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Mini-review

Toxic neuropathies: Mechanistic insights based on a chemical perspective

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HIGHLIGHTS

- We reviewed the neurotoxicity and toxicodynamics of γ-diketone neuropathy.
- Axonal swellings and degeneration are epiphenomena related to dose-rate.
- Axonal atrophy was shown to be the primary HD-induced neuropathological feature.
- HD causes atrophy by forming adducts with lysine residues on cytoskeletal proteins.
- This mechanism is based on γ-diketone chemistry and using a quantitative approach.

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ABSTRACT

2,5-Hexanedione (HD) and acrylamide (ACR) are considered to be prototypical among chemical toxicants that cause central–peripheral axonopathies characterized by distal axon swelling and degeneration. Because the demise of distal regions was assumed to be causally related to the onset of neurotoxicity, substantial effort was devoted to deciphering the respective mechanisms. Continued research, however, revealed that expression of the presumed hallmark morphological features was dependent upon the daily rate of toxicant exposure. Indeed, many studies reported that the corresponding axonopathic changes were late developing effects that occurred independent of behavioral and/or functional neurotoxicity. This suggested that the toxic axonopathy classification might be based on epiphenomena related to dose-rate. Therefore, the goal of this mini-review is to discuss how quantitative morphometric analyses and the establishment of dose-dependent relationships helped distinguish primary, mechanistically relevant toxicant effects from non-specific consequences. Perhaps more importantly, we will discuss how knowledge of neurotoxicant chemical nature can guide molecular-level research toward a better, more rational understanding of mechanism. Our discussion will focus on HD, the neurotoxic γ-diketone metabolite of the industrial solvents n-hexane and methyl-n-butyl ketone. Early investigations suggested that HD caused giant neurofilamentous axonal swellings and eventual degeneration in CNS and PNS. However, as our review will point out, this interpretation underwent several iterations as the understanding of γ-diketone chemistry improved and more quantitative experimental approaches were implemented. The chemical concepts and design strategies discussed in this mini-review are broadly applicable to the mechanistic studies of other chemicals (e.g., n-propyl bromine, methyl methacrylate) that cause toxic neuropathies.

Abbreviations: CNS, central nervous system; PNS, peripheral nervous system; HD, 2,5-hexanedione; ACR, acrylamide; NF, neurofilament; NFM, medium molecular weight neurofilament; NFH, heavy molecular weight neurofilament; KSP, lysine-serine-proline motif; P_1, low-speed pellet; P_2, high speed pellet; S_1, low-speed supernatant; S_2, high-speed supernatant; MAP, microtubule associated proteins; HSAB, hard and soft, acids and bases; BSA, bovine serum albumin.

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Contents

1. Introduction

Subchronic exposure to a variety of chemical pollutants in the atmosphere, diet, drinking water and occupational setting (Table 1) can result in nerve damage that has been traditionally classified as a central–peripheral distal axonopathy [1,2]. Based on early morphological studies, the primary neuropathological manifestation of this neuropathy appeared to be retrograde myelinated axon degeneration in the peripheral (PNS) and central (CNS) nervous systems. Although a causal relationship has not been established, this "dying back" degeneration presumably mediated the characteristic toxicant-induced neurological deficits in humans and experimental animals; e.g., uncoordinated gait, skeletal muscle weakness and foot drop. Axon degeneration was often preceded by multifocal paranodal giant axonal swellings and, depending upon the toxicant, these swellings contained accumulations of cytoskeletal components (e.g., neurofilaments), fragments of the smooth endoplasmic reticulum and degenerating mitochondria [1]. Among the compounds listed in Table 1, acrylamide (ACR) and 2,5-hexanedione (HD) are considered to be prototypical toxicants that produce distal axonopathy. ACR is a water-soluble α,β-unsaturated carbonyl derivative of the type-2 alkene chemical class and has extensive manufacturing applications; e.g., paper, textile and fabric industries [2]. HD is the common neurotoxic γ-diketone metabolite of n-hexane and methyl-n-butyl ketone. Both parent compounds are used in fabric manufacturing and have been associated with several human outbreaks of neuropathy following subchronic occupational exposure [1].

Early studies of HD and ACR neurotoxicity were based on the premise that distal axon regions were sites of toxicant action and that axonopathy was the pathognomonic outcome of a specific mechanism; e.g., inhibition of axolemmal Na pumps [3,4]. Because axonal swellings and degeneration were assumed to be causally related to the onset of neurotoxicity, substantial effort was devoted to deciphering the respective mechanisms [5,6]. However, ensuing research indicated that the expression of these traditional hallmark features was dependent upon the rate of toxicant exposure. For example, ACR caused axon degeneration at low subchronic exposure rates (10–20 mg/kg/d), whereas higher daily dose-rates (50 mg/kg/d) produced neurotoxicity in the absence of degeneration [7,8]. This observation lead to quantitative morphometric analyses which showed that nerve terminals in the PNS and CNS were primary sites of ACR action [2,6]. However, the molecular mechanism of presynaptic toxicity remained elusive until ACR, a α,β-unsaturated carbonyl compound, was recognized to be a soft (polarizable) electrophile (electron deficient species). Moreover, the principles of hard and soft, acids and bases theory (see ahead) suggested that ACR would preferentially form adducts with soft nucleophiles (polarizable, electron rich species), which in biological systems are anionic sulfhydryl thiolate (RS^-) groups on protein cysteine residues. Indeed, subsequent neurochemical, enzymatic and proteomic studies provided corroborative evidence that ACR caused presynaptic toxicity by inhibiting the function of key nerve terminal proteins (e.g., N-ethylmaleimide sensitive factor) through targeting of regulatory cysteine thiolate sites [2,6].

Defining molecular mechanisms is a critical step toward reducing the black box nature of toxic neuropathies. However, as the preceding synopsis of ACR research illustrates, this level of understanding requires identifying primary neurotoxicologically relevant effects and, in particular, a detailed knowledge of the corresponding toxicant chemistry. Therefore, the goal of this review is to discuss how a similar approach involving determination of dose-rate specificity, quantitative morphometric analyses and application of chemical principles was used to clarify the neuropathological character and molecular mechanism of γ-diketone neuropathy. As will be evident, ACR and HD are significantly different chemicals with correspondingly different mechanisms of action. This highlights the fact that the chemical concepts and design strategies discussed in this review can be applied to studies of the diverse chemicals that cause toxic neuropathies (Table 1).

Table 1
For each compound, respective orbital energies (E_{LUMO}, E_{HOMO}) were obtained from ground state equilibrium geometries with DF B3LYP-6-31G* in vacuum from 6-31G* initial geometries and were used to calculate softness (σ) and the electrophilic index (ω) as described in LoPachin et al. [35].

Chemical	Softness (σ, $\times 10^{-3}$ eV^{-1})	Electrophilicity (ω, eV)
Carbon disulfide	359	3.97
Methyl acrylate	315	3.22
Acrylamide	315	2.62
2,5-Hexanedione	310	2.09
n-Propyl bromide	270	1.93
Vinyl chloride	277	1.71

2. γ-Diketone neuropathy – morphometric analyses

Giant multifocal swellings of large myelinated axons in the CNS and PNS have been historically considered the hallmark feature of γ-diketone neuropathy [1]. Accordingly, research conducted over the past 35 years has been directed toward discerning the molecular mechanism of these swellings. Since the swellings contained neurofilament (NF) masses, it was proposed that they resulted from direct HD modification of NF proteins. Covalent NF–NF crosslinks were thought to be formed by autooxidation of the pyrrole rings that result from reaction of ε-amino groups on NF lysine residues with γ-diketones like HD [9]. Theoretically, nascent NF subunits would undergo chemical modification as they progressed along the axon and eventually the resulting cross-linked NFs would accumulate at narrow distal nodes of Ranvier where their anterograde transport is impeded. The subsequent neurofilamentous swellings would initiate axon degeneration and characteristic neurological deficits [4,10]. Other studies however identified axon atrophy as a significant feature of γ-diketone neuropathy [e.g., see 11,12]. To resolve this apparent conflict, we conducted a series of quantitative morphometric studies to characterize the spatiotemporal expression of axonal swelling, atrophy and degeneration in conventionally fixed central [13,14] and peripheral [15,16] nerves of HD-intoxicated rats. Results showed that swollen axons were an exclusive but infrequent product of long-term (307–98 days) HD intoxication at lower daily dose-rates (100–175 mg/kg/d; respectively; Fig. 1). Higher dose-rates (400 mg/kg/d) produced