Hypothesis

Topical pyridostigmine for ocular myasthenia gravis: a translational hypothesis

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ABSTRACT

Background: Myasthenia gravis (MG) is an autoimmune disorder of the neuromuscular junction predominantly mediated by antibodies that target the acetylcholine receptors in the skeletal muscles. Ocular involvement of the levator palpebrae superioris and extraocular muscles is common, resulting in ptosis and diplopia. Oral pyridostigmine, an acetylcholinesterase, is typically the first-line therapy, but its administration is frequently limited by systemic cholinergic adverse effects and inconsistent relief of ocular symptoms.

Hypothesis: We hypothesize that pyridostigmine can be reformulated as a topical ocular/periocular formulation to directly enhance the neuromuscular transmission of the levator palpebrae superioris and extraocular muscles, thereby improving ptosis and strabismus while minimizing systemic side effects. The levator palpebrae superioris and extraocular muscles underlie the conjunctiva. Pyridostigmine bromide is a small, hydrophilic quaternary ammonium compound with topical application properties that favor conjunctival permeability while limiting transcorneal penetration. Its formulation pH closely approximates that of conjunctival tissue. Clinical precedent from alpha-adrenergic eye drops (e.g., apraclonidine) that elevate the eyelid via Müller's muscle support the plausibility of a topical approach. Although limited residence time on the ocular surface is a potential barrier, advances in delivery systems, including hydrogels, nanoparticles, and ionic liquids, may enhance retention and conjunctival penetration. Periocular transdermal delivery may further exploit the thin periocular skin to achieve localized vascular access. Based on the pharmacological mechanism of acetylcholinesterase inhibition, we propose that *ex vivo* animal model ocular permeability and tissue integrity be examined to determine the feasibility of topical pyridostigmine formulation providing effective localized neuromuscular transmission enhancement in patients with ocular MG.

Conclusions: If preliminary *ex vivo* animal model observations find that topical pyridostigmine formulations are stable with appropriate conjunctival penetration and preserved epithelial integrity, it would support progression to *in vivo* animal studies and translational modelling. The feasibility of topical or periocular pyridostigmine as a localized treatment strategy for ocular MG deserves further investigation. If validated, this approach could shift management toward targeted, better-tolerated topical therapies with reduced systemic risk.

KEYWORDS

generalized myasthenia gravis, ocular myasthenia gravis, pyridostigmine, mestinon, ophthalmic absorption, drug delivery system, hydrogel, neuromuscular junctions, eyelid ptosis

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INTRODUCTION

Acquired myasthenia gravis (MG) is a heterogeneous autoimmune disorder of the postsynaptic neuromuscular junction, primarily mediated by antibodies that target the nicotinic acetylcholine receptor. These antibodies impair muscle activation through several mechanisms: complement-mediated lysis of the junctional folds, accelerated receptor endocytosis, and direct functional blockade [1, 2]. Ocular involvement is common with MG, involving variable fatigue of the levator palpebrae superioris and extraocular muscles, causing ptosis and diplopia. Ocular manifestations are the initial presentation in many patients with MG, and in 55–85% of patient's symptoms may remain clinically confined to the ocular muscles for prolonged periods [3, 4].

Pyridostigmine is an acetylcholinesterase inhibitor. Its oral formulation is the most commonly used drug for the symptomatic treatment of MG [5, 6]. Pyridostigmine increases acetylcholine availability at the neuromuscular junction, and can alleviate ptosis and diplopia, but the response is inconsistent [7]. Unfortunately, systemic administration of pyridostigmine is frequently limited by dose-dependent cholinergic adverse effects, including diarrhea, flatulence, vomiting, hyperhidrosis, hypersalivation, urinary urgency, incontinence, and muscle cramps [7, 8]. These adverse effects represent a major barrier to dose escalation of oral pyridostigmine and long-term adherence in patients with MG [8].

Compared to the oral intake of drugs, topical formulations can provide targeted therapy, avoid the hepatic first-pass effect, and decrease systemic absorption and potential systemic side effects of drugs [9]. We could only locate one case report on the use of nebulized topical pyridostigmine for the oropharyngeal symptoms of MG [10]. At present, no topical cholinergic therapy exists for the treatment of ocular MG. Neostigimine is also an acetylcholinesterase inhibitor, and neostigmine eye drops have been proposed for the diagnosis of ocular MG though not for its treatment [11]. However, neostigmine causes more cardiac inhibition, hypersalivation, and cholinergic side effects than pyridostigmine, and has a shorter duration of action than pyridostigmine. Pyridostigmine requires less frequent dosing and has a better tolerance profile than neostigmine [7, 12].

Alpha-agonist eye drops can ameliorate small amounts of ptosis via activation of the sympathetically innervated Müller's muscle, but their prolonged use may be limited by rebound conjunctival hyperemia [13]. These therapeutic gaps underscore our exploration of topical/periocular pyridostigmine for ocular MG.

HYPOTHESIS

We hypothesize that pyridostigmine can be reformulated topically as an eye drop, gel, or periocular transdermal patch to target the levator palpebrae superioris and extraocular muscles in patients with MG with ptosis or diplopia. We postulate that periocular topical agents can achieve high periocular concentration with low systemic absorption and fewer systemic side effects than oral administration [14].

Pyridostigmine bromide is a small molecule with a molecular weight of 261.12 Daltons (Da) and a hydrophilic physicochemical profile [15, 16]. The conjunctiva has pore sizes of 3–4.9 nm in the bulbar and palpebral regions, permitting the passage of molecules up to 5–10 kDa [17]. The conjunctiva is considered the preferred route for delivering hydrophilic drugs to deeper ocular and periocular tissues [17, 18]. Compared with the cornea, the conjunctiva has a much larger surface area, larger paracellular spaces, and greater permeability to hydrophilic solutes [18].

Pyridostigmine has a low molecular weight and high water solubility, which are advantageous physiochemical properties for transconjunctival drug delivery pathways that are increasingly recognized as critical for targeting periocular, posterior segment, and orbital tissues [18].

Potential limitations of topical ocular therapy include drug dilution by the tear film, inadequate drug penetration of pyridostigmine to the levator and extraocular muscles through the conjunctiva, and drug loss from tear turnover, nasolacrimal drainage, and conjunctival vessels, which significantly reduces residence time on the ocular surface [19, 20]. Differences between the pH of ophthalmic formulations and that of the ocular surface can increase reflex tearing [21]. Typically, only 1–5% of an applied ophthalmic dose penetrates ocular tissues due to the aforementioned factors [19].

Pyridostigmine bromide has a pH range of 5–7 [22], which closely approximates the pH of the tear film and conjunctiva (6.5–7.6) [23]. Optimization of formulation pH and buffering may therefore reduce reflex tearing and improve conjunctival penetration [19, 24].

Topical drug formulation strategies to prolong ocular surface residence time include increasing viscosity through hydrogels, incorporating mucoadhesive polymers, and employing sustained-release nanocarriers that can substantially enhance drug retention and local tissue exposure [20, 25–28].

Pyridostigmine bromide is a hydrophilic positively charged quaternary ammonium compound [15]. Although there is little specific comparative data on the corneal versus conjunctival penetration of pyridostigmine, an established tenet in ocular pharmacology is that the conjunctiva is more permeable to hydrophilic compounds than the lipophilic corneal epithelium [29]. The preferential conjunctival absorption of pyridostigmine over the cornea is advantageous because the conjunctiva overlies the levator palpebrae and extraocular muscles [30]. The hydrophilic nature of pyridostigmine and the lipophilic corneal epithelium may lower the risk of ocular absorption and the potential side effects of blurred vision from increased accommodation and miosis [8].

The systemic absorption of topical ocular drugs is unavoidable, as a fraction of the drug is absorbed via nasolacrimal drainage into the nasal mucosa or through conjunctival and episcleral vasculature [19]. Accordingly, early pharmacokinetic studies would monitor plasma cholinesterase inhibition and systemic muscarinic effects to characterize the therapeutic index [31].

Collectively, the pharmacologic rationale, anatomical feasibility, and emerging formulation technologies suggest that this hypothesis warrants systematic experimental evaluation.

EVALUATION OF THE HYPOTHESIS

Our hypothesis is amenable to systematic evaluation through a staged experimental framework that progresses from formulation development and *ex vivo* tissue testing to *in vivo* animal models, and, ultimately, early-phase clinical investigation [32–34].

Initial *in vitro* formulation studies are required to establish physicochemical compatibility between pyridostigmine and candidate delivery platforms, including hydrogels, nanoparticle systems, and ionic-liquid formulations [33]. These studies would enable optimization of formulation parameters such as pH buffering, viscosity, osmolarity, and release kinetics, all of which influence ocular surface residence time and drug availability [34]. Sustained-release and mucoadhesive formulations are of particular interest, as they may mitigate rapid tear-mediated clearance and enhance conjunctival absorption [35, 36].

Following formulation optimization, *ex vivo* penetration studies using porcine eyes and conjunctiva provide a well-established method to assess ocular barrier traversal [37, 38]. This model would allow quantification of pyridostigmine transport across conjunctival pathways, estimation of tissue recovery and permeability under controlled conditions, early pharmacokinetic and stability data, and examination for corneal intolerance.

In vivo evaluation in animal models would then be performed [39] with eye drop and transdermal formulation in parallel. Skin-to-muscle absorption studies could provide an additional mechanistic validation of periocular delivery [40, 41]. An initial murine model [42] is proposed, where instead of subcutaneous drug administration topical pyridostigmine is applied to the gastrocnemius, with subsequent quantification of drug concentrations [42] in the gastrocnemius, soleus, and plantaris muscles at defined post-application intervals. Detection of pyridostigmine in these muscles provides a mechanistic proxy for the feasibility of transdermal diffusion to underlying neuromuscular tissue [42]. We could not locate a reference on transdermal pyridostigmine for the treatment of myasthenia in the literature, but there is a case report of topical nebulized pyridostigmine to the oropharynx for bulbar myasthenia [10].

Neostigmine is also an acetylcholinesterase inhibitor, but causes more cardioinhibitory and salivation side effects and has a shorter duration of action than pyridostigmine [12]. Topical neostigmine drops have been proposed for the diagnosis of ocular MG though not for its treatment [11]. An animal study investigated the safety and effect of topical ocular neostigmine but concentrated on miosis [43].

Animal models of MG with ptosis [44–46] can further assess local pharmacodynamic effects following topical or periocular pyridostigmine administration. Proposed endpoints may include quantitative eyelid elevation, blink dynamics, ocular motility measurements, and acetylcholinesterase activity [46] in the levator or extraocular muscles. Concurrent plasma sampling would allow assessment of systemic absorption and monitoring for muscarinic adverse effects [47, 48], thereby defining the therapeutic window.

Building on rodent feasibility data, periocular administration studies in rabbits offer a more translationally relevant intermediate model that more closely approximates human orbital anatomy and tissue thickness [43, 48, 49]. As in the proposed rodent testing, rabbit experimentation could evaluate topical periocular pyridostigmine formulations applied

adjacent to the eyelid, with serial tissue assays quantifying drug concentrations in the levator palpebrae superioris and selected extraocular muscles. Plasma drug levels would be measured concurrently to assess systemic exposure. Functional endpoints would include eyelid position [4], blink rate, ocular surface tolerance, intraocular pressure, and intraocular inflammation, alongside targeted monitoring for cholinergic toxicity.

If preclinical studies demonstrate localized efficacy with minimal side effects, progression to early-phase clinical studies in patients with ocular MG would be justified [50]. Initial clinical investigations would prioritize safety, tolerability, and pharmacodynamic outcomes, including standardized assessments of ptosis, diplopia, and patient-reported symptom burden. Careful monitoring for systemic cholinergic effects would be essential to confirm that topical or periocular delivery offers a therapeutic advantage over oral administration.

Collectively, this tiered experimental strategy allows incremental testing of the pyridostigmine hypothesis across formulation, anatomical, pharmacokinetic, and functional domains [47, 48]. By systematically addressing feasibility, efficacy, and safety at each stage, this approach provides a rational pathway [51, 52] for evaluating whether topical pyridostigmine can serve as a clinically useful localized therapy for ocular MG.

Translational Considerations

Pyridostigmine exhibits favorable human pharmacokinetics [53] supporting the feasibility of targeted periocular delivery to achieve therapeutic muscle concentrations. Encapsulation within biodegradable nanoparticles [26, 27, 54–56], such as polylactic acid-based systems [57], could protect pyridostigmine from premature degradation and facilitate controlled release. *In vivo* pharmacokinetic and pharmacodynamic validation and formal dose escalation studies remain essential prior to human application [19].

Using Boolean logic ("pyridostigmine" AND "glaucoma"), a literature review of PubMed in February 2026 identified no documented cases of pyridostigmine-induced glaucoma. Pyridostigmine is a quaternary amine, and pilocarpine is a tertiary amine. Pilocarpine is often used to treat angle-closure glaucoma, but it can also induce the condition if there is anteriorization of the lens iris diaphragm [58]. Pyridostigmine is a quaternary amine and can also cause pupillary constriction [58], though the risk of angle-closure glaucoma with topical pyridostigmine is not established.

Translational Pharmacokinetic Challenges

An *ex vivo* permeability data experiment could support the technical feasibility for topical pyridostigmine delivery, yet providing insufficient quantitative parameters for human-dose extrapolation or sophisticated modeling. Localized periocular pharmacokinetics differs fundamentally from systemic routes [59, 60]. Key translational uncertainties include regional blood flow differences, human tear clearance rates, variable regional perfusion of the eyelid versus orbital vasculature, local enzymatic clearance pathways, and complex diffusion gradients from the skin or conjunctiva to the levator and extraocular muscles [60].

Required preclinical validation includes rabbit *in vivo* tissue distribution studies (serial levator and extraocular muscle biopsies, plasma sampling) to establish tissue/plasma ratios, functional pharmacokinetic/pharmacodynamic correlations (eyelid elevation vs tissue concentrations), and human microdosing studies confirming bioavailability before dose escalation [48, 49, 51, 52].

DISSCUSSION

Although there are many other medications for the treatment of MG [61–63], pyridostigmine is the most commonly used drug for the symptomatic treatment [5, 6], and as such the focus of this paper. We suggest that *ex vivo* porcine studies be done to see if pyridostigmine bromide can be formulated into stable ophthalmic preparations with measurable permeability and no overt epithelial toxicity. If the former are successful, future research should focus on refining formulations to maximize ocular residence, systematically assessing conjunctival penetration and progressing toward *in vivo* validation in animal models and ocular side effects before systemic clinical validation [51, 52, 64]. Ultimately, these studies would provide a strong preclinical foundation for exploring localized, better-tolerated pyridostigmine therapy for ocular MG. If validated, this hypothesis could substantially shift OMG management. Compared with oral pyridostigmine, topical pyridostigmine could theoretically provide targeted neuromuscular enhancement, and, similarly to other topical agents, bypass the gastrointestinal tract and hepatic first-pass effect with reduced systemic drug circulation to non-target organs [9]. This would mitigate the systemic cholinergic adverse effects which include gastrointestinal discomfort, hypersalivation, muscle cramps, and urinary urgency that cause the 26% discontinuation rate of oral pyridostigmine treatment [8]. If transdermal pyridostigmine proves effective for ocular MG, the approach could extend beyond ocular MG to perhaps proximal muscle groups of systemic myasthenia [5, 7].

By enabling localized delivery, clinicians could titrate therapy according to ocular symptom severity rather than systemic tolerance [8], potentially improving adherence and functional outcomes. Prior clinical evidence supporting the concept that topical formulations can be used to diagnose or improve ptosis include topical alpha agonist eyedrops for the short-term treatment of ptosis [13, 65] and neostigmine [11] for the diagnosis of OMG. However, topical pyridostigmine merits further investigation because alpha agonists stimulate the sympathetically innervated Müller's muscle, which is a weaker elevator of the eyelid than the levator [66]; and compared to pyridostigmine, neostigmine has a shorter duration of action plus more cardiac and gastrointestinal side effects [7].

Beyond OMG, the successful development of a topical cholinergic therapy could enable a broader class of localized neuromuscular ocular interventions. Potential applications of topical pyridostigmine include the correction of transient ptosis secondary to botulinum toxin injection [67]. This cholinergic approach targeting the levator palpebrae superioris [66] complements established alpha-adrenergic agents that elevate the eyelid via Müller's muscle stimulation [30, 65], establishing a dual pharmacological framework to expand non-surgical options for ptosis correction.

From a translational perspective, conceptual feasibility exists for pyridostigmine penetration of the conjunctiva [18] to reach periocular muscle tissue based on its physicochemical properties and established systemic efficacy [53]. While direct *in vivo* periocular exposure data for pyridostigmine remain to be established, these findings suggest a plausible pathway for hydrophilic small molecules, with broader implications for ocular pharmacology. Formulation strategies using biocompatible carriers such as hydrogels, ionic liquids [33], and nanoparticle suspensions [54] offer general potential to optimize ocular residence time and tissue targeting for a variety of therapeutic agents [34]. Even if subsequent studies reveal limitations in pyridostigmine penetration, these experiments will provide valuable mechanistic insights into ocular drug delivery, including identification of physicochemical boundaries for periocular diffusion [28]. They will also inform alternative strategies such as punctal plugs, drug-eluting contact lenses, periocular depot injections, transdermal microdelivery, and localized gene modulation approaches [68]. Importantly, systemic absorption of topical pyridostigmine drops through the nasal mucosa is expected to be minimal compared with oral or parenteral routes with punctal occlusion [69]. Future research will benefit from interdisciplinary collaboration among ophthalmologists, pharmacists, and biomaterials scientists [55]. Ophthalmologists provide clinical insight into disease-specific endpoints, ocular anatomy, and treatment feasibility; pharmacists contribute expertise in formulation development, drug stability, and pharmacokinetics; and materials scientists enable the design of sustained-release carriers that overcome washout and enhance tissue penetration. Together, these disciplines can accelerate the translation of this hypothesis into clinically viable therapies.

CONCLUSIONS

In summary, the conceptual framework proposes that periocular pyridostigmine delivery could theoretically achieve targeted levator palpebrae superioris and extraocular muscle concentrations to address ptosis and diplopia in patients with MG. This hypothesis leverages established ocular pharmacokinetic barriers, anatomical targeting precision, and nanoparticle encapsulation precedents, but requires comprehensive *in vivo* pharmacokinetic, pharmacodynamic, and safety validation prior to clinical consideration.

ETHICAL DECLARATIONS

Ethical approval: No human or live animal experimentation was conducted. Literature synthesis followed institutional research ethics policies.

Conflict of interest: None.

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