported by a grant from McNeil
Consumer and Specialty Pharmaceuticals to KAC.

References

[1] L. Avery, H.R. Horvitz, Effects of starvation and neuroactive drugs on feeding in


2028–2033.


Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and

MPTP: contribution to the apoptotic theory in Parkinson’s disease, Prog. Neu-


from cellular stresses to dopaminergic neurons of c. elegans, J. Neurosci.


Xu, K.E. Strathern, F. Liu, S. Cao, K.A. Caldwell, G.A. Caldwell, G. Marsischky,

R.D. Kolodner, J. Lahaer, J.C. Rochet, N.M. Bonini, S. Lindquist, Alpha-synuclein

blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson’s models,


[10] K. Evasion, C. Huang, I. Yamben, D.F. Covey, K. Korfeld, Anticonvulsant medica-


Maragone, K. Gwinn-Hardy, Z. Wszeolek, D. Dickson, J.W. Langston,

Comparison of kindreds with Parkinsonism and alpha-synuclein genomic mul-


G.A. Caldwell, C. elegans blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson’s models,


MPTP: contribution to the apoptotic theory in Parkinson’s disease, Prog. Neu-


drugs have potent anti-fibrillogenic and fibril-stabilizing effects for a-

[15] O. Hornykiewicz, Dopamine (3-hydroxytyramine) and brain function, Pharma-


3–29.


Acetaminophen and aspirin inhibit superoxide anion generation and lipid

peroxidation, and protect against l-methyl-4-phenyl pyridinium-induced

[18] M.C. McKhann, G. Jorgensen, H.R. Horvitz, Genes required for GABA function in


Hulihan, T. Peuralinna, A. Dutra, R. Nussbaum, S. Lincoln, A. Crawley, M. Hanson,


