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Beyond Muscle Relaxation: The Multi-Target Neuroprotective Mechanism of Orphenadrine in Excitotoxic Brain Injury

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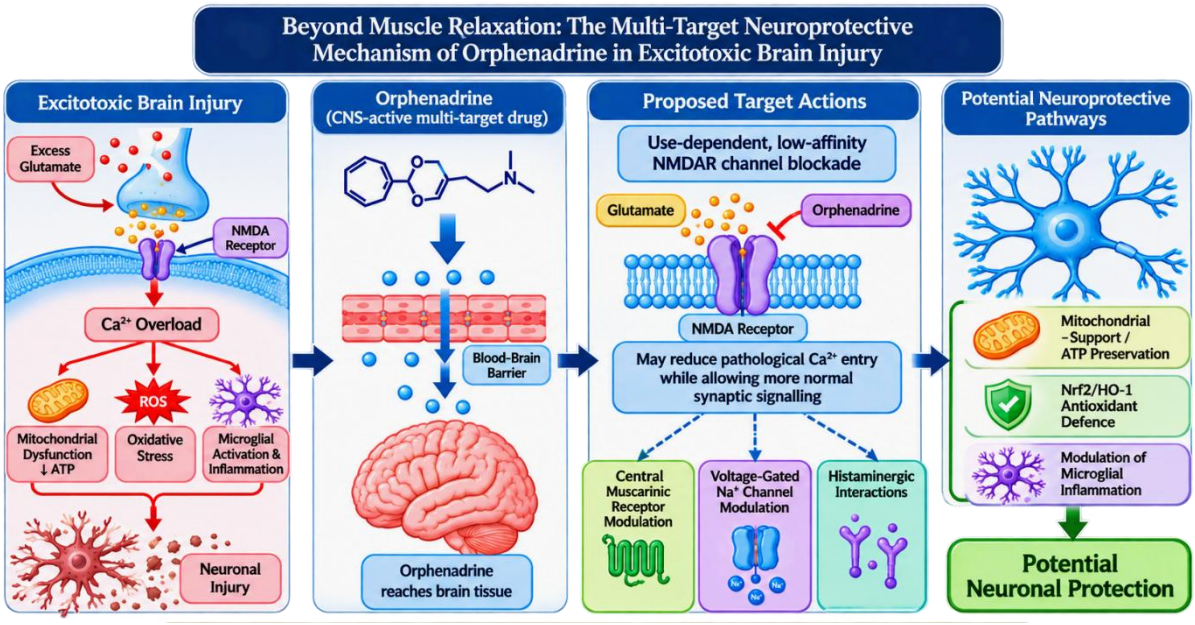
Abstract

Excitotoxicity is one of the major pathogenic processes involved in several acute and chronic neurological disorders such as ischemic brain injury, traumatic brain injury, epilepsy and neurodegenerative diseases. Excessive activation of glutamate receptors, particularly N-methyl-D-aspartate receptors (NMDARs), causes increased calcium entry, oxidative stress, mitochondrial dysfunction and activation of inflammatory pathways which ultimately lead to injury or death of neurons. NMDAR antagonism is an attractive strategy to reduce excitotoxic neuronal damage. However, several high-affinity NMDAR antagonists have had negative consequences, as substantial inhibition of physiological glutamatergic neurotransmission may interfere with normal neuronal communication. Orphenadrine is a centrally acting anticholinergic, used for many years as a skeletal muscle relaxant and adjunct analgesic. It also has a pharmacological profile, including antagonistic activity at several neuronal targets including NMDA receptors, in addition to its musculoskeletal activity. Its use-dependent, relatively low affinity for binding to the NMDA receptor channel provides a molecular basis to investigate whether it can relieve pathological excitotoxic signaling while maintaining some normal neurotransmission. The proposed multi-target neuroprotective paradigm involves further effects on oxidative stress, mitochondrial function, neuroinflammation and intracellular stress responses. But these pathways are best considered as preclinical or mechanistic theories rather than established clinical indications for neuroprotection. The present study describes the pharmacological basis, possible neuroprotective mechanisms, translational opportunities and limits of orphenadrine in excitotoxic brain injury.

Keywords: Glutamate, polypharmacology, neuroprotection, excitotoxicity, NMDA receptor, oxidative stress, neuroinflammation, mitochondria, neurodegeneration.



Graphical Abstract

Highlights

1. Orphenadrine exhibits a multi-target pharmacological profile beyond skeletal muscle relaxation.
2. Its low-affinity, use-dependent NMDA receptor blockade may help reduce excitotoxic neuronal damage.
3. Orphenadrine may modulate oxidative stress, mitochondrial dysfunction and neuroinflammatory pathways.
4. Its central nervous system activity and blood–brain barrier penetration support further investigation of its neuroprotective potential.
5. Preclinical validation and clinical studies are required to establish its neuroprotective efficacy and safety.

1. Introduction

Excitotoxicity is damage to neurons caused by prolonged or excessive activation of excitatory neurotransmitter systems, especially glutamatergic signalling. Glutamate is the predominant excitatory neurotransmitter in the mammalian central nervous system and is critical for many physiological processes, including memory, learning, synaptic plasticity and neuronal communication. However, under pathological conditions, high levels of extracellular glutamate may cause prolonged activation of ionotropic glutamate receptors, particularly NMDA receptors [1]. This causes an excess of calcium and sodium to enter neurons, and a cascade is triggered that includes calcium-dependent enzymes, mitochondrial dysfunction, generation of reactive oxygen species, membrane damage, and activation of cell-death pathways. Because NMDA receptors are highly permeable to calcium, overactivation of these receptors is a major mediator of excitotoxic neuronal injury [2]. Excess calcium activates proteases, phospholipases, nitric oxide synthase and other intracellular enzymes. Mitochondria at the same time are overloaded with calcium, which impairs oxidative phosphorylation and increases the generation of reactive oxygen species. Oxidative stress can harm lipids, proteins and nucleic acids[3] .These processes are inter-related and demonstrate why excitotoxicity should be conceptualized as a complex network of excitatory neurotransmission, calcium homeostasis, mitochondrial function, oxidative stress and inflammation rather than a single receptor event. Excitotoxic neuronal damage may be aggravated by inflammatory signalling and microglial activation. In the CNS, injured neurons release endogenous danger signals that activate microglia and other immune components [4]. Activated microglia can generate reactive species and inflammatory mediators which produce a local milieu conducive to further neuronal dysfunction. Therefore, instead of completely blocking a single receptor, a good neuroprotective strategy would ideally have to regulate multiple pathogenic pathways [5].

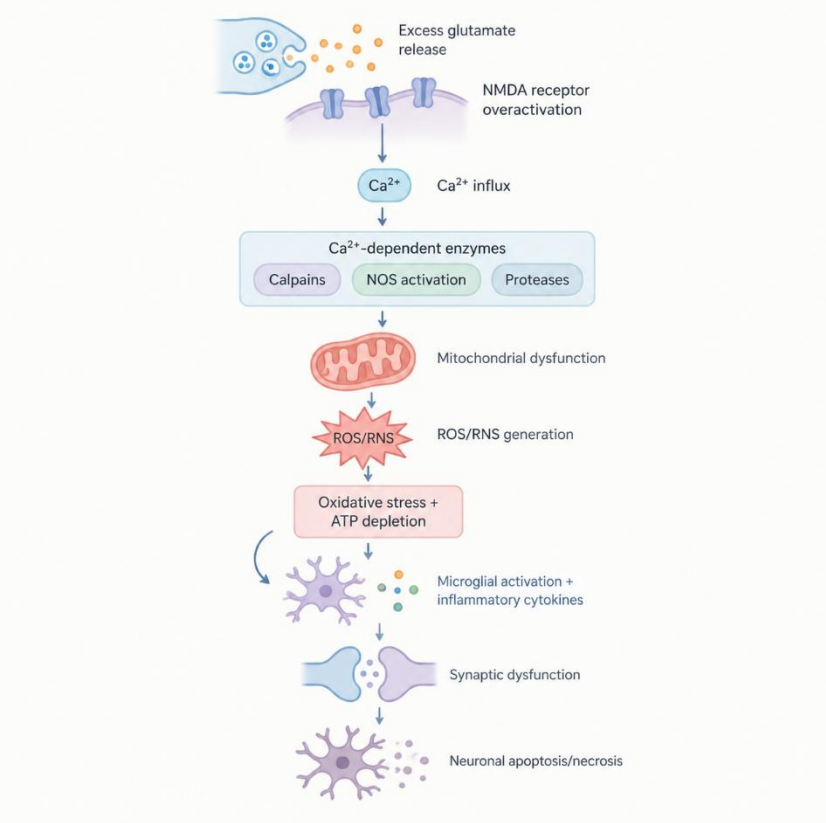


Figure 1: Excitotoxicity cascade

Table 1. Pathophysiological Mechanisms of Excitotoxic Brain Injury. [6-8]

Mechanism	Major molecular event	Consequence
Glutamate excess	Excessive extracellular glutamate accumulation and NMDAR overactivation	Excessive Na^+ and Ca^{2+} influx
Ca^{2+} dysregulation	Intracellular Ca^{2+} overload activates calpains, phospholipases, proteases, and nitric oxide synthase (NOS)	Cytoskeletal damage, membrane injury, and cellular dysfunction
Mitochondrial dysfunction	Ca^{2+} accumulation within mitochondria, impaired electron transport, and reduced oxidative phosphorylation	ATP depletion and cellular energy failure
Oxidative stress	Excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)	Lipid peroxidation and oxidative damage to proteins and DNA
Neuroinflammation	Microglial activation and stimulation of pro-inflammatory signalling pathways	Release of inflammatory mediators and secondary neuronal injury
Synaptic dysfunction	Altered glutamatergic and other neurotransmitter signalling	Impaired neuronal communication and synaptic homeostasis
Endoplasmic reticulum stress	Disruption of protein folding and activation of the unfolded protein response	Cellular stress and activation of apoptotic pathways
Cell death	Activation of apoptotic, necrotic, and other regulated cell-death pathways	Progressive neuronal loss

1.2 Clinical Limitations of High-Affinity NMDAR Blockade

N-methyl-D-aspartate receptors (NMDARs), a class of ionotropic glutamate receptors, play important roles in excitatory neurotransmission, learning, memory, synaptic plasticity and neuronal survival. However, over-activation of NMDARs can lead to glutamate-mediated excitotoxicity, resulting in excessive calcium (Ca2+) influx, oxidative stress, mitochondrial dysfunction, protease activation and eventually neuronal damage or death [9].Therefore, NMDAR antagonism has been investigated as a therapeutic target for a variety of neurological disorders including Parkinson's disease, epilepsy, ischemic brain injury, traumatic brain injury, and other neurodegenerative diseases. However, potent NMDAR antagonists, particularly high-affinity, channel-blocking antagonists, have not translated to the clinic [10]. A major limitation is that NMDARs are not just pathogenic receptors. In normal environments, their activation is required for normal synaptic transmission and neuronal plasticity. Thus, both high and normal NMDAR signalling can be blocked by high affinity antagonists. This widespread blockage may impair learning, memory formation, cognition and adaptive neural responses. Thus, the therapeutic challenge is to reduce excessive receptor activity without abolishing physiological glutamatergic neurotransmission [11] .This difference between pathogenic and normal NMDAR activity is especially relevant for long-term treatment of chronic neurodegenerative diseases. Another important limitation is the emergence of side effects on the central nervous system. Strong NMDAR antagonists such as phencyclidine and other channel blockers have been associated with changes in perception, cognition, behaviour, and motor function. Dose-dependent neurological or psychiatric effects of clinical NMDAR antagonists, even those that may be beneficial [12]. For example, ketamine has dissociative and perceptual effects. Memantine is relatively better tolerated due to its pharmacology, but still may cause dizziness, disorientation or other CNS related side effects in some patients. Such effects may limit the safe dose and therefore narrow the therapeutic window [13].

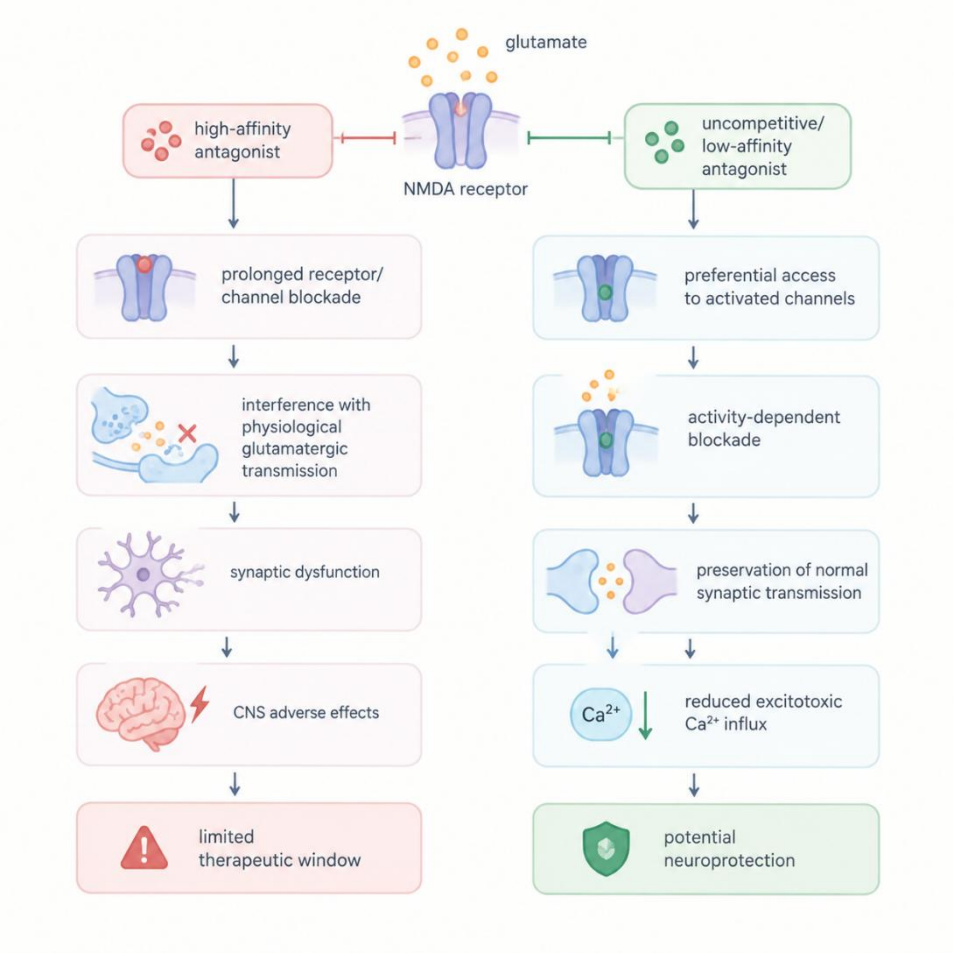


Figure 2. High- versus low-affinity NMDAR channel blockade

1.3 Uncompetitive, Low-Affinity Channel Antagonists as Potential Neuroprotective Agents

N-methyl-D-aspartate receptors (NMDARs) are key glutamate-gated ion channels mediating excitatory neurotransmission, synaptic plasticity, learning and memory. Excessive and sustained activation of NMDARs can result in excitotoxicity characterized by excessive calcium influx, mitochondrial dysfunction, generation of reactive oxygen species (ROS), oxidative stress and activation of neuronal death pathways. NMDAR activity plays physiological and pathological roles, so complete inhibition may have unintended consequences [14]. A number of uncompetitive, low-affinity NMDAR channel antagonists have been the focus of considerable interest as potential neuroprotective drugs because they can selectively inhibit excessive receptor activation while permitting a greater degree of normal neurotransmission. Pharmacological properties of conventional competitive antagonists and uncompetitive antagonists differ [15]. Non-competitive NMDAR antagonists act on the ion channel associated with the receptor after it has been activated. Conversely, competitive antagonists usually bind or compete at the recognition site of the neurotransmitter. These receptor channels are more likely to be blocked if they are activated regularly or consistently, making their activity use-dependent or activity-dependent [16]. This feature is of great potential advantage for neuroprotection, because it allows the inhibition of abnormal glutamatergic activity without completely inhibiting normally functioning NMDARs. Very important are the low affinity and fast dissociation properties of these antagonists. When the pathogenic stimulation decreases, a low affinity antagonist can unbind rapidly and does not stay abnormally long in receptor coupling [17]. This enables normal synaptic signalling to proceed more successfully than high affinity channel blockers. This pharmacological behaviour may expand the therapeutic window and reduce the adverse effects caused by excessive inhibition of NMDAR. Thus, the aim is to reduce excessive receptor-mediated calcium entry in pathological conditions without impairing normal neuronal transmission, rather than to block the receptor per se [18].

1.4 Historical Context and Re-evaluation of Orphenadrine

Orphenadrine is a synthetic, centrally acting drug derived from the diphenhydramine chemical class. It is mainly used in the clinical setting for the treatment of severe musculoskeletal disorders and muscular spasms. Pharmacologically it possesses strong antimuscarinic, antihistaminic and centrally acting actions [19]. Thus, its historical clinical use has been largely associated with skeletal muscle relaxation and symptomatic therapy of musculoskeletal problems, rather than with the direct protection of neurons. Such a conventional classification has led to the relative lack of investigation of orphenadrine as a possible treatment for neurodegenerative diseases. The pharmacological history of orphenadrine is very interesting because it does more than relax skeletal muscles [20]. Orphenadrine may act on neuronal targets such as ion channels and muscarinic cholinergic receptors. It has also been shown experimentally that orphenadrine may also influence excitatory neurotransmission. These other pharmacological properties have prompted researchers to reconsider the drug's action on the central nervous system for potential applications beyond its known clinical indications [21]. The multi-target profile of orphenadrine provides a basis for investigating its potential effects on neuronal excitability, synaptic transmission and pathways associated with neuronal damage rather than being considered purely as a centrally acting muscle relaxant. Orphenadrine's history also illustrates a key concept in today's pharmacology: drug repurposing. Medications developed for a single therapeutic indication may have other molecular activities that were not fully understood when the medications were first developed [22]. Receptor pharmacology, molecular docking, electrophysiology, neurochemistry and computational drug discovery have enabled the evaluation of previously characterized medications for biological effects beyond their classical therapeutic classification. Orphenadrine is an interesting candidate in this respect, as it has demonstrated CNS activity and interaction with multiple neuronal targets, suggesting a rationale for investigating potential neuroprotective mechanisms [23].

2. Multi-Target Polypharmacology Profile & Chemical Structure

2.1. Structural Architecture: Diphenhydramine Derivatives and BBB Permeation

Orphenadrine is closely related to diphenhydramine in that it belongs to this chemical family and contains the distinctive diaryl ether and aminoalcohol architecture. Orphenadrine can be considered as a substituted derivative, whose physicochemical and pharmacological properties are determined to a large extent by the structural constituents of the aromatic and tertiary amino alkyl [24]. The molecule contains a tertiary amine, ether linkage, aromatic rings and a relatively lipophilic carbon skeleton. Passive diffusion through the BBB is generally favoured by drugs with sufficient lipid solubility and a suitable ratio of ionized and non-ionized species, hence the significance of the association of basic amines with lipophilic aromatic moieties for CNS penetration. At physiological pH, the tertiary amine of orphenadrine is partially protonated and partially uncharged [25]. The protonated fraction is important for ionic and receptor interactions, but the non-ionized fraction partitions more readily into lipid membranes and facilitates BBB penetration. The balance between ionization and lipophilicity is one of the main factors affecting CNS exposure. Thus, the structural characteristics of orphenadrine are responsible for the explanation why the drug has significant central pharmacological effects not only in peripheral tissues[26].

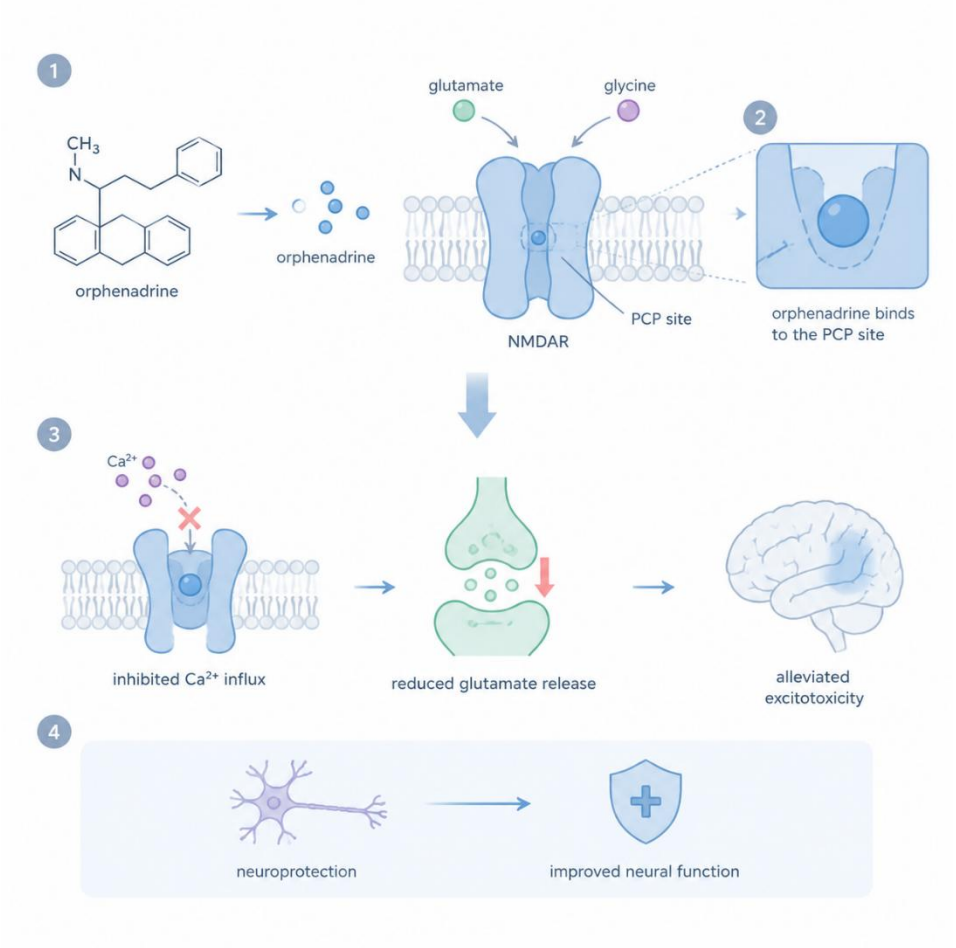


Figure 3. Orphenadrine chemical structure and CNS/BBB penetration.

Table 2. Structural and Pharmacological Characteristics of Orphenadrine. [27-29]

Feature	Description	Potential relevance
Chemical class	Centrally acting ethanolamine-related compound structurally related to diphenhydramine	Contributes to central pharmacological activity
Aromatic groups	Contains aromatic rings within its diaryl ether framework	Increases lipophilic character and facilitates hydrophobic interactions with biological targets
Tertiary amine	Ionizable basic nitrogen that exists in protonated and non-protonated forms depending on pH	Important for receptor and ion-channel interactions and contributes to physicochemical behaviour
Ether linkage	Ether group connecting the aromatic-containing portions of the molecule	Influences molecular conformation, polarity, and target-binding interactions
Lipophilicity	Relatively lipophilic molecular character	Promotes partitioning into biological membranes
BBB permeability	Physicochemical properties are compatible with CNS exposure	Allows access to neuronal and CNS-associated molecular targets
CNS penetration	Orphenadrine produces centrally mediated pharmacological effects	Provides a basis for investigating CNS mechanisms and potential neuroprotective activity
Structural relationship to diphenhydramine	Shares a diaryl ether/ethanolamine-related scaffold with diphenhydramine	Helps explain similarities in central pharmacological properties

Multi-target pharmacology	Can interact with several receptor and ion-channel systems	Provides a rationale for investigating polypharmacology neuroprotection
Neuronal target accessibility	CNS exposure permits interaction with neuronal molecular systems	Relevant to investigation of NMDA receptor, muscarinic, histaminergic, and ion-channel mechanisms.

2.2 Kinetics of NMDA Receptor Blockade

Glutamate-gated ion channels called N-methyl-D-aspartate receptors (NMDARs) are essential for excitatory neurotransmission and synaptic plasticity. Through prolonged Ca2+ influx, mitochondrial malfunction, oxidative stress, and activation of cell-death pathways, their excessive activation can result in excitotoxic neuronal injury. An NMDAR channel blocker's pharmacological impact is dependent on the kinetics of receptor attachment and dissociation in addition to its binding affinity. The degree to which normal vs pathological NMDAR signalling is suppressed depends on factors like the rate of channel entry, resident time within the channel, voltage dependency, frequency of receptor activation, and recovery from blockade [30]. Channel-blocking antagonists often interact with the open ion channel pore, in contrast to competitive antagonists that stop glutamate or glycine from activating the receptor. As a result, receptor activation is necessary for their access to the binding site. This results in a blockage that is either activity-dependent or use-dependent. More antagonist molecules may enter and occupy the open channels when NMDAR channels are stimulated frequently or continuously. There are fewer channels that can be blocked when receptor activity is low. Because excessive glutamatergic activity linked to diseased situations may be suppressed more potently than normal, transitory synaptic signalling, this feature is especially appealing for neuroprotection. While the dissociation rate indicates how quickly an antagonist exits the channel, the association rate indicates how quickly an antagonist enters and blocks an activated NMDAR. Together, these factors affect how long receptor inhibition lasts. Long-lasting inhibition of NMDAR-mediated signalling can result from high-affinity antagonists with slow dissociation staying inside the channel. Strong blocking of this kind can successfully lessen excitotoxicity, but it can also disrupt normal NMDAR functions. On the other hand, as pathogenic stimulation diminishes, substances with more quick dissociation can allow receptor function to recover[31].

2.3 Central Muscarinic and Histaminergic Receptor Interactions

The pharmacological profile of orphenadrine includes important interactions with central muscarinic cholinergic receptors in addition to its effects on excitatory neurotransmission. Many of the physiological effects of acetylcholine in the central and peripheral nervous systems are mediated by muscarinic receptors, which are G-protein-coupled receptors. There are five main subtypes, M1–M5, with M2 and M4 primarily related to Gi/o proteins and M1, M3, and M5 often coupled to Gq/11 proteins[32]. These receptors are involved in learning, memory, motor control, synaptic transmission, neuronal excitability, and attention within the central nervous system. Thus, an essential part of the primary pharmacological profile of orphenadrine is its antimuscarinic effect. Antimuscarinic activity is particularly relevant given the historical use of orphenadrine as a centrally acting skeletal muscle relaxant. Orphenadrine may alter neuronal activity by reducing muscarinic cholinergic signalling in brain areas that are involved in motor control [33]. In Parkinsonian diseases the balance between cholinergic and dopaminergic neurotransmission is of particular importance. Dopamine depletion in the basal ganglia has been associated with an imbalance between dopaminergic and cholinergic signalling, and modulation of cholinergic activity has been explored as a possible treatment for some parkinsonian symptoms. Thus, orphenadrine’s effects on motor circuits may be mediated through its anticholinergic properties, but this anticholinergic activity should be distinguished from a demonstrated neuroprotective or disease-modifying effect. Furthermore, central muscarinic receptors modulate glutamatergic neurotransmission and neuronal excitability [34]. The interaction between cholinergic and glutamatergic signalling pathways can modulate the release of glutamate and the response of neurons to acetylcholine. Thus, the modulation of muscarinic receptors may have an indirect effect on excitatory neuronal activity. This interaction can be important in excitotoxicity, as excess glutamatergic activation can lead to

increased intracellular Ca2+ and promote oxidative and mitochondrial stress. The combination antimuscarinic and excitatory-neurotransmission actions of orphenadrine can be viewed in this context as part of a broader multi-target framework. Antimuscarinic activity is of particular interest due to the historical use of orphenadrine as a centrally acting skeletal muscle relaxant[35] .Orphenadrine may modify neuronal activity by decreasing muscarinic cholinergic signalling in brain regions implicated in motor control. The balance between cholinergic and dopaminergic neurotransmission is of particular importance when dealing with Parkinsonian diseases. Dopamine loss in the basal ganglia has been linked to an imbalance between dopaminergic and cholinergic signalling and modulation of cholinergic activity has been studied as a potential therapy for some parkinsonian symptoms. Thus, the effects of orphenadrine on motor circuits may be mediated by its anticholinergic effects, but this anticholinergic activity should be distinguished from a demonstrated neuroprotective or disease-modifying effect. In addition, glutamatergic neurotransmission and neuronal excitability are modulated by central muscarinic receptors. Cholinergic and glutamatergic signalling pathways may modulate the release of glutamate and the neuronal response to acetylcholine. Thus, the activity of excitatory neurons can be indirectly affected via muscarinic receptor modulation. This interaction can be important in excitotoxicity because excessive glutamatergic activation can lead to increased intracellular Ca2+ and promote oxidative and mitochondrial stress. The multireceptor model is a useful way to think of the combination of orphenadrine's antimuscarinic and excitatory neurotransmission effects[36].

2.4 Modulation of Voltage-Gated Sodium Channels

Voltage-gated sodium channels (VGSCs) are essential membrane proteins responsible for generating and propagating action potentials in neurons. These channels open quickly when the membrane depolarizes, and sodium ions (Na+) flood into the cell, creating the rising phase of the action potential. VGSCs rapidly inactivate after opening and recovering to a closed state. And this tightly regulated cycle allows for effective neuronal communication[37]. In contrast, excessive neuronal firing or aberrant sodium-channel activity can result in disordered neural signalling, increased metabolic demand and neuronal hyperexcitability. Thus, modulation of VGSCs is a major component in the pharmacology of a number of CNS-active drugs. Besides its muscarinic, histaminergic and NMDAR-related actions, Orphenadrine has been shown to have interaction with voltage-gated sodium channels. Neuronal excitability is largely dependent on sodium channel availability and activity. Orphenadrine’s ability to modulate sodium channel activity is particularly relevant to its known effects on the central nervous system. A sodium-channel modulator may stabilize neuronal membranes and reduce recurrent firing of neurons by attenuating excessive sodium-channel-mediated depolarization[38]. This mechanism may contribute to orphenadrine overall pharmacological effects on the central nervous system. The regulation of sodium channels is commonly described as state-dependent or use-dependent block. VGSCs have three functional states: resting, activated/open and inactivated. Some channel-blocking agents interact with activated or inactivated channel states preferentially and not with all channel states equally. During repetitive firing of the neurons, sodium channels cycle through these states over and over again, increasing the chance that a use-dependent blocker would interact with them. Thus, regular, low-frequency activity might not have such a detrimental impact on neurons as highly active ones. This drug property may be useful in conditions where a reduction of abnormal neuronal hyperexcitability is desired without affecting normal neural activity [39].

Table 3. Proposed Multi-Target Pharmacological Profile of Orphenadrine. [40-41]

Target/Pathway	Proposed Interaction	Potential Consequence
NMDA receptor/channel	Uncompetitive, use-dependent channel blockade	↓ Excessive Ca^{2+} influx and excitotoxic signalling
Muscarinic receptors	Central antimuscarinic/anticholinergic interaction	Modulation of central cholinergic signalling
Histaminergic system	Interaction with histamine receptor systems	Modulation of CNS neurotransmission
Voltage-gated Na^+ channels	Modulation of voltage-gated sodium-channel activity	↓ Neuronal hyperexcitability and abnormal electrical activity
Mitochondrial pathways	Potential preservation of mitochondrial function during cellular stress	↓ ATP depletion and metabolic injury
Nrf2/HO-1 antioxidant pathway	Proposed modulation of endogenous antioxidant defence	↓ ROS-mediated oxidative stress
Microglial pathways	Potential modulation of microglial activation	↓ Neuroinflammatory signalling

3. Mitigating Primary Excitotoxicity: The NMDA Interface

3.1 Voltage-Dependent Fast Off-Rate Kinetics and Synaptic Homeostasis

N-methyl-D-aspartate receptors (NMDARs) are important glutamate-gated ion channels mediating excitatory neurotransmission, synaptic plasticity, learning, memory and neural adaptability. Under physiological conditions NMDAR activation is tightly controlled and mediates normal synaptic transmission. However, prolonged or excessive activation can result in excitotoxicity, primarily via an excessive influx of Ca2+. Elevated intracellular Ca2+ can activate calcium-dependent enzymes, mitochondrial dysfunction, increased reactive oxygen species (ROS) and pathways leading to neuronal dysfunction and cell death [42]. Inhibition of NMDAR activity is a key strategy to prevent excitotoxic neuronal death. Voltage-dependent blockade is a key pharmacologic property of certain NMDAR channel blockers. NMDARs contain an ion channel that allows cations (e.g., Na+ and Ca2+) to flow into neurons. When the channel is open, molecules that block channels can enter the pore of the receptor. The strength of their interaction could depend on the membrane potential. Changes in the membrane voltage alter the electrochemical environment inside the channel and thus influence the binding and mobility of charged blocker molecules. This voltage dependence allows various channel antagonists to modulate NMDAR activity in an activity-dependent manner [43]. The concept of a fast off rate is important when dissociating persistent high affinity antagonists from potentially tolerated NMDAR channel blockers. The speed with which a drug leaves its receptor or channel binding site is called the off rate. A fast-off-rate antagonist can block an active NMDAR, but as receptor activity decreases the antagonist dissociates fairly quickly. This way the receptor can return to its normal activity faster than if you use a blocker that remains bound for a long time. Such reversible control may provide a better balance between preventing pathological excitation and preserving normal NMDAR signalling. Rapid dissociation, voltage dependence and use dependence can all be combined to produce a pharmacological profile that targets highly active populations of receptors. Severe glutamatergic stimulation may cause NMDAR channels to remain open or open repeatedly, increasing the possibility of channel blocking. However, in normal synaptic transmission, receptor activation is often transient and under temporal control. Thus, the effect of a rapidly reversible blocker on physiological signalling may be more short-lived. This notion represents an important basis for the development of NMDAR-targeted neuroprotective strategies [44].

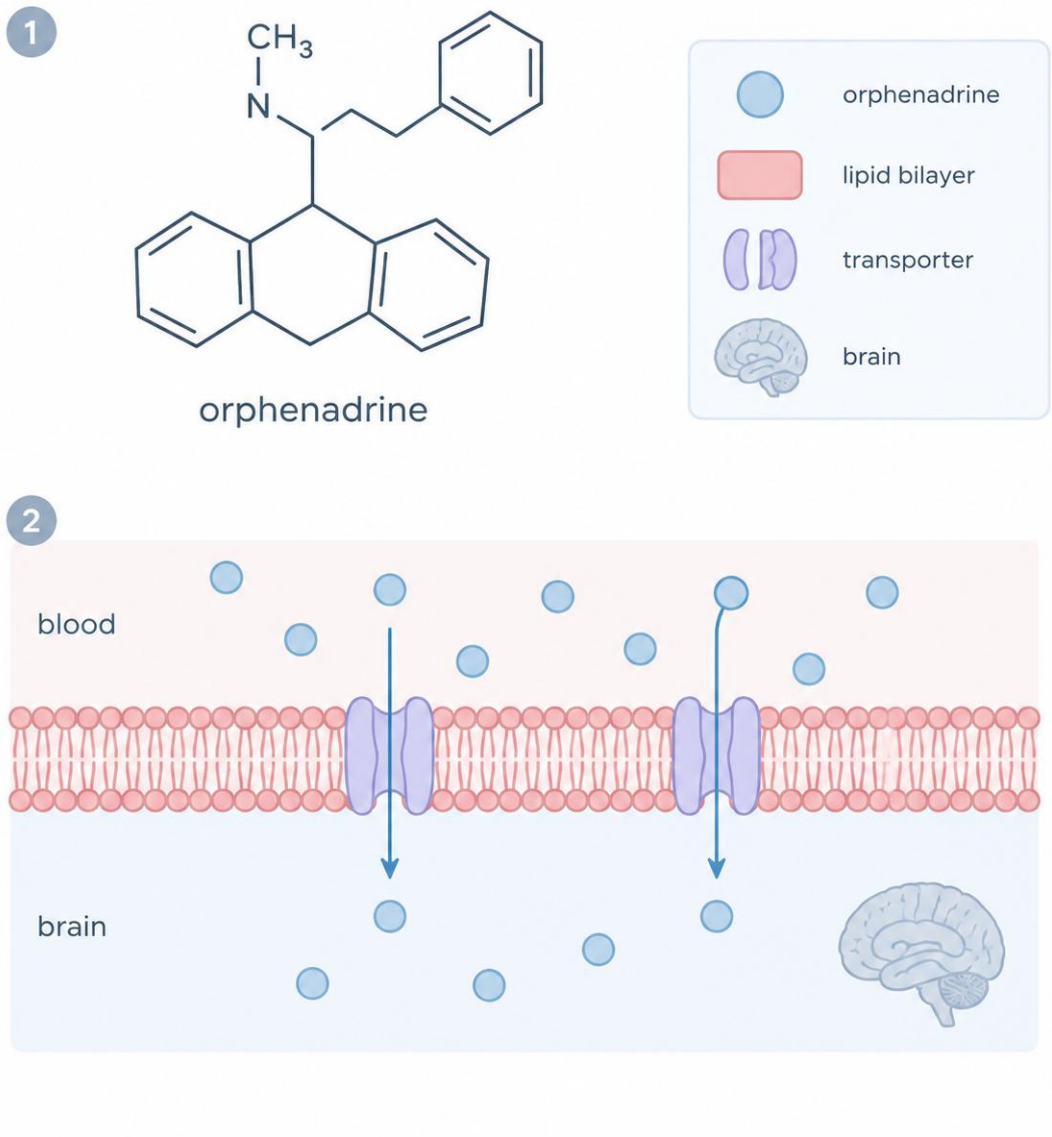


Figure 4. Proposed orphenadrine interaction with the NMDAR channel/PCP-associated site.

3.2 In Vitro Protective Efficacy in Cerebellar Granule Cells

The cerebellar granule neuron (CGN) is a widely employed in vitro neuronal model to study mechanisms of excitotoxicity and neuronal survival. These cells express functional glutamatergic receptors including N-methyl-D-aspartate receptors (NMDARs) and their viability is highly dependent on intracellular Ca2+ homeostasis, glutamate signalling, extracellular potassium concentration and mitochondrial activity [45]. This paradigm has been particularly useful in studies directed to compounds that modulate excitotoxic signalling, and for the evaluation of possible mechanisms of neuroprotection, as excessive glutamatergic stimulation can lead to neuronal injury in CGN cells. In experimental settings, excitotoxic damage of cerebellar granule cells can be induced by changing the ionic composition of the environment surrounding the neurons or by overstimulation of glutamatergic pathways. Excessive NMDAR activation leads to increased Ca2+ influx which results in calcium-dependent enzyme activation, mitochondrial dysfunction, oxidative stress and activation of cell-death pathways. Furthermore, neuronal damage can be mediated by necrotic and apoptotic pathways, depending on the experimental conditions [46]. Thus, the CGN model provides a regulated cellular system to study the effects of pharmaceutical drugs on the cascade of events linking excessive excitatory stimulation to neuronal death. To assess the protective effects of a compound that modulates NMDAR several complementary endpoints can be used. Cell viability tests (e.g. MTT, resazurin or other metabolic assays) can provide an estimate of survival of the cells after an excitotoxic stimulus. Neuronal shrinkage, neurite loss, membrane damage and other features associated with cellular injury can also be revealed by morphological

evaluation. Measuring intracellular Ca2+, mitochondrial membrane potential, ROS production, and apoptotic markers may provide mechanistic details of how a test chemical modifies excitotoxic neuronal damage [47].

Table 4. Experimental evidence for the potential neuroprotective effects of orphenadrine. [48-49]

Model	Experimental insult	Tissue/cell system	Orphenadrine intervention/dose	Major outcome	Mechanistic endpoint	Evidence level	Reference
NMDAR-mediated excitotoxicity	NMDA (100 μM)	Dissociated rat cerebellar granule cells	10^{-8}–10^{-4} M; IC_{50} 11.6 ± 4.7 μM for mitochondrial membrane-potential protection and 13.5 ± 2.3 μM for cytoplasmic membrane-potential protection	Prevented NMDA-induced decreases in mitochondrial and cytoplasmic membrane potentials; protected CGCs from glutamate-induced neurotoxicity	Mitochondrial membrane potential; cytoplasmic membrane potential; glutamate-induced neuronal injury	Experimental	Sureda et al., 1999, *Neuropharmacology* 38:671–677, DOI: 10.1016/S0028-3908(98)00228-7
Kainic-acid excitotoxicity	Kainic acid, 10 mg/kg	Rat hippocampus	10 mg/kg, pretreatment	Reduced kainic-acid-induced increase in PBR density and prevented induction of HSP72	PBR density as an indirect marker of microglial activation; HSP72 expression as a cellular stress/astroglial response	Experimental	Sureda et al., 1999, *Neuropharmacology* 38:671–677, DOI: 10.1016/S0028-3908(98)00228-7
3-Nitropropionic acid (3-	3-NP, 100 μM	Primary rat cerebella	Orphenadrine pretreatme	Increased cell survival	Neutral-red viability;	Experimental	Pubill et al., 2001, *British Journal of*

NP) neurotoxicity		r granule cells	nt; maximal protection at 6 μM; concentrations >24 μM lost protection and ≥48 μM potentiated toxicity	and reduced 3-NP-induced neuronal morphological injury	propidium-iodide staining; neuronal morphology		*Pharmacology* 132:693–702, DOI: 10.1038/sj.bjp.0703869
3-NP-induced striatal neurotoxicity	3-NP, 30 mg/kg/day for 3 days	Rat brain, predominantly striatum	30 mg/kg/day for 3 days; 10 and 20 mg/kg/day were not protective	Reduced 3-NP-associated mortality; prevented increases in PBR binding and HSP27 expression in the striatum	[³H]-PK11195 binding; HSP27 expression; mortality/body-weight changes	Experimental in vivo	Pubill et al., 2001, *British Journal of Pharmacology* 132:693–702, DOI: 10.1038/sj.bjp.0703869
Focal cerebral ischemia/reperfusion	90-min middle cerebral artery occlusion followed by reperfusion	Male rats	10 or 30 mg/kg, i.p., beginning 1 h after reperfusion and continued once daily for 21 days	Improved neurological function, beam-walk performance and locomotor activity; reduced infarct volume; improved biochemical and histological	MDA, nitrite, glutathione, catalase, IL-6, TNF-α, BBB integrity, HIF-1α, TFAM, caspase-3 and Nrf2/HO-1	Experimental in vivo; recent independent evidence	Balhara et al., 2026, *Toxicology and Applied Pharmacology* 510:117757, DOI: 10.1016/j.taap.2026.117757

				measures of injury			

3.3 Suppression of 3-Nitropropionic Acid-Induced Metabolic Toxicity

3-Nitropropionic acid (3-NP) is an experimental neurotoxic and naturally occurring hazardous substance that is well known to study the mechanisms of excitotoxicity, oxidative stress, brain damage and mitochondrial dysfunction. The neurotoxic effects are intimately associated with the inhibition of succinate dehydrogenase (SDH; complex II of the mitochondrial electron transport chain). Inhibition of this enzyme leads to disruption of tricarboxylic acid cycle and mitochondrial respiration, which results in decreased generation of ATP and impairment of cellular energy consumption [50]. Mitochondrial metabolic disorders can lead to rapid neuronal dysfunction and promote neuronal degeneration because neurons have a high and constant energy demand. 3-NP inhibits succinate dehydrogenase causing poor electron transport and disruption of oxidative phosphorylation creating a metabolic state. A reduction in the availability of ATP compromises energy-dependent functions such as the maintenance of ionic gradients, the membrane potential, recycling of neurotransmitters and intracellular calcium control. Failure of these homeostatic processes can result in neuronal depolarization and an increased susceptibility to excitotoxic damage [51]. Thus 3-NP toxicity provides an experimental paradigm in which neuronal damage results from the interplay of excitotoxic events and metabolic dysfunction. 3-NP-induced mitochondrial dysfunction is also associated with the generation of reactive oxygen species (ROS). Impairment of electron transport can result in electron leakage and oxidative stress, leading to an increase in the production of superoxide and other reactive species. Besides impairing respiratory function, excess ROS can damage proteins, lipids, nucleic acids and mitochondrial membranes. This leads to a positive feedback loop whereby mitochondrial dysfunction increases oxidative stress, and oxidative damage further impairs mitochondrial function[52]. Therefore, antioxidant and mitochondrial-stabilizing systems are important parts of the cellular response to 3-NP-induced toxicity. Another aspect of 3-NP-induced neuronal damage is glutamatergic excitotoxicity. Mitochondrial dysfunction reduces the neurons' capacity to buffer intracellular Ca2+, but energy failure may disrupt ion homeostasis and enhance neuronal excitability. Thus, increased glutamatergic signalling and NMDAR activation may result in excessive Ca2+ influx. The increased intracellular Ca2+ stimulates oxidative and apoptotic processes, increases mitochondrial stress and activates calcium-dependent enzymes. 3-NP is a useful model to study interconnected pathways of neurodegeneration due to the interplay of mitochondrial energy failure, calcium dysregulation and excitatory neurotransmission[53].

3.4 Kainic Acid-Induced Striatal and Hippocampal Injury

Kainic acid (KA) is a potent glutamatergic neurotoxin widely used in experimental models of oxidative stress, inflammation, excitotoxic neuronal damage and neurodegeneration associated with seizures. Kainic acid acts as an agonist on kainite-type ionotropic glutamate receptors and indirectly enhances glutamatergic neurotransmission by stimulating neural networks. Excessive stimulation of glutamate receptors can lead to sustained neuronal depolarization, elevated intracellular calcium levels, generation of reactive oxygen species (ROS), impaired mitochondrial function, and initiation of pathways leading to cell death[54]. Since excitotoxic neurodegeneration includes mechanisms that may be important for excessive neuronal excitation and protection of vulnerable neuronal populations, KA-induced damage provides a useful experimental model for the investigation of compounds that could modulate these mechanisms. The hippocampus is particularly susceptible to excitotoxicity induced by kainic acid. The excessive activation of the glutamatergic system following KA exposure can lead to abnormal neuronal firing and a prolonged calcium influx, particularly in the hippocampal circuits. Increased intracellular calcium activates calcium-dependent enzymes such as proteases, phospholipases and endonucleases that can injure mitochondria, DNA, cytoskeletal proteins and cell membranes[55]. Mitochondrial impairment leads to a vicious circle of energy failure and oxidative stress leading to further reduction of ATP synthesis and increase of ROS formation. Histologically, KA exposure can lead to neuronal loss and degeneration in vulnerable areas of the hippocampus. This paradigm is useful for studying processes of excitotoxic neuronal death. In addition to the hippocampal lesion, kainic acid can induce

pathological changes in the striatum through secondary excitotoxic mechanisms and increased glutamatergic activity. The striatum receives abundant glutamatergic input from cortical and other brain regions, and it is important to understand the relationship between excitatory neurotransmission, dopaminergic signalling, mitochondrial dysfunction and neuronal survival. Overactive glutamate signalling not only increases calcium-dependent oxidative and metabolic stress but also disrupts the physiological balance of excitatory/inhibitory neurotransmission. Although KA-induced injury should be regarded as a mechanistic model of excitotoxicity rather than an exact model of Parkinson's disease pathology, these pathways are relevant to neurodegenerative diseases in which striatal neuronal activity is compromised[56].

4.1 Mitochondrial Preservation

Neurons are highly and continuously dependent on ATP for maintenance of membrane potential, neurotransmission, axonal transport and intracellular ion homeostasis. Thus, mitochondrial dysfunction is an important pathogenic event in neurodegenerative diseases and neuronal injury. Mitochondria normally generate ATP by oxidative phosphorylation; they also act as calcium buffers, maintain redox balance and relay apoptotic signals. But intracellular calcium and metabolic demand from excessive neural stimulation can profoundly disrupt mitochondrial function [57]. Chronic calcium accumulation can cause damage to the electron transport chain, reduce ATP synthesis, increase reactive oxygen species (ROS), and promote depolarization of the mitochondrial membrane. Thus, maintaining mitochondrial function is a key secondary consideration for neuroprotective therapies after an initial excitotoxic insult. Mitochondrial dysfunction and excitotoxicity are mechanistically intimately linked. These Ca2+ transients can result from over-activation of glutamatergic receptors and are buffered by mitochondria. Hypertrophy-induced excess calcium deposition may impair mitochondrial membrane potential and facilitate mitochondrial ROS generation. Moderate calcium intake may improve metabolic flexibility. Excess ROS can also damage mitochondrial DNA, membrane lipids and proteins, impairing oxidative phosphorylation [58]. This results in a self-perpetuating cycle in which excitotoxic stimulation leads to mitochondrial dysfunction, which in turn further impairs the ability of neurons to maintain ionic and metabolic homeostasis. Thus, one of the main targets of neuroprotective pharmacology is to break this vicious circle. Mitochondria also play a direct role in neuronal cell-death signalling. Severe injury to the mitochondrion can result in the release of pro-apoptotic factors such as cytochrome c into the cytoplasm. Downstream, activation of caspase-dependent apoptotic pathways by cytochrome c may lead to neuronal death. Thus, mitochondrial permeability transition, loss of membrane potential, ATP depletion and severe oxidative stress may determine the outcome of reversible excitotoxic insult to irreversible neuronal injury. After the initial excitatory onslaught, agents that can maintain mitochondrial membrane integrity, reduce excessive calcium-related mitochondrial stress, and limit ROS generation may then exert a secondary protective effect [59]. Mitochondrial dysfunction is particularly relevant to Parkinson's disease, because the susceptibility of dopaminergic neurons is related to defects in mitochondrial bioenergetics, oxidative stress, and cellular quality control mechanisms. The dopaminergic neurons in the substantia nigra are energy demanding and have extensive axonal arborization, therefore, adequate mitochondrial function is important for neuronal survival. Impairment of mitochondrial complexes may result in reduced ATP synthesis and increased oxidative stress. In addition, mitochondrial dysfunction can interact with alpha-synuclein aggregation, impaired protein clearance, neuroinflammation and altered calcium handling. Therefore, mitochondrial preservation is gaining more and more consideration as a therapeutic target in addition to controlling glutamatergic excitotoxicity and dopamine metabolism [60].

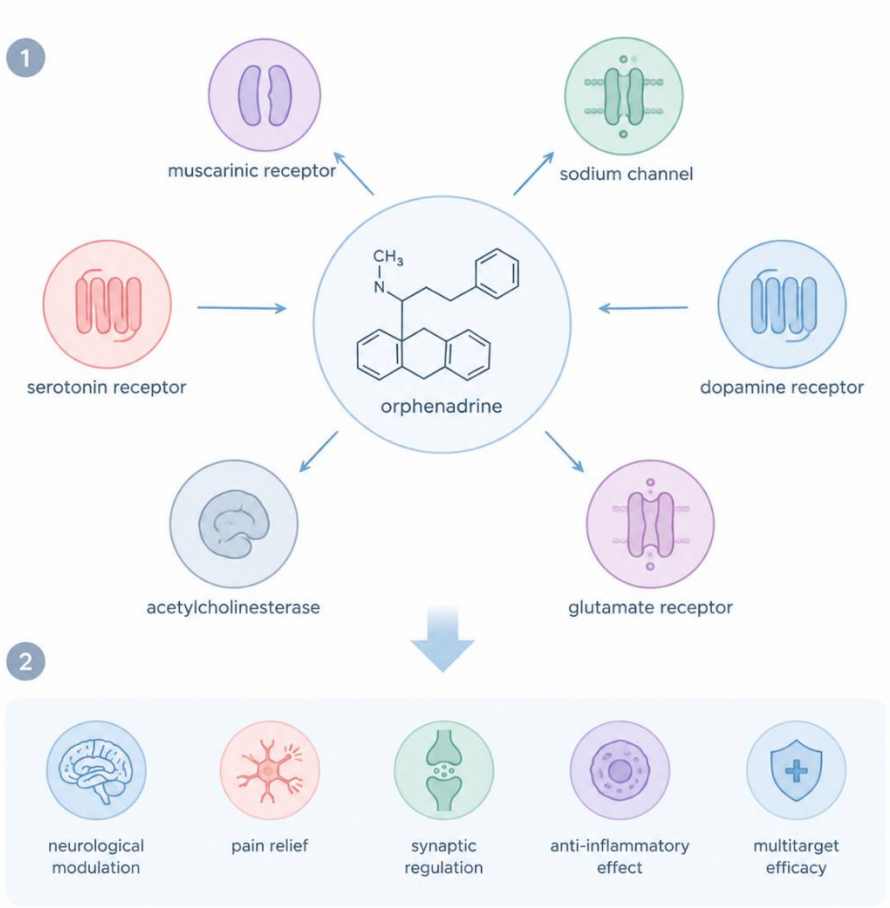


Figure 5. Multi-target polypharmacology network of orphenadrine.

4.2 Antioxidant Systems: Nrf2/HO-1 Pathway

Oxidative stress is an important secondary mechanism in neurodegenerative diseases and in neuronal damage. Neurons have a high metabolic activity, a high oxygen consumption, a high content of polyunsaturated fatty acids and a very low potential for regeneration and are therefore very vulnerable to oxidative injury. An overproduction of reactive oxygen species (ROS) and reactive nitrogen species can damage cellular lipids, proteins, DNA, and mitochondria. Under normal physiological conditions, these reactive species are regulated by endogenous antioxidant mechanisms. However, ROS formation can exceed the cell antioxidant capacity during ecotoxicity, mitochondrial failure, neuroinflammation and neurotoxic chemicals such as aluminum compounds. Activation of the pathways that define the endogenous antioxidant is an integral part of secondary neuroprotection[61]. The nuclear factor erythroid 2-related factor 2 (Nrf2) pathway is a key endogenous system for maintaining cellular redox homeostasis. Under basal conditions, Nrf2 is negatively regulated by Kelch-like ECH-associated protein 1 (Keap1), which promotes Nrf2 ubiquitination and proteasomal degradation. Oxidative or electrophilic stress modifies key cysteine residues in Keap1, resulting in decreased degradation of Nrf2 and allowing Nrf2 to accumulate and translocate to the nucleus. In the nucleus, Nrf2 binds to antioxidant response elements (AREs) in the promoter regions of a number of cytoprotective genes. This transcriptional response enhances the ability of cells to remove reactive species and maintain intracellular redox homeostasis [62]. Heme oxygenase-1 (HO-1) is a cytoprotective inducible enzyme, encoded by the HMOX1 gene, and a major downstream effector of the Nrf2 signalling pathway. Nrf2-mediated transcription is induced by oxidative stress and HO-1 expression is up-regulated. HO-1 breaks down heme to biliverdin, CO, and ferrous iron Then the biliverdin is converted into bilirubin. Nevertheless, HO-1 overactivation or prolonged activation can have context-dependent effects. Iron released in heme catabolism can perform oxidative chemistry if not properly sequestered. These products are involved in cellular stress responses and antioxidant defence. Therefore, the Nrf2/HO-1 signalling is not an inexhaustible antioxidant mechanism but a regulated cytoprotective response. Besides HO-1, the Nrf2 pathway controls many more antioxidants. The

activation of Nrf2 can lead to the induction of an up-regulation of enzymes and proteins involved in glutathione biosynthesis, detoxification, ROS metabolism and cellular redox regulation [63]. The glutathione-dependent detoxification systems include glutamate-cysteine ligase, glutathione-related enzymes and NAD(P)H quinone oxidoreductase 1 (NQO1). Neurons also rely on glutathione, which helps them clear hydrogen peroxide and other oxidizing chemicals. This is especially important for neurons in terms of availability of glutathione. Thus, Nrf2 signalling activation may lead to a coordinated antioxidant defence rather than a single ROS scavenging enzyme. The Nrf2/HO-1 pathway is closely associated with mitochondrial protection. Overproduction of ROS leads to damages to mitochondrial DNA, lipids and proteins thus disturbing oxidative phosphorylation and decreasing production of ATP. Dysfunctional mitochondria, in contrast, may produce excess ROS and set off a vicious cycle of metabolic and oxidative stress. Nrf2 can induce antioxidant and detoxifying pathways that may counteract this oxidative stress and restore mitochondrial homeostasis. So, Nrf2 signalling is a critical second line of defence after excitotoxic or metabolic injury [64].

4.3 Anti-Inflammatory Effects and Microglial Activation

Parkinson's disease is one of many neurodegenerative diseases that are worsened by neuroinflammation, a major secondary component of neuronal injury. Inflammation is initially a protective response to remove the damaged cells and restore tissue homeostasis. However, excessive or persistent activation of inflammatory pathways can be detrimental to neurons. Microglia, the main immune cells of the central nervous system, are sensitive sensors of neuronal damage. Microglia can undergo morphologic and functional changes and release inflammatory mediators following exposure to excitotoxic, oxidative, metabolic or chemical stress. Chronic microglial activation may therefore create a pathogenic milieu in which oxidative stress, inflammatory signalling, mitochondrial dysfunction, and neuronal degeneration are self-perpetuating [65]. Microglia rapidly respond to changes in the neural environment. Under normal conditions these cells constantly survey the surrounding tissue and support neuronal homeostasis, the removal of debris and the maintenance of synapses. Microglial responses to brain injury can be initiated by extracellular ATP, damaged cellular contents, altered neurotransmitter signalling and other danger-associated chemical signals. Activated microglia can also release reactive oxygen and nitrogen species, as well as cytokines and chemokines, including tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). These mediators may however help in tissue healing, but their long-term synthesis may trigger neuronal oxidative stress and increase cellular damage [66]. Excitotoxicity and microglial activation have a reciprocal interaction. In addition to releasing intracellular chemicals that activate microglia, excessive glutamatergic stimulation can damage neurons. Activated microglia can then emit reactive species and inflammatory mediators that make neurons more susceptible to further excitotoxic stimulation. This produces a positive feedback loop whereby inflammation further weakens neuronal resilience to excitotoxic and oxidative stress, while neuronal excitement increases inflammation. Therefore, rather than being a stand-alone anti-inflammatory impact, reduction of excessive microglial activation is a crucial part of a larger neuroprotective strategy. One of the main transcriptional regulators involved in inflammatory responses is nuclear factor kappa B (NF-κB)[67]. NF-κB activation and its translocation to the nucleus, where it controls the expression of many inflammatory genes, can be facilitated by the activation of inflammatory receptors and intracellular signalling pathways. TNF-α, IL-1β, IL-6, inducible nitric oxide synthase (iNOS), and other mediators linked to neuroinflammation can be produced as a result of increased NF-κB signalling. Proteins, mitochondria, and neuronal membranes can all be further harmed by excessive nitric oxide and reactive oxygen species. Therefore, it may be possible to lessen the amplification of neuroinflammatory injury by modulating NF-κB-associated inflammatory signalling[68].

4.4 Intracellular Stress Proteins

An essential endogenous defence mechanism that aids neurons in withstanding harsh environmental and metabolic circumstances is made up of intracellular stress proteins. Physiological stressors related to high metabolic activity, oxidative phosphorylation, neurotransmission, calcium flow, and protein turnover are constantly present in neural cells. Cellular proteins may experience misfolding, oxidation, aggregation, or

other types of structural damage under pathological situations such excitotoxicity, oxidative stress, mitochondrial dysfunction, inflammation, and exposure to neurotoxic chemicals. In order to restore protein homeostasis, stabilize cellular structures, aid in repair, and enhance survival, stress-responsive proteins are thereby stimulated or activated[69]. After an initial neuronal insult, these pathways play a significant role in the later neuroprotective response. Heat shock proteins (HSPs), including as HSP70, HSP90, HSP27, and associated molecular chaperones, are among the most significant intracellular stress proteins. Heat shock proteins aid in the proper folding of freshly made proteins as well as the removal or refolding of damaged proteins during cellular stress. For instance, HSP70 can interact with misfolded or unfolded proteins to stop them from aggregating improperly. Because the buildup of aberrant proteins can disrupt intracellular transport, mitochondrial activity, and synaptic function, this chaperone activity is especially crucial in neurons. Therefore, improving molecular chaperone systems may help protect neurons from oxidative and excitotoxic damage [70]. Additionally, HSPs are involved in controlling cell-death pathways. Apoptotic signalling can be triggered by oxidative damage and mitochondrial malfunction in situations of extreme cellular stress. Apoptotic pathway components, including as interactions with apoptozole-related proteins and caspase activation, can be disrupted by specific stress proteins. Although its effects are largely dependent on the cellular context and the degree and duration of stress, HSP70 has been studied for its capacity to influence multiple processes involved in apoptosis. Therefore, when cellular damage is still within a possibly reversible range, increased production of stress proteins may be an adaptive response that helps preserve neuronal life [71].

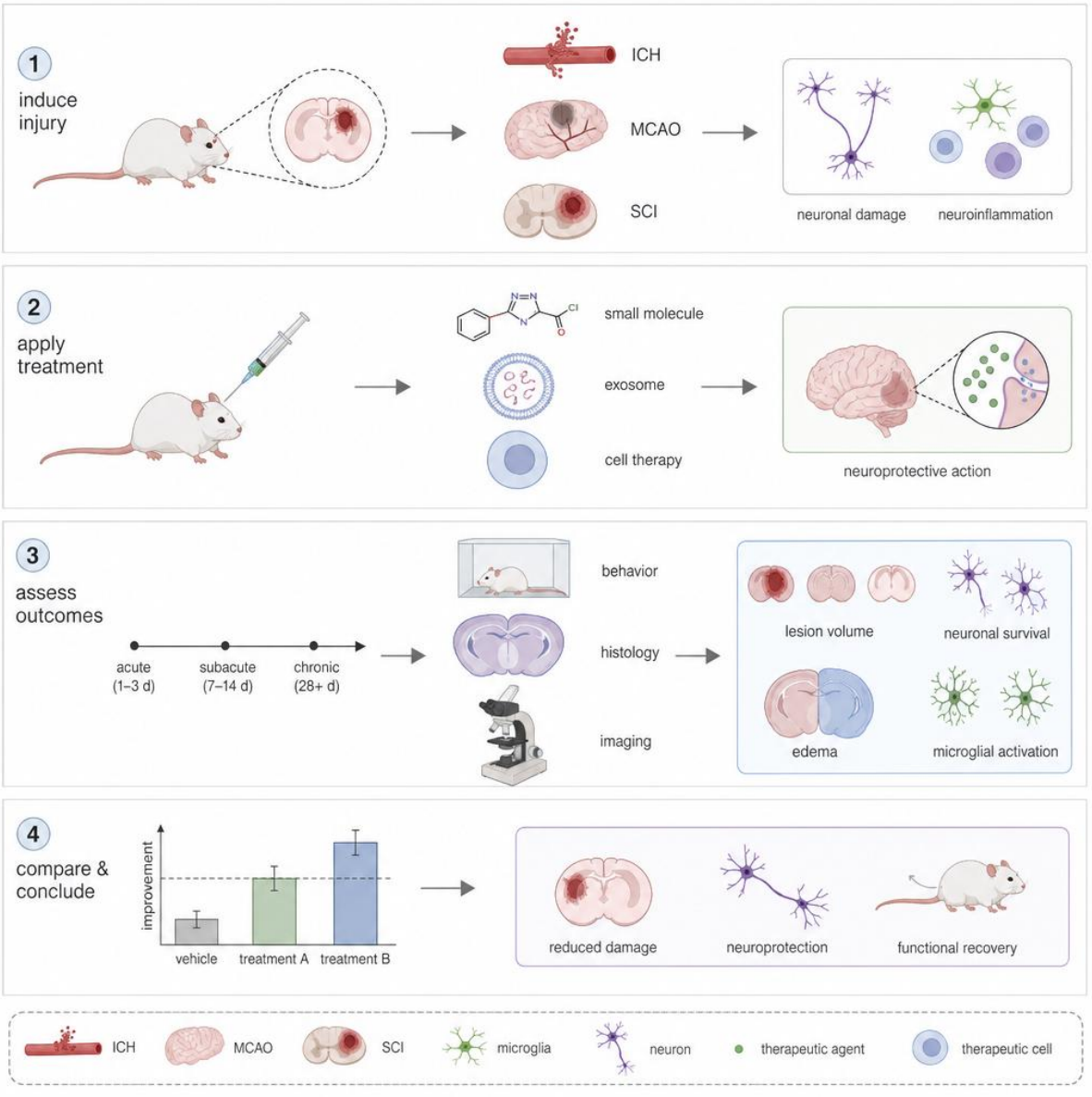


Figure 6. Integrated secondary neuroprotective pathways associated with orphenadrine.

Table 5. Secondary Neuroprotective Pathways Associated with Orphenadrine. [71-72]

Pathway	Marker/Target	Expected Biological Effect
Mitochondrial pathway	TFAM (Transcription Factor A, Mitochondrial)	Maintenance of mitochondrial DNA and mitochondrial biogenesis; potential preservation of cellular energy metabolism
Hypoxia-response pathway	HIF-1α (Hypoxia-Inducible Factor-1α)	Activation of adaptive cellular responses under metabolic or hypoxic stress
Antioxidant pathway	Nrf2/HO-1	Enhancement of endogenous antioxidant defences and reduction of ROS-mediated cellular damage
Neuroinflammatory pathway	Microglial activation	Potential reduction in excessive microglial-mediated inflammatory responses
Cytokine pathway	IL-6	Potential attenuation of pro-inflammatory signalling and secondary neuronal injury
Cytokine pathway	TNF-α	Potential reduction in inflammatory signalling and cytokine-mediated neuronal damage
Cellular stress-response pathway	HSP72	Modulation of cellular stress responses and support of protein homeostasis
Cellular stress-response pathway	HSP27	Potential enhancement of cellular stress tolerance and protection against stress-induced injury

5. Translational Opportunities & The Geriatric Paradox

5.1 Repurposing Strategy, Safety Profiling and Human Tolerability

Investigating an established medication for a novel therapeutic application is known as drug repurposing. Because traditional medication development is linked to significant time, expense, and uncertainty, this method can be very helpful in the study of neurodegenerative diseases. An existing foundation of knowledge about a compound's pharmacology, formulation, pharmacokinetics, side effects, and human exposure can be obtained by repurposing it. Because orphenadrine has a long history of clinical usage as a centrally acting muscle relaxant with antimuscarinic and antihistaminic qualities, repurposing it is mechanistically intriguing. Its well-established pharmacological profile offers a foundation for examining whether other neuronal activities may be relevant to neuroprotection [73]. However, rather than relying just on prior clinical use, evidence for a repurposed indication must be created especially for the new illness context. The availability of pre-existing safety information is a significant benefit of a repurposing strategy. Future neuroprotective research can take into account the known adverse-effect profile of orphenadrine due to its recognized clinical pharmacology. Dry mouth, tachycardia, blurred vision, urinary hesitancy or retention, constipation, dizziness, drowsiness, and, less frequently, CNS effects like confusion, agitation, hallucinations, or tremor are among the side effects that are mainly linked to its anticholinergic activity, according to prescribing information. Additionally, the prescribing advice advises adequate monitoring when prolonged treatment is utilized and points out that safety during continuous long-term therapy has not been established [74]. Since Parkinson's disease is a disease that primarily affects the elderly, who often have several co-morbidities and may be taking many drugs at the same time, the safety profile of orphenadrine is of particular importance when considering its possible use for neurodegenerative disorders. Aging can lead to physiologic changes that affect drug distribution, metabolism, clearance and sensitivities to CNS and anticholinergic effects. So a drug that is well tolerated by younger people may have different clinical effects in older people. Therefore, the repurposing studies should consider age-related changes in pharmacokinetics, polypharmacy, renal and hepatic function, cardiovascular condition and vulnerability to CNS side effects in addition to the pharmacological efficacy of orphenadrine. The geriatric component is especially pertinent since anticholinergic exposure may be problematic in the elderly. The American Geriatrics Society Beers Criteria 2023 is an evidence-based tool to

identify potentially inappropriate medications for patients age 65 years or older based on adverse effects, drug-drug interactions, disease-specific risk, and renal function. Most importantly, however, just because a medication is on the Beers Criteria list does not mean it is always inappropriate, but it does mean that a more thorough, individualized assessment of its potential risks and benefits is warranted [75]. This concept is of importance for any future studies of orphenadrine as a potential neuroprotective or disease-modifying agent. The concept of a geriatric paradox therefore becomes important in the proposed translational strategy. The same pharmacological properties that may contribute to a potential neuroprotective mechanism can coexist with adverse effects that become more clinically significant with aging. For example, central anticholinergic activity may theoretically influence neurotransmitter balance but can also produce unwanted effects such as dry mouth, blurred vision, constipation, urinary retention, dizziness, and cognitive or behavioural disturbances. The objective of repurposing should consequently not be to assume that an established drug is automatically suitable for chronic neurodegenerative treatment, but rather to determine whether a therapeutically meaningful exposure can be achieved with an acceptable safety margin [76].

Table 6. Translational Considerations for the Potential Neuroprotective Repurposing of Orphenadrine. [77-79]

Aspect	Potential Opportunity	Important Consideration
Drug repurposing	Existing pharmacological and clinical experience with orphenadrine may facilitate preclinical evaluation for new indications	Established adverse-effect profile and contraindications must be considered
CNS penetration	Pharmacological CNS activity indicates potential access to brain targets	Quantitative brain exposure, BBB permeability, and CNS concentration require confirmation
Acute neuroprotection	Modulation of excitotoxic signalling may provide a basis for investigating acute neuronal protection	Therapeutic window, dose-response relationship, and duration of treatment require investigation
Aging population	Potential relevance to disorders involving neuronal excitotoxicity and neurodegeneration	Anticholinergic burden and age-related pharmacokinetic/pharmacodynamic changes require careful assessment
Combination therapy	Multi-target activity could provide opportunities for combination with other neuroprotective or antiepileptic approaches	Pharmacodynamic and pharmacokinetic drug–drug interactions must be evaluated
Dosing	Existing human pharmacological data provide a starting point for dose-range investigations	A dose producing experimental neuroprotection may differ from established therapeutic doses
Safety assessment	Previous human exposure provides useful background for designing further studies	New neuroprotective indications require indication-specific safety and tolerability evaluation
Clinical translation	Existing human experience can support development of testable translational hypotheses	Dedicated clinical studies are required to establish efficacy and safety for neuroprotection
Patient selection	Specific neurological conditions characterized by excitotoxicity could be investigated	Disease stage, comorbidities, concomitant medications, and individual susceptibility may influence outcomes
Biomarker development	Molecular markers such as oxidative stress, inflammatory cytokines, and mitochondrial	Biomarkers must be validated and correlated with meaningful functional outcomes

	markers may help assess pharmacological effects	

5.2 Acute Neuroprotection Versus Long-Term Anticholinergic Burden

An important translational question regarding the balance between acute neuroprotective activity and the effect of prolonged anticholinergic exposure is raised by the possible neuroprotective application of orphenadrine. While many experimental models assess drug effects over relatively short exposure periods, neurodegenerative disorders like Parkinson's disease are chronic conditions that may require long-term pharmacological management. A substance may show positive effects against oxidative stress, acute excitotoxicity, or neuronal damage without necessarily being appropriate for long-term use. Orphenadrine's potential protective mechanisms and the safety consequences of prolonged exposure should therefore be taken into account when evaluating its therapeutic relevance [80]. Several of the pharmacological effects of orphenadrine are attributed to its clinically proven antimuscarinic action. Muscarinic acetylcholine receptors are involved in motor network function, autonomic regulation, memory, and cognition. In the basal ganglia, cholinergic signalling interacts closely with dopaminergic neurotransmission, and alterations in the cholinergic–dopaminergic balance are relevant to Parkinsonian motor circuitry. Consequently, modulation of muscarinic signalling may influence neuronal activity and potentially contribute to the proposed multi-target neuroprotective profile of orphenadrine. However, the same antimuscarinic activity can produce adverse effects, particularly when exposure is prolonged or when patients are vulnerable to anticholinergic effects. Acute neuroprotection and chronic pharmacological exposure should therefore be viewed as distinct therapeutic questions [81]. In an experimental excitotoxicity model, transient modulation of excessive neuronal activity may theoretically reduce calcium overload, mitochondrial dysfunction, ROS generation, and subsequent neuronal death. Such an effect could be beneficial if the drug is administered during a period of acute neuronal stress. However, chronic administration introduces additional considerations because continuous receptor blockade may alter physiological neurotransmission and produce cumulative or persistent adverse effects. Demonstrating protection in an acute experimental model therefore does not automatically establish suitability for long-term neuroprotective therapy. The anticholinergic burden becomes particularly relevant in older adults. Aging is associated with increased susceptibility to adverse drug reactions because of changes in pharmacokinetics, pharmacodynamics, physiological reserve, and the prevalence of multiple comorbidities and medications [82]. Central anticholinergic effects may include cognitive impairment, confusion, drowsiness, dizziness, and other CNS effects, while peripheral effects can include dry mouth, constipation, blurred vision, tachycardia, and urinary retention. The clinical importance of these effects depends on dose, duration, individual susceptibility, concomitant medications, and underlying disease. Therefore, a potential neuroprotective indication would require careful evaluation of whether the desired CNS effect can be achieved without producing unacceptable anticholinergic effects. This problem is compounded by the link between Parkinson's disease and cholinergic signalling. The out-of-balance dopaminergic and cholinergic signalling in basal-ganglia circuits in Parkinsonian disease. Anticholinergic drugs have traditionally been used to treat certain Parkinsonian symptoms such as tremor, but their therapeutic efficacy is limited by side effects and concerns about cognitive tolerance in the elderly. Thus, the antimuscarinic mechanism of orphenadrine provides both a potential pharmacological rationale and a possible obstacle for the development of this agent as a neuroprotective drug [83]. Therapeutic goal, in addition to optimizing anticholinergic activity, would be to determine if neuroprotective effects can be distinguished from excessive muscarinic blockade. So, the concept of a therapeutic window is crucial for the evaluation of orphenadrine. A relevant translational profile would require demonstration of neuroprotective activity at an exposure remaining within a clinically tolerable range. The mechanism might have limited therapeutic relevance if the concentration required for protection against oxidative damage or excitotoxicity is substantially higher than tolerated human exposure. In contrast, if protection could be demonstrated at pharmacologically relevant dosages with no significant additional anticholinergic effects, then the results would strengthen the case for continued research. Exposure-response studies are therefore essential to bridge clinically relevant

pharmacology and experimental neuroprotection. Patients on multiple medications might also be particularly susceptible to the long-term effects of anticholinergic exposure [84]. Polypharmacy can increase the total anticholinergic load when many medications have anticholinergic effects. This may increase the risk of adverse effects and make it harder to understand treatment outcomes. Thus, concurrent antiparkinsonian medications and other centrally active treatments would need to be considered in any future research on orphenadrine for Parkinson's disease. Drug-drug interactions and cumulative CNS effects should be a significant component of the safety assessment, not viewed as secondary factors. It is also important to consider the potential differences between neuroprotection and symptomatic pharmacotherapy. The antimuscarinic effect could alter nerve communication and movement symptoms, but it may not halt the neurodegenerative process. To show a true disease-modifying or neuroprotective effect, one must show preservation of neural structure or function, and slowing of the progression of pathology, rather than just transient behavioural effects [85]. This differentiation can be studied in experimental settings by combining behavioural data with neural markers, oxidative-stress measures, mitochondrial functionality, inflammatory mediators, apoptosis-related proteins, and histological assessment. This distinction provides a useful framework for the analysis of experimental results for the proposed orphenadrine-AlCl3-Danio rerio study. Orphenadrine may enhance locomotor activity through several mechanisms, including direct effects on motor circuitry, reduction in neurotoxicity, or even actual preservation of neuronal function. Thus, behavioural improvement per se cannot be taken as definitive evidence of neuroprotection. A demonstration of parallel improvements in oxidative stress, mitochondrial integrity, neural markers, inflammatory responses and other mechanistic endpoints would provide stronger evidence that the observed behavioural impact is related to cellular protection [86].

5.3 Aging Brain, Delirium and Cognitive Considerations

Neurotransmission, neural connection, cerebral blood flow, synaptic plasticity and physiological reserve change gradually with brain aging and the brain becomes more vulnerable to side effects from centrally acting drugs. These modifications are particularly relevant to the re-purposing of orphenadrine as a potential neuroprotective agent for age-related neurodegenerative diseases. The central antimuscarinic activity may interfere with normal cholinergic neurotransmission, but modulation of neuronal excitability and cholinergic signalling may theoretically contribute to therapeutic effects in specific clinical settings [87]. Therefore, the neuroprotective effects of orphenadrine and its potential influence on cognition and brain function should be considered when it is examined in an aging population. Acetylcholine is a key player in attention, learning, memory, arousal and cortical information processing. Widespread cortical and hippocampal activity is modulated by cholinergic projections, mainly from basal forebrain areas. Changes in cholinergic neurotransmission during normal aging could diminish the capacity of the brain to adapt to additional pharmacologic or pathologic stress. The antimuscarinic drugs block muscarinic acetylcholine receptors, causing additional reduction of cholinergic signalling [88]. The clinical outcomes vary from person to person and depend on drug potency, dosage, CNS penetration, length of treatment, concurrent drugs and baseline cognitive reserve. These considerations are especially pertinent when considering a centrally acting drug for long-term use in the elderly. One of the most important clinical problems related to compromised cholinergic function in susceptible elderly people is delirium. It is a sudden change in the level of consciousness and attention which can develop rapidly and is often associated with an underlying medical condition, hospitalization, metabolic disturbance, infection, surgery or drug exposure [89]. Pharmacological suppression of central cholinergic activity is a known risk factor for delirium, particularly in the presence of other risk factors. Therefore, potential for central anticholinergic effects should be considered when assessing orphenadrine in the elderly. This emphasizes the need for individual assessment of vulnerability and cumulative medication burden but does not imply that all patients on an anticholinergic medication would develop delirium. Furthermore, the aging brain may be more sensitive to the sedative and central nervous system effects of medications with central action. Sleepiness, dizziness, poor attention and altered psychomotor performance may also have effects on other domains than cognition as they may increase the risk of falls and functional impairment [90]. This is even more relevant in the case of Parkinson’s disease

where gait irregularities, postural instability and balance impairment may already be present. Even if a drug improves another aspect of neuronal physiology, it can reduce functional outcome if it causes excessive drowsiness or poor attention. Therefore, in future studies on the neuroprotective potential of orphenadrine, functional and behavioural tolerability should be used in addition to molecular endpoints. Cognitive issues are particularly important, since Parkinson's can lead to a range of non-motor symptoms including cognitive impairment, sleep disturbances, mood swings and autonomic dysfunction. People can have cognitive impairment at different times and to different degrees [91]. Ideally, a prospective neuroprotective treatment in this situation would preserve neuronal function without increasing the cognitive load. The cholinergic system is so intimately involved in cognitive processing that the presence of an antimuscarinic mechanism is a matter of concern when considering orphenadrine. Therefore, future clinical research should be designed to isolate disease-related cognitive decline from the cognitive effects of treatment. In the elderly, the concept of cumulative anticholinergic burden is particularly relevant. Parkinson's disease patients receive a variety of drugs, some of which may possess anticholinergic properties even if they are not anticholinergic drugs. Concurrent exposure can increase the total anticholinergic burden and increase the risk of peripheral and central nervous system adverse effects. Thus, evaluation of orphenadrine alone may underestimate its clinical hazards related to polypharmacy in the real world. Its long-term safety should be investigated in translational studies taking into account the complete pharmacological profile, concomitant exposure to anticholinergics, renal and hepatic function, and individual susceptibility [92].

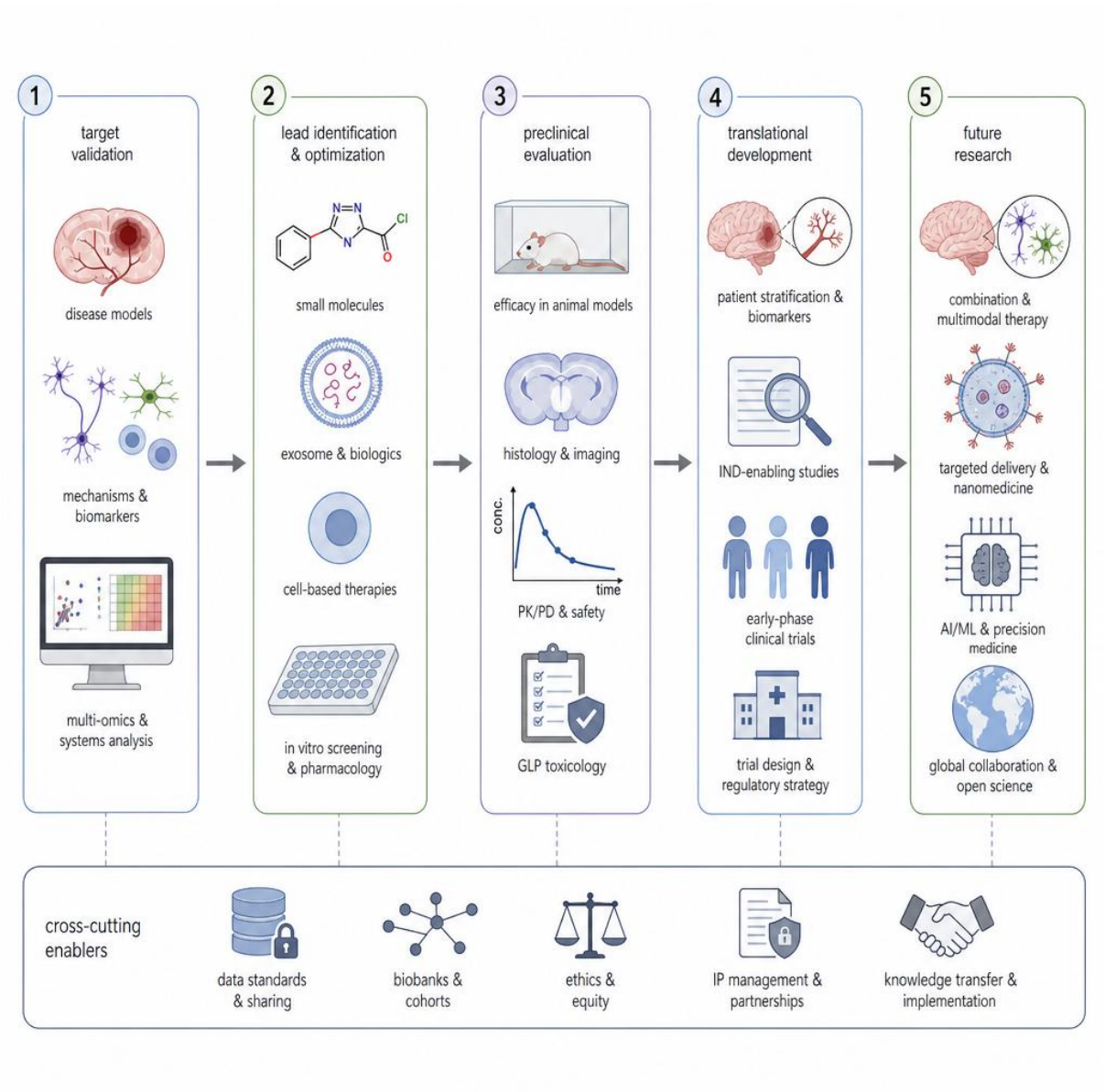


Figure 7. The geriatric paradox and therapeutic window of orphenadrine repurposing.

5.4 Combination Strategies with Antiepileptic or Neuroprotective Agents

As neuronal injury is often mediated by a number of interlinked processes rather than a single biochemical pathway, combination therapy is a potentially effective technique for examining the neuroprotective capabilities of orphenadrine. Neurodegenerative and acute neurological diseases may coexist with excitotoxicity, excessive glutamatergic signalling, aberrant neuronal firing, calcium overload, mitochondrial dysfunction, oxidative stress and neuroinflammation [93]. Orphenadrine has been shown to interact with neuronal ion channels besides its pharmacological effects on central muscarinic and histaminergic receptors. Hence, in theory, the co-administration of orphenadrine with drugs acting via complimentary mechanisms may allow for more extensive control of neuronal hyperexcitability and cellular stress than targeting a single pathway alone. But combinations such as these should now be considered an exploratory research technique, rather than a proven neuroprotective medication. They provide an important pharmacological rationale for the assessment of this concept, as many antiepileptic agents reduce excessive neuronal excitability by mechanisms mechanistically similar to excitotoxic damage [94]. Drugs such as levetiracetam, lamotrigine, carbamazepine, valproate and gabapentin/pregabalin affect neuronal excitability through a variety of molecular targets including synaptic vesicle proteins, voltage-gated sodium channels, calcium channels and neurotransmitter systems. Excessive neuronal activity can trigger increased glutamate release and subsequent calcium-dependent damage. Thus, orphenadrine can increase the ability of a substance to modulate NMDA receptors or ion channels by reducing neuronal hyperexcitability. Reduction in neuronal activity does not necessarily result in neuroprotection in chronic neurodegenerative disease, and the utility of this approach should be tested experimentally. A very interesting strategy would be the combination of orphenadrine with substances that modulate voltage-gated sodium channels or other factors that modulate action-potential generation. Excessive sodium-channel activation causes increased metabolic demand and prolonged neural firing. Neural stimulation induces a persistent increase in reactive oxygen species generation, ATP consumption, calcium entry and mitochondrial burden [95].

6. Summary, Strategic Knowledge Gaps, & Future Directions

6.1 Multi-Target Neuroprotection

Parkinson's disease and other neurodegenerative diseases are complex multifactorial situations where a number of pathogenic processes interact to cause neuronal malfunction and cell death. Excitotoxicity, mitochondrial dysfunction, oxidative stress, neuroinflammation, aberrant protein accumulation, poor neurotransmission and alteration of neuronal ion homeostasis are several factors that may lead to progressive neuronal damage. Thus, conventional treatments that target a single cause of disease may not be sufficient to address the complex biological milieu associated with neurodegeneration [96]. The concept of multi-target neuroprotection has received much attention because of the potential for increased cellular protection through simultaneous regulation of interconnected disease pathways. Orphenadrine has known central antimuscarinic and antihistaminic activity and interactions with neuronal ion channels have been reported in the experimental literature. Thus, it presents an interesting possibility for further investigation in this paradigm. Modulation of neuronal excitability is one of the key processes considered in orphenadrine's neuroprotective potential. Sustained receptor activation, intracellular calcium accumulation, mitochondrial dysfunction and subsequent oxidative damage can lead to excitotoxic neuronal injury due to excessive glutamatergic activity [97]. Thus, agents capable of reducing over excitation of neurons can stop the destructive cascade at an early stage. Its known interaction with neuronal ion channels provides a molecular basis for investigating the possibility that orphenadrine may alter excitability in the face of excessive stimulation. Nevertheless, molecular docking alone is not enough to prove functional channel blocking or neuroprotective efficacy, and the exact role of different ion-channel targets in neuroprotection has to be determined experimentally. Another pharmacological aspect of the potential multi-target efficacy of orphenadrine is its antimuscarinic properties [98]. Central cholinergic signalling regulates arousal, motor control, attention, and other aspects of cognitive function. Changes in cholinergic-dopaminergic interactions are particularly relevant to parkinsonian illnesses. Historically, centrally acting anticholinergic medications have been used to ameliorate some of the motor symptoms of

Parkinsonism, including tremor, although their efficacy may be limited by peripheral and cognitive side effects. Thus, the cholinergic component of orphenadrine pharmacology may be pertinent to some neural circuits but should not be interpreted literally as evidence of disease modifying neuroprotection. Another element of the proposed multi-target architecture is mitochondrial preservation. Mitochondria are particularly sensitive to oxidative stress and high intracellular calcium [99]. Mitochondrial dysfunction results in decreased ATP production, impaired neuronal energy metabolism, increased production of reactive oxygen species, and activation of cell death pathways. Mitochondrial dysfunction may improve the effects of excitotoxic stimulation. Neurons have high energy requirements and limited capacity for regeneration. Thus, an assessment of the potential for orphenadrine to indirectly support mitochondrial function during excitotoxic or toxicant-induced neuronal stress may yield important insights into processes outside of the primary receptor pharmacology[100].

6.2 Preclinical Disconnects and Methodological Gaps

The pharmacological properties of orphenadrine render it an interesting candidate to study neuroprotection. However, there are still large gaps between the known pharmacology, experimental findings, computational predictions and the criteria used to demonstrate disease-modifying neuroprotection. A large part of the proposed neuroprotective framework is supported by mechanistic interpretation, rather than concrete data from verifiable models of Parkinson's disease. This distinction is very crucial since neuroprotection, symptomatic relief, reduction of neuronal excitability and overall decrease of toxic damage have unique therapeutic outcomes [101]. A major issue for future research will be to determine whether orphenadrine truly modifies mechanisms of neuronal degeneration or merely produces secondary changes in behavioural or cellular end points. A major methodological gap is the lack of direct evidence linking orphenadrine to neuroprotection specific to Parkinson’s disease. Parkinson’s disease is characterized by progressive loss of dopaminergic neurons in the substantia nigra, changes in the circuitry of the basal ganglia, α-synuclein pathology, oxidative stress, mitochondrial dysfunction and neuroinflammation. Although they cannot fully recapitulate the real disease, experimental models recapitulating only one aspect of this pathology may provide valuable mechanistic insights. Data from excitotoxicity, metabolic-toxin or general neurotoxicity models are not always adequate to take as proof of Parkinson's disease change [102]. Future studies should be combined with complementing models representing different diseased components and include disease-relevant endpoints, such as dopaminergic neuronal survival, striatal dopamine levels, α-synuclein-related alterations, mitochondrial function, oxidative stress and motor behaviour. Another associated gap is the interpretation of AlCl 3 -induced neurotoxicity as a model of Parkinsonian pathology. Aluminum exposure is important in studying processes of toxic brain injury as it can induce oxidative, mitochondrial, inflammatory and neurological disruption in experimental systems. However, the idiopathic Parkinson's disease and the AlCl 3 -induced model are not comparable. Some of the pathways involved in neurodegeneration may overlap with the processes underlying aluminum-associated neurotoxicity, but without replicating the entire clinical range of Parkinson's disease [103]. Thus, results from an $AlCl_3$-exposed Danio rerio model should be interpreted as evidence of protection against a specific neurotoxic challenge rather than as definitive evidence of disease change in Parkinson's disease. There is a large gap between functional pharmacology and molecular docking as well. Docking studies are useful to predict a potential orientation, or relative binding affinity of a ligand in a protein binding site, but they cannot determine whether the interaction occurs in a living creature, or if it produces a useful pharmacological response.[104] This constraint is crucial for ion channels and membrane proteins, where the drug's effect may be influenced by receptor conformation, the membrane environment, voltage dependency, channel state and kinetic properties. This makes that the expected interactions of orphenadrine with proteins related to Parkinson disease must be considered as theories needing experimental verification. Electrophysiological studies, receptor binding assays, enzyme assays, and cellular functional assays would provide better support for target engagement [105].

6.3 Future Research Directions

Future studies of the neuroprotective potential of orphenadrine will need to extend beyond computational predictions and early pharmacological observations to rigorous mechanistic validation. Although it is centrally pharmacologically effective, the possible use of orphenadrine as a neuroprotective drug in neurodegenerative diseases is not well understood. Whether its effects on neurotransmitter systems, oxidative stress, mitochondrial function, neuroinflammatory pathways, and neuronal excitability can all act synergistically to produce measurable protection against neuronal degeneration needs to be determined in future studies [106]. A more robust body of evidence would arise from a multidisciplinary approach involving computational pharmacology, molecular biology, electrophysiology, animal models, pharmacokinetics and behavioural neuroscience. A future direction is the systematic identification and validation of molecular targets associated with neuroprotection mediated by orphenadrine. At the beginning, docking studies can be used to study the interaction with the proteins related to Parkinson's disease such as monoamine oxidase-B, dopamine transporter, α-synuclein-associated targets and specific cholinergic receptors [107]. However, computational predictions should be validated by molecular dynamics simulations, binding assays, enzyme inhibition studies, receptor-function experiments and cellular models. Since docking alone does not prove channel-state dependence, voltage dependence, kinetics, and membrane environment, electrophysiological techniques such as patch-clamp recording would be especially useful for ion channel targets. Further studies are needed to investigate the relationship between orphenadrine and the principal mechanisms involved in neuronal degeneration. Excitotoxicity, calcium dysregulation, mitochondrial dysfunction, oxidative stress and neuroinflammation should be regarded as related, rather than separate, pathways. Intracellular calcium, mitochondrial membrane potential, ATP production, reactive oxygen species, lipid peroxidation, glutathione status, antioxidant enzymes, inflammatory cytokines and neuronal viability provide a mechanistic profile of the response to treatment. These studies would help to determine whether the neuroprotective effects of orphenadrine are primarily due to reduced neuronal excitability or whether secondary effects on cellular stress pathways are also at play [108]. It is worth considering the proposed function of antioxidants and cellular stress response pathways. Further studies may investigate the influence of orphenadrine on transcriptional pathways such as Nrf2/HO-1 and endogenous antioxidant defences in conditions of induced oxidative stress. But these routes should be studied as hypotheses rather than presumptive mechanisms. Gene-expression analysis, protein-expression studies, enzyme activity assays, and pathway-specific pharmacological or genetic interventions can be used to determine if these signalling systems are causally involved in any observed neuroprotective effect. The Danio rerio model provides an intermediate platform suitable for screening and mechanistic inquiry because behavioural, biochemical and molecular endpoints can be explored within the same experimental framework [109]. Future zebrafish studies should include orphenadrine with multiple treatment groups such as vehicle control, toxin control, positive reference treatment, and orphenadrine-treated groups along with well-characterized AlCl 3 exposure protocols. Behavioural parameters should be accompanied by measurements of dopaminergic function, oxidative stress, mitochondrial state, inflammatory markers and neuronal integrity. Such an approach would assist in differentiating between nonspecific effects on locomotor activity or general CNS activity and true neuroprotection [110].

Table 7. Knowledge Gaps, Future Research Priorities, and Potential Combination Strategies for Orphenadrine-Mediated Neuroprotection. [111-115]

Knowledge Gap / Research Priority	Why It Matters	Suggested Experimental Approach	Potential Translational Relevance	
Exact molecular target profile	Identification of the principal molecular targets is essential for establishing the mechanism underlying potential neuroprotective activity.		Receptor-binding assays, functional assays, molecular docking, and molecular dynamics studies.	Establishes direct molecular targets and distinguishes experimentally validated interactions from predicted mechanisms.
NMDAR interaction kinetics	Determining affinity, use dependence, voltage dependence, and dissociation kinetics may define the pharmacological window for modulation of excitotoxic signalling while minimizing interference with physiological NMDAR function.		Electrophysiological recordings, patch-clamp studies, receptor-channel kinetic analysis, and binding studies.	Provides a mechanistic basis for optimizing dose and therapeutic window.
BBB exposure	Establishing whether therapeutically relevant concentrations reach the brain is essential for CNS translation.		Pharmacokinetic studies, brain-to-plasma concentration measurements, CNS exposure studies, and BBB permeability assessment.	Determines whether systemic dosing can achieve pharmacologically relevant CNS concentrations.
Nrf2/HO-1 involvement	Demonstrating whether this pathway contributes directly to antioxidant effects would strengthen the proposed secondary neuroprotective mechanism.		Nrf2/HO-1 expression studies, pathway inhibition, gene-silencing/knockdown approaches, and oxidative-stress assays.	Validates or refines the proposed antioxidant mechanism.
Mitochondrial effects	Clarifying mitochondrial actions may establish a mechanistic link between excitotoxicity, energy failure, oxidative stress, and neuronal survival.		Assessment of TFAM, ATP levels, mitochondrial membrane potential, ROS production, mitochondrial respiration, and related mitochondrial markers.	Identifies whether mitochondrial protection contributes independently to the neuroprotective phenotype.
Microglial effects	Determining whether orphenadrine directly modulates microglial activation is important for establishing its		Microglial activation models, immunohistochemistry, inflammatory-marker	Clarifies whether modulation of neuroinflammation

	proposed anti-inflammatory activity.	analysis, and cytokine assays.	represents a direct or secondary effect.
Dose–response relationship	Defining the relationship between exposure and neuroprotective effects is necessary to identify an appropriate experimental therapeutic range.	Preclinical dose-ranging studies and pharmacokinetic–pharmacodynamic analysis.	Supports rational dose selection and therapeutic-window determination.
Long-term safety	Chronic CNS exposure may produce adverse effects that are not apparent in short-term neuroprotection experiments.	Long-term behavioural, cognitive, neurological, pathological, and toxicological studies.	Essential for evaluating the feasibility of chronic or repeated use in neurodegenerative disorders.
Age-dependent effects	Aging can alter pharmacokinetics, CNS sensitivity, and vulnerability to anticholinergic adverse effects.	Studies in aged animal models with behavioural, cognitive, pharmacokinetic, and safety assessments.	Particularly relevant to the older populations most affected by neurodegenerative diseases.
Combination strategies: Orphenadrine + NMDAR-selective/modulatory agent	Complementary modulation of excessive glutamatergic signalling may enhance suppression of excitotoxic injury while potentially preserving physiological NMDAR activity.	Combination studies using electrophysiology, neuronal-survival assays, dose–response analysis, and pharmacodynamic interaction assessment.	Potentially improved control of excitotoxicity; requires assessment of excessive NMDAR inhibition and neurological adverse effects.
Combination strategies: Orphenadrine + antioxidant/Nrf2 activator	Addresses excitotoxicity together with downstream ROS generation and impaired antioxidant defences.	Combination treatment in oxidative-stress and excitotoxicity models with Nrf2/HO-1, ROS, mitochondrial, and cell-survival endpoints.	May provide complementary redox and mitochondrial protection.
Combination strategies: Orphenadrine + mitochondrial protective agent	Targets mitochondrial dysfunction as a major downstream consequence of Ca^{2+} overload and excitotoxic stress.	Combination studies assessing mitochondrial membrane potential, ATP, ROS, respiration, and neuronal survival.	Could enhance mitochondrial resilience and neuronal survival.
Combination strategies: Orphenadrine + anti-inflammatory/NF-κB-modulating agent	Combines suppression of excitotoxic injury with modulation of inflammatory amplification.	Neuroinflammatory models with NF-κB, cytokine, microglial, and neuronal-injury endpoints.	May reduce secondary inflammatory neuronal damage.

Combination strategies: Orphenadrine + microglial-targeted therapy	May limit the transition from acute neuronal injury to sustained neuroinflammation.	Microglial activation models, cytokine profiling, imaging, and neuronal–microglial co-culture systems.	Provides a strategy for simultaneously targeting neuronal excitotoxicity and inflammatory amplification.
Combination strategies: Orphenadrine + anti-apoptotic/neurotrophic strategy	Targets downstream cell-death pathways not directly addressed by NMDAR modulation.	Combination studies assessing apoptotic markers, neurotrophic signalling, neuronal survival, and functional outcomes.	May improve neuronal survival following excitotoxic or metabolic injury.
Combination with standard disease-modifying therapy	Combining the proposed effects of orphenadrine with established disease-specific treatments could provide complementary pharmacological effects.	Combination studies, pharmacodynamic interaction analysis, PK studies, and disease-relevant animal models.	Potentially supports rational repurposing while requiring careful evaluation of polypharmacy and drug–drug interactions.
Additive, synergistic, or antagonistic interactions	Determining whether combinations genuinely improve efficacy rather than simply increasing drug exposure is essential for rational combination therapy.	Dose-matrix studies, is bolographic analysis, combination-index approaches, and pharmacodynamic interaction studies.	Identifies combinations with genuine therapeutic advantages and minimizes unnecessary polypharmacy.
Translation to human disease	Experimental models may not fully reproduce the complexity of human excitotoxic and neurodegenerative disorders.	Validation across multiple cellular and animal models, followed by appropriately designed translational and clinical studies.	Determines whether experimentally observed neuroprotection can be reproduced in human disease.

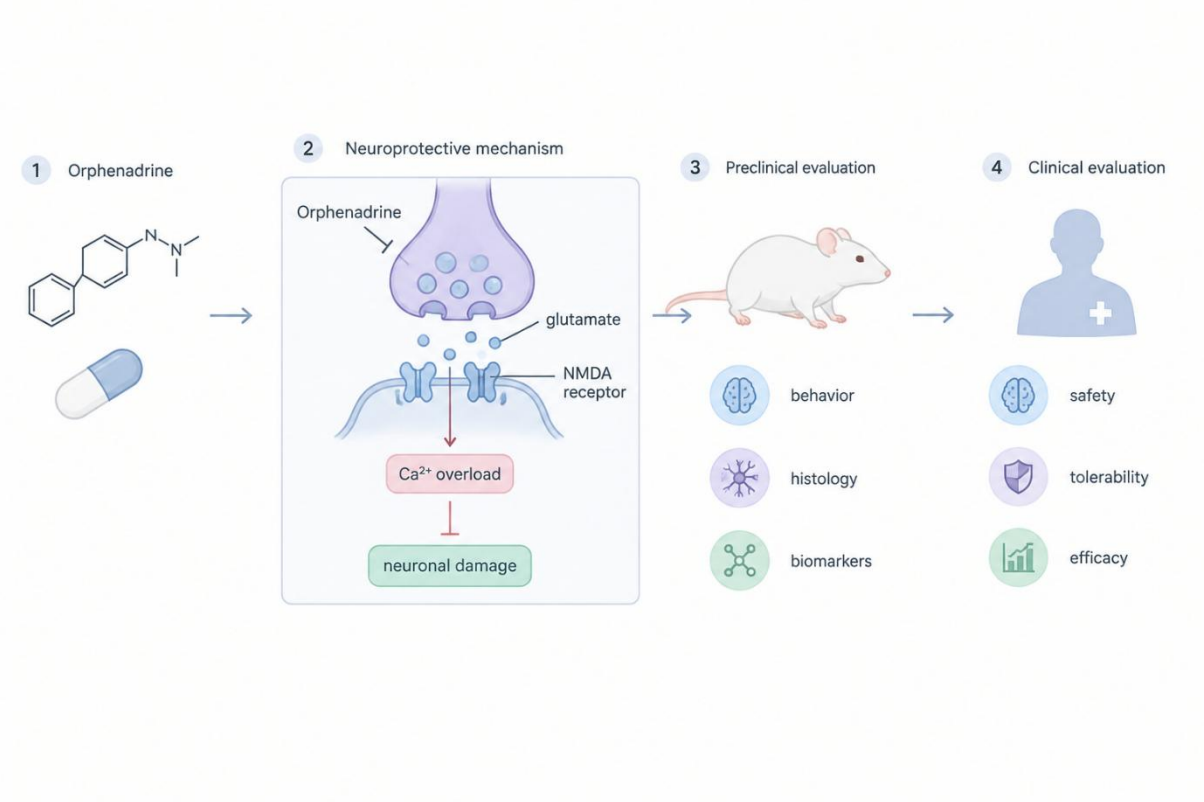


Figure 8. Translational roadmap for evaluating orphenadrine as a potential neuroprotective agent.

Conclusion

Orphenadrine, a drug long considered a centrally acting muscle relaxant, has a much more complex pharmacological profile than that reflected by its current clinical use. Its ability to act as a low affinity, use-dependent NMDA receptor antagonist has the potential to reduce excitotoxic neuronal damage without completely interfering with normal glutamatergic transmission. Orphenadrine's effects on NMDA regulation, muscarinic receptors, histaminergic systems, voltage-gated sodium channels and mitochondrial pathways exemplify a polypharmacology framework that could address the multifactorial nature of excitotoxicity. According to the data we reviewed, orphenadrine could have the potential to reduce neuroinflammation, oxidative stress, calcium overload and mitochondrial dysfunction, which are the main causes of neuronal death. Importantly, its pharmacokinetic properties, including rapid off-rate kinetics and blood–brain barrier penetration, support the possibility of achieving a fine balance between neuroprotection and preservation of normal synaptic functioning. But these results are still mostly mechanistic and pre-clinical. Orphenadrine has anticholinergic side effects and clinical translation requires rigorous safety, tolerability, and therapeutic window assessment. This use of the drug for neuroprotection is an example of the wider concept of looking at established drugs for new indications. However, it is important to do clinical trials to fully evaluate its role in excitotoxic brain injury.

List of abbreviations

Abbreviation	Full Form
ATP	Adenosine Triphosphate
BBB	Blood–Brain Barrier
Ca^{2+}	Calcium Ion
CGN	Cerebellar Granule Neuron
CNS	Central Nervous System
CO	Carbon Monoxide
DNA	Deoxyribonucleic Acid
3-NP	3-Nitropropionic Acid
HIF-1α	Hypoxia-Inducible Factor-1 Alpha

HO-1	Heme Oxygenase-1
HSP	Heat Shock Protein
HSP27	Heat Shock Protein 27
HSP70	Heat Shock Protein 70
HSP72	Heat Shock Protein 72
HSP90	Heat Shock Protein 90
IL-1β	Interleukin-1 Beta
IL-6	Interleukin-6
iNOS	Inducible Nitric Oxide Synthase
KA	Kainic Acid
Keap1	Kelch-like ECH-Associated Protein 1
MDA	Malondialdehyde
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
Na^+	Sodium Ion
NF-κB	Nuclear Factor Kappa B
NMDA	N-Methyl-D-Aspartate
NMDAR	N-Methyl-D-Aspartate Receptor
NOS	Nitric Oxide Synthase
Nrf2	Nuclear Factor Erythroid 2-Related Factor 2
NQO1	NAD(P)H Quinone Oxidoreductase 1
PBR	Peripheral Benzodiazepine Receptor
PCP	Phencyclidine
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
SDH	Succinate Dehydrogenase
TFAM	Transcription Factor A, Mitochondrial
TNF-α	Tumour Necrosis Factor Alpha
VGSC	Voltage-Gated Sodium Channel
VGSCs	Voltage-Gated Sodium Channels
HMOX1	Heme Oxygenase-1 Gene

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