Published in final edited form as:

Parkinsonism Relat Disord. 2014 January ; 20(0 1): S73–S75. doi:10.1016/S1353-8020(13)70019-1.

Aldehyde dehydrogenase inhibition generates a reactive dopamine metabolite autotoxic to dopamine neurons

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SUMMARY

The neurotransmitter dopamine (DA) is important for numerous biological functions, including control of movement. Oxidation of DA to highly toxic and reactive species has been hypothesized to contribute to the selective neurodegeneration observed in Parkinson's disease (PD). DA catabolism is initiated by oxidative deamination via monoamine oxidase to yield 3,4-dihydroxyphenylacetaldehyde (DOPAL). Such metabolism can be problematic as it greatly increases the toxicity of DA by production of DOPAL, known to be a toxic and reactive intermediate. DOPAL undergoes carbonyl metabolism primarily via aldehyde dehydrogenase (ALDH) enzymes to a less toxic acid product. Previous studies from our laboratory have shown that cellular ALDH enzymes are sensitive towards products of oxidative stress and lipid peroxidation, which are thought to be elevated during PD pathogenesis. Inhibition of ALDH and the resulting accumulation of DOPAL are concerning as DOPAL is toxic to dopaminergic cells, readily modifies proteins and causes protein aggregation. In addition, pesticides with association between exposure and PD incidence can interfere with DA metabolism and trafficking and/or ALDH activity, directly or indirectly, yielding elevation of DOPAL. Therefore, impairment of carbonyl metabolism is a potential mechanistic link between cellular insult and generation of a toxic and reactive intermediate endogenous to dopamine neurons.

Keywords

Aldehyde dehydrogenase; Dopamine; 3,4-Dihydroxyphenylacetaldehyde; Pesticides

1. Introductory remarks

Parkinson's disease is a common neurodegenerative condition involving loss of neurons such as those that produce dopamine (DA). The precise mechanism of PD pathogenesis is not known but thought to involve several key components, including exposure to environmental toxicants (e.g., pesticides) and oxidative stress [1]. Numerous epidemiological studies have provided evidence demonstrating a significant and positive correlation between exposure to various pesticides (e.g., dieldrin, benomyl) and the incidence of PD [2]. Many of these pesticides were shown to produce cellular oxidative stress and to be toxic to dopamine neurons. Of question, then, is how does exposure to such environmental agents, which may distribute widely throughout the human body, initiate events or mechanisms leading to PD, a specific disease? What is the mechanistic link

between pesticide/environmental agent exposure and neurodegenerative disease? Why are DA neurons susceptible targets?

A clue to the puzzle might lie in the fact that DA neurons have the unique property of synthesizing, trafficking, metabolizing and utilizing DA, the latter referring to synaptic transmission. DA is an important neurotransmitter that requires some attention to detail by neurons given the labile catechol group, which can auto-oxidize to a quinone and produce reactive oxygen species [3]. Several studies, both in vitro and in vivo, point to DA as a source of damage especially in the event that trafficking or vesicular storage is disrupted [4,5].

One other problematic feature of DA is its metabolism via monoamine oxidase (MAO), which produces hydrogen peroxide, a reactive oxygen species, plus the reactive and toxic 3,4-dihydroxyphenylacetaldehyde (DOPAL) (Fig. 1) [6]. The latter product, DOPAL, undergoes further biotransformation to the acid product 3,4-dihydroxyphenylacetic acid (DOPAC) by aldehyde dehydrogenase (ALDH) enzymes (Fig. 1). Inhibition of aldehyde oxidation was proposed to occur competitively via acetaldehyde generated from ethanol, and such ALDH impairment was hypothesized to contribute to ethanol addiction by production of DOPAL, a DA metabolite that generates products with unique pharmacological activity [7,8]. Results of subsequent work did not support such a hypothesis but revealed the presence of numerous ALDH enzymes, both low and high K_m sources of cytosolic and mitochondrial DOPAL oxidation [9]. As a minor and perhaps compensatory pathway, reduction of DOPAL to the corresponding alcohol 3,4-dihydroxyphenylethanol (DOPET) is catalyzed by cytosolic aldehyde or aldose reductase (AR) (Fig. 1). Of note, the ALDH enzymes are known to be sensitive to products of oxidative stress and several pesticides [2,10].

Should these carbonyl metabolizing systems be inhibited, the intermediate DOPAL is predicted to elevate to harmful levels and contribute to the loss of DA-containing neurons according to the “catecholaldehyde hypothesis” [11]. The mechanism of toxicity for DOPAL, the MAO metabolite of DA, may include several mechanisms, including: protein modification and aggregation, production of reactive oxygen species, mitochondrial damage and oxidation to a highly reactive quinone [12–15].

2. Inhibition of ALDH by products of oxidative stress

Using several models for DA catabolism, it has been shown that products of oxidative stress at physiologic levels inhibit DOPAL biotransformation by ALDH and AR enzymes. 4-Hydroxy-2-nonenal (4HNE) and malondialdehyde (MDA) represent major products of lipid peroxidation and therefore were used as model compounds for lipid aldehydes generated via oxidative stress [16].

In several model DA metabolism systems, including isolated mitochondria, synaptosomes and dopaminergic cells, the addition of 4HNE at physiologic levels yielded inhibition of ALDH activity towards DOPAL [10,17,18]. Such a finding was also true for synaptosomes and cells treated with MDA [17,18]. There were several key findings to these studies (Fig. 1).

First, 4HNE and MDA at physiologic levels significantly inhibited the complete battery of ALDH enzymes, which was different than the case for acetaldehyde as noted above [17,18]. It appears that 4HNE and MDA target all ALDHs involved with DOPAL oxidation such that no compensatory ALDH activity exists. Exogenous treatment of dopaminergic PC6-3 cells with 4HNE or MDA yielded ALDH inhibition, which is impressive given that the

intracellular levels of these compounds at target sites may be much lower than the measured extracellular concentration.

Second, the AR activity was inhibited by physiologic levels of MDA but not 4HNE [17,18]. Such a result for 4HNE is not a surprise given previous work demonstrating the lipid peroxidation product to be a substrate for AR [19]; however, MDA inhibition of AR was not expected.

Third, MAO was not inhibited by 4HNE or MDA [10,17,18]. The finding indicates that these major lipid peroxidation products target carbonyl metabolism and not MAO, with the net effect being a potential increase in cellular DOPAL levels.

3. Environmental agents and DA metabolism

The organochlorine pesticide dieldrin was found to be elevated in PD patients compared to controls, and previous work has shown dieldrin to affect a number of cellular events important for DA neurons, the net effect being oxidative stress, alterations in the metabolism and trafficking of dopamine, mitochondrial dysfunction and apoptosis [20]. Short treatment (1 h) of differentiated dopaminergic PC6-3 cells with a non-toxic level of the organochlorine pesticide (25 μ M) yielded elevation in reactive oxygen species production [21]. In addition, incubation of these cells with a range of dieldrin concentrations (5–100 μ M; no less than 80% viability as determined via the MTT assay) for 72 h resulted in dose-dependent generation of the ROS- and lipid peroxidation-generated 4HNE (data not shown). Treatment of the PC6-3 cells with dieldrin yielded a dose-dependent increase in the level of DOPAL, and while the organochlorine was shown to increase oxidative stress for these cells, it is not clear if lipid peroxidation products are responsible for DOPAL's elevation [21].

Results of recent work demonstrate thiocarbamate pesticides to be toxic to DA neurons and implicate these pesticides as potential risk factors for PD [22]. The thiocarbamate molinate was found to be an inhibitor of ALDH, and more specifically, molinate and its sulfoxide and sulfone metabolites were found to inactivate human ALDH2 [23,24]. Enzyme inhibition was shown to result from covalent modification of the active site Cys302.

Fitzmaurice et al. recently presented evidence for a positive correlation between exposure to the fungicide benomyl and the risk of PD [2]. Benomyl and/or its bioactivated metabolites are potent inhibitors of ALDH2 that cause selective damage to DA neurons [2, 24]. The fungicide was demonstrated to disrupt DA homeostasis and addition of a MAO inhibitor (pargyline) afforded some protection, implicating DOPAL as a causative agent [2]. Preliminary studies indicate cytotoxicity for the combination of benomyl plus DA for dopaminergic SH-SY5Y cells differentiated with phorbol 12-myristate 13-acetate as measured via the MTT assay (data not shown). Work is in progress to measure DOPAL levels and determine the role of DOPAL for the observed cell death. Such a result implicates DA metabolism, and specifically the intermediate DOPAL, as a mediator of benomyl's toxicity to DA neurons.

4. Discussion and conclusion

Much data have been generated demonstrating the importance of fidelity for DA metabolism and trafficking as such pathways produce reactive intermediates and toxic byproducts. In regard to DA metabolism, carbonyl biotransformation appears to be the weak link as the ALDH and AR enzymes rely on production of cofactors (e.g., NAD) and are sensitive to oxidative stress [6,10,17,18]. The major lipid peroxidation products 4HNE and MDA, with 4HNE known to be elevated in brains of those afflicted with PD [25], target the complete battery of ALDH activity for DOPAL but do not inhibit MAO [6,10,17,18]. In addition, AR,

predicted to compensate in the event of ALDH impairment, is inhibited by MDA [18]. Pesticides, such as dieldrin, molinate and benomyl, may directly or indirectly contribute to impairment of ALDH activity or to other mechanisms responsible for elevation of DOPAL in DA neurons from normal to harmful levels.

While 4HNE and MDA have been used as model for lipid peroxidation products, it is conceivable that other lipid aldehydes produced via oxidative stress may contribute to ALDH inhibition [16]. The highly reactive 4-oxo-2-nonenal and its oxidation product, 4-oxononenoic acid, were found to be potent, irreversible inhibitors of human ALDH2 that covalently modified the enzyme's active site [26].

One other contributing factor to ALDH as a “weak link” in detoxifying reactive aldehydes is that the activity of the enzyme and afforded cytoprotection may be compromised with age. Previous work has suggested an age-dependent slight decrease in ALDH activity towards various aldehydes [27]. In addition, decreased expression of the isoform of ALDH, ALDH1A1, was observed for PD patients [28].

As noted above, AR can compensate in the event of ALDH inhibition, and elevated DOPET was observed for ALDH1A1 and ALDH2 knockout animals, compared to control [11]. However, the lipid peroxidation product MDA not only inhibits ALDH but also AR [18]. In addition, a recent study concluded that AR is inefficient at DOPAL detoxication and compensating for ALDH inhibition [11].

Other environmental agents/contaminants may contribute to ALDH inhibition and elevated DOPAL levels. Recent work has demonstrated a significant association between exposure to the industrial solvent trichloroethylene (TCE) and PD incidence, and an animal model of chronic TCE dosing revealed damage to DA neurons [29]. Interestingly, TCE is metabolized via cytochrome P450 to trichloroacetaldehyde, which is a competitive and reversible ALDH inhibitor.

While previous studies have focused on DOPAL and DA neurons, there is little known about the interaction (e.g., toxicity, activation, metabolism, protection of DA neurons) of DOPAL with non-neuronal cells, such as astrocytes and microglia. Astrocytes contribute to DA metabolism and DOPAL production, and interestingly, mice with elevated astrocyte MAO-B demonstrated PD-like pathology [30]. It is possible the electrophile DOPAL activates microglia, which would yield release of pro-inflammatory mediators.

Therefore, ALDH inhibition may be a mechanistic link between pesticide exposure (i.e., producing oxidative stress or interacting directly with ALDH) and pathologic events related to PD as shown in Fig. 2. Confirmation of such a prediction could yield new and novel means to treat or prevent PD, such as use of carbonyl scavengers to trap reactive species or activation of ALDH activity to more efficiently clear toxic aldehydes such as DOPAL.

Acknowledgments

This work was supported by grants NIH R01 ES15507 (J.A.D.), NIH T32 GM067795 (B.C.V.) and NIH T32 GM008365 (J.H.S.).

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Fig. 1.

Oxidative deamination of DA via MAO yields DOPAL, which undergoes oxidation to an acid (DOPAC) or, as a minor pathway, an alcohol (DOPET), by ALDH and AR, respectively. The lipid peroxidation products 4HNE and MDA do not target MAO but potently inhibit carbonyl metabolism.

Fig. 2.
Pesticides and oxidative stress yield elevated DOPAL and adverse effects related to PD.