

POINT-COUNTERPOINT

In the following point-counterpoint article, internationally-acclaimed myopia researchers were challenged to defend the two opposing sides of the topic defined by the title; their contributions, which appear in the order Point followed by Counterpoint, were peer-reviewed by both the editorial team and an external reviewer. Independently of the invited authors, the named member of the editorial team provided an Introduction and Summary, both of which were reviewed by the other members of the editorial team. By their nature, views expressed in each section of the Point-Counterpoint article are those of the author concerned and may not reflect the views of all of the authors.

How does atropine exert its anti-myopia effects?

Atropine, a non-specific muscarinic receptor antagonist, is to date the oldest and most effective pharmacological treatment to inhibit the development of myopia. It is used by school-age children in many Asian countries where myopia has become epidemic; for example, in Taiwan, it is widely prescribed for children with a diagnosis of myopia. However, there has been general resistance to its adoption in the US and Europe, where myopia prevalence figures are lower and greater weight is given to its side-effects of cycloplegia and photophobia, with both practical visual implications and unknown implications for ocular health and accommodation function. For example, its long-term use could cause premature presbyopia, predispose to cataracts, or even cause light damage to the retina. Pirenzepine, a partially-selective m1/m4 receptor antagonist, showed partial inhibition of myopia progression in Phase II trials with minimal side-effects, although its development for clinical application has been terminated. The site of action of atropine was initially thought to be the receptors in the ciliary muscle, in concert with the then prevailing hypothesis that excessive accommodation was the root cause of myopia. However, the findings from a number of animal studies refuted this hypothesis; for example, atropine is effective in preventing myopia in chicks, in which accommodation is mediated by nicotinic receptors, leaving open the question of atropine's site and mode of action. Important questions remain concerning whether atropine's effect involves muscarinic receptors at all, if so, which ones (m1, m2, m3, m4, m5), and where are they located (retina, choroid, sclera)? It is clear that a more thorough understanding of the mechanisms mediating the effects of muscarinic agents in preventing myopia is crucial to developing effective treatment therapies. Here Neville McBrien debates William Stell and his graduate student Brittany Carr, reviewing the evidence for and against atropine's achieving its anti-myopia effects via muscarinic versus non-muscarinic receptors, and whether the retina is the site of atropine's action.

Citation information: McBrien NA*, Stell WK* & Carr B*. Point-counterpoint. How does atropine exert its anti-myopia effects? *Ophthalmic Physiol Opt* 2013, **33**, 373–378.

*All authors contributed equally to this paper.

Point



Neville A. McBrien
*Department of Optometry &
Vision Sciences, University of
Melbourne, Melbourne,
Australia*

E-mail: nmcbrien@unimelb.edu.au

Atropine is a broad-band muscarinic acetylcholine (mAChR) receptor antagonist which demonstrates high affinity for all five muscarinic acetylcholine receptors (M1-M5). Atropine has consistently been demonstrated to inhibit axial myopia progression in both humans and animal models. Yet it has not found widespread clinical application for myopia control outside South East Asia due to ocular side-effects of cycloplegia and pupil dilation with the related risks of increased light exposure. Although there is increasing evidence that the mode of action by which

atropine prevents myopia via muscarinic receptors, most likely based in the retina, the evidence is not yet conclusive.

Point 1: Atropine prevents myopia via a non-accommodative mechanism.

Atropine was first used for myopia control on the premise that ocular accommodation was the causative factor and atropine was a strong cycloplegic drug. Atropine 1% daily has been repeatedly shown to be effective at stopping myopia progression in children.¹ However, the finding that atropine prevents myopia in an animal model (chick) that possesses a striated ciliary muscle, which is innervated by nicotinic receptors rather than muscarinic receptors, demonstrates that atropine prevents myopia via a non-accommodative mechanism² and explains the reason why optical approaches to reduce accommodation (e.g. bifocals, progressive addition lenses) have proved ineffective at stopping myopia.³ These findings have shifted research to the posterior segment of the eye as the locus of action for atropine's anti-myopia effect, with the retina the most likely tissue.

Point 2: Evidence that atropine's anti-myopia effect is based at a retinal site of action.

Although there is strong evidence from animal models of experimentally-induced myopia that the neurochemical signalling cascade causing myopia begins at the retina level with, for example, sign of defocus changes found in the amacrine cells of the retina,⁴ there is a view that muscarinic antagonist control of myopia is initiated at the sclera.⁵ Previous studies have suggested that, because relatively high doses of atropine are needed to prevent myopia in experimental studies, the site of action is more likely the sclera than the retina. However, a recent study has demonstrated that very low doses of atropine (0.01%) applied topically to adolescent children was nearly as effective at stopping myopia progression as doses (1%) a 100 times higher;⁶ this finding follows earlier studies that found 0.1% atropine to reduce myopia progression by 60% compared to placebo-treated Taiwanese children.⁷ Thus lower doses of atropine (0.1% to 0.01%) are effective in inhibiting myopia.

In addition, recent evidence on a mammalian model of myopia has demonstrated that the highly-selective muscarinic antagonists MT3 (M_4 receptor antagonist) and MT7 (M_1 receptor antagonist) are effective in preventing experimentally-induced myopia at low nanomolar concentrations, which are close to the receptor affinity constants for the two antagonists (see Figure 1; Arumugam & McBrien,⁸). This finding supports a retinal site of action for muscarinic antagonist anti-myopia effects as the low concentration of the applied intravitreal doses would likely be in the picomolar range at choroidal or scleral muscarinic receptors (see Cottrell *et al.*⁹) and thus below functional levels at these tissues. Further evidence for a retinal site of action of atropine in

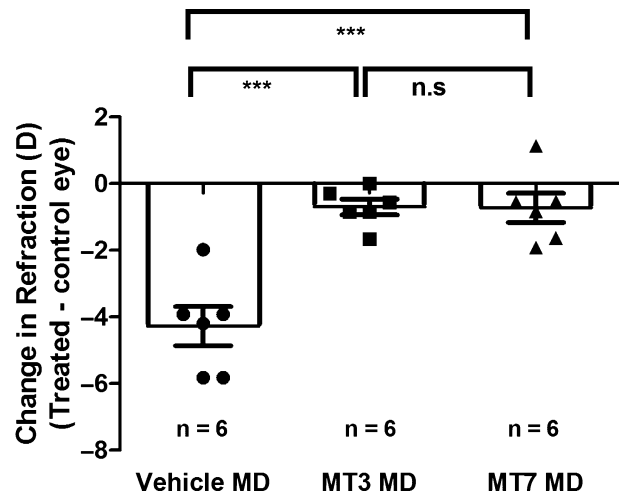


Figure 1. Daily intravitreal application of MT3 (M_4 muscarinic antagonist) or MT7 (M_1 muscarinic antagonist) prevents experimentally-induced myopia [via monocular form deprivation (MD)] in tree shrews when compared to saline vehicle treated animals. The dose applied resulted in a concentration of approximately 200 nM at the vitreal/retinal interface. $N = 6$ in each group. *** $p < 0.01$. (Reproduced with permission from Arumugam and McBrien, 2012 Association for Research in Vision and Ophthalmology).

preventing myopia comes from recent findings that MT3 (M_4 receptor antagonist) inhibition of myopia in chicks also prevents the choroidal thinning normally associated with the induced myopia.¹⁰ By preventing a response of a tissue to myopia development when that tissue is earlier in the drug pathway than the sclera argues against a scleral site of action.

Another argument made for a scleral site of action was the finding that very high doses of pirenzepine (M_1/M_4 partially-selective antagonist) directly applied to isolated cultures of avian sclera cells (chondrocytes) inhibited glycosaminoglycan synthesis, which is a marker for scleral expansion.⁵ However, such high (mM) doses applied directly to the cultured cells would likely be toxic to the cell and it is this likely toxicity that inhibited the cellular function of glycosaminoglycan synthesis and not, as suggested, that pirenzepine was working via muscarinic receptors on the isolated sclera cell, which of course would have no acetylcholine, its neurotransmitter, present to antagonize.

Thus the above arguments are weak as evidence of a scleral site of action for muscarinic antagonist control of myopia and the most likely site of action is in the retina.

Point 3: Muscarinic antagonist control of myopia is mediated via M_1 and M_4 muscarinic receptors.

Atropine is a non-selective muscarinic antagonist with high affinity for all five muscarinic receptors. The fact that a partially-selective M_1/M_4 antagonist has been shown to prevent myopia progression by approximately 50% in children¹¹ implicates M_1 and M_4 receptors. The advent of highly-selective or in some cases specific muscarinic antagonists (see

Näreoja *et al.*,¹²) provides the opportunity to dissect out specific muscarinic receptor involvement in atropine's inhibition of myopia. In particular MT7 (M1 antagonist with over 1000 fold higher affinity for M1 over other receptors) and MT3 (M4 antagonist with over 100 fold higher selectivity for M4 than M1) have recently been demonstrated to inhibit axial myopia in a mammalian model of myopia using physiologically relevant nanomolar doses at the retina (see figure 1; Arumugam & McBrien,⁸). Although MT3 has recently been reported to also have high affinity with some adrenergic receptors as well as the M4 receptor, the same study found MT7 to be specific for the M1 muscarinic receptor, with no cross-reactivity to other receptors.¹² The above findings, demonstrating that an M1 specific antagonist and a highly-selective M4 antagonist inhibit myopia, strongly implicate both the M1 and M4 muscarinic receptor signalling pathways in the mechanism by which atropine prevents myopia. Thus, the development of highly selective muscarinic antagonists which target just M1 and M4 muscarinic receptors offers future possibilities for myopia control in human.

Conclusion

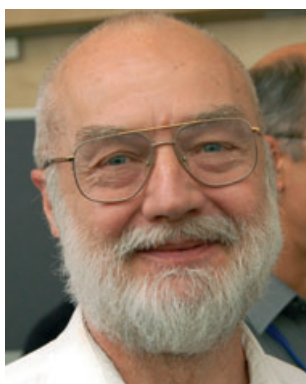
There are still unanswered questions as to the full signalling pathway by which atropine prevents axial myopia in human and animal models and further investigations are required to be conclusive. However, the recent evidence favours a retinal based mechanism mediated via muscarinic receptor signalling.

Counterpoint

William K. Stell

*Department of Cell Biology and Anatomy;
Hotchkiss Brain Institute and Alberta
Children's Hospital Research Institute;
Lions' Sight Centre;
University of Calgary Faculty of Medicine,
Calgary, AB, Canada*

E-mail: wstell@ucalgary.ca



Brittany Carr

*Lions' Sight Centre;
Graduate Department of Neuroscience;
University of Calgary Faculty of Medicine
Calgary, AB
Canada*



Atropine, a broad-spectrum muscarinic acetylcholine (mAChR) receptor antagonist, is known to retard the development of myopia in both human and animal models. Although atropine is widely believed to prevent myopia specifically by acting via muscarinic receptors, probably in the retina, the evidence for this is meagre. Here we review the evidence that atropine may prevent myopia via a non-muscarinic or non-retinal mechanism, and we argue in favour of this view.

Argument 1: Atropine prevents myopia without requiring a competing of acetylcholine (ACh).

Atropine is a competitive antagonist of ACh at orthosteric sites on mAChRs. Classically a competitive antagonist should block the action of the agonist, in this case ACh, but should have no action on its own. However, atropine was found to inhibit glycosaminoglycan synthesis (and thus presumably scleral expansion) by isolated chicken scleral chondrocytes, in the absence of any apparent source of ACh.⁵ Atropine and another competitive mAChR-antagonist, pirenzepine, inhibit form-deprivation myopia in chicks whose retinas lack 90% of normal ChAT⁺ (choline acetyltransferase-positive: acetylcholine-synthesizing) activity, as a result of ablating most of the ChAT⁺ amacrine cells.¹³

Argument 2: Many other mAChR antagonists, which should prevent myopia if atropine does so by acting at mAChRs, fail to do so.

Atropine, a broad-spectrum drug, presumably is capable of acting at any chick mAChR; therefore, its anti-myopia action

should be replicated by any other broad-spectrum muscarinic antagonist, as well as by certain subtype-specific antagonists. However, its anti-myopia action in chicks is replicated by only a minority of agents tested; other than atropine and pirenzepine,^{13,14} only himbacine (M2/4-preferring),¹⁵ oxyphenonium (pan-specific)¹⁶ and MT3 (M4)¹⁰ have been found to inhibit myopia development strongly in chicks and mammals. In contrast, a number of antagonists that are expected to act preferentially via M1 (benztropine, dicyclomine), M2 (AF-DX-116, methoctramine, gallamine), M3 (4-DAMP, HHSiD, pf-HHSiD), or via all mAChRs (mepenzolate, procyclidine) including M4 and M5, were ineffective.^{14,16} Since most of these studies have been carried out in the chick, one might argue that the negative results were due to insufficient cross-reactivity or specificity of drugs at the orthologous chick receptors, since the binding and actions of most agents have been characterized only with mammalian mAChRs. Furthermore, it is equally plausible that some positive results were due to binding of muscarinic ligands at receptors other than the ones to which they are known to act in mammals, i.e., at other isoforms of cholinergic receptors. A case in point is pirenzepine, a relatively selective mammalian M1 receptor antagonist: in chicks, which appear to have no M1 receptor, pirenzepine binds to a genetically modified M2 receptor.¹⁷ Both chick m2 and m4 genes, stably transfected into Chinese hamster ovary (CHO) cells, express receptors that bind the nonspecific muscarinic antagonists quinuclidinyl benzilate (QNB) and atropine, as well as pirenzepine and AF-DX 116, with high affinity;¹⁷ yet QNB and AF-DX 116 were found to be ineffective against form-deprivation myopia in chicks.^{14,16}

Argument 3: Atropine may affect eye growth via receptors other than mAChRs.

Atropine is known to be capable of acting on targets other than muscarinic receptors. One example is non-competitive inhibition of cholinergic muscle end-plate currents (nicotinic receptors) with half-maximal inhibition at 2×10^{-5} M (see Feltz *et al.*,¹⁸ for data & early summary). Still other actions, involving modulation of K^+ and Ca^{2+} channels, have been reported; these, however, could be mediated by the molecular target(s) of atropine, including muscarinic receptors, and not due to direct actions of atropine on those channels.

Prevention of myopia by nonspecific or non-retinal actions of atropine is especially likely, because it must be used at very high concentrations (typically approaching millimolar [10^{-3} M]; e.g., Fischer *et al.*,¹³) to prevent myopia in chicks. In contrast, in typical muscarinic receptor test systems such as chicken heart or ileum, and mammalian tissues, atropine is effective in the near-nanomolar range [10^{-9} – 10^{-8} M]. It is beyond reason that

the calculated concentrations of atropine reaching muscarinic receptors in the retina could have been in error by a factor $\geq 10^4$ [i.e., 10^{-4} – 10^{-3} vs 10^{-9} – 10^{-8}]. It follows, therefore, that intravitreal atropine (and other agents found to be at least somewhat effective at similarly high concentrations¹⁶) exert their myopia-suppressive effects either (1) upon non-muscarinic receptors in retina/RPE, to which they bind with much lower affinity than to muscarinic receptors; or (2) upon high-affinity receptors in (e.g.) choroid or sclera, which they reach only after diffusion and dilution.

Argument 4: Pharmacology of atropine and other muscarinic antagonists has not been sufficiently well characterized in the most-studied animal models of myopia.

It remains to be demonstrated that the prevention of myopia by presumed m4-antagonists, himbacine and MT3, is mediated by binding to m4 in chicks. In contrast, cross-reactivity of some muscarinic antagonists with adrenergic receptors is likely, given the common molecular origins and high degree of homology of these two receptor families.¹⁹ In fact, MT3 has been shown to bind with high affinity to α -adrenergic receptors in mammals.¹² If this is true of MT3, it may be also be true of atropine or any other muscarinic receptor antagonist. Even in mammalian (especially human) test systems, in which most pharmacological characterizations have been performed, specificity is determined entirely with reference to a particular type of receptor and its binding of (or activation by) a particular one or few ligands. One would not know whether MT3 binds non-specifically to adrenergic receptors with high affinity, without first inquiring whether MT3 interacts with any other type of receptor in the retina/eye that may instead be responsible for control of eye growth. This question is rarely asked; therefore, claims that an agent is 'highly specific' for any particular receptor in chicken, tree shrew, or even human retina, are unfounded. To date there is no compelling reason to believe that atropine binds, or prevents myopia development, exclusively via muscarinic receptors.

Conclusion

The results of many studies are consistent with the interpretation that atropine and other muscarinic antagonists prevent myopia by action on m4-mAChRs. However, the critical data are too meagre and there are too many unanswered questions for these interpretations to be conclusive. Therefore, any claims that a pharmacological agent or its mechanism of action is 'highly specific' for a particular molecular target, should be viewed with scepticism.

Acknowledgement

WKS gratefully acknowledges the support of a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) and support for graduate student training from the Foundation Fighting Blindness (Canada)/EYEGEYE Research Training Fund and an Odd Fellow/Rebekah Visual Research Student Award (University of Calgary) to BC.

SUMMARY

Debora Nickla

*Department of Biomedical Sciences and Disease,
New England College of Optometry, Boston, USA*

E-mail: NicklaD@neco.edu

The use of atropine to slow the progression of myopia in Asian children has been in practice for decades, despite deleterious side effects such as photophobia and cycloplegia. Both sides of this debate agree on the value of more research to determine the mode and site of action of atropine.

POINTS OF AGREEMENT

- Atropine, a non-selective muscarinic acetylcholine antagonist, inhibits myopia development in both humans and animal models.
- In humans, atropine causes cycloplegia, however, the mechanism whereby it slows myopia progression is *not* via paralyzing accommodation.
- There is the potential that anti-myopiagenic effects similar to that of atropine but with fewer attendant adverse side effects may be achieved with anti-muscarinic drugs having narrower selectivity.

ISSUES TO BE RESOLVED

- The ineffectiveness of many nonselective muscarinic receptor antagonists in animal models begs the question of which receptor subtype(s) mediates the anti-myopiagenic effect in children. In the tree shrew, a mammal, evidence supports an M1-mediated mechanism. In the chick, one of the most widely used models, the selectivities of the various anti-muscarinic drugs are largely unknown, making interpretation problematic.
- It is not known to what extent atropine binds non-muscarinic receptors, and whether such binding augments, or accounts for, its anti-myopiagenic effects. Dr. Stell points out that MT-3 shows high-affinity binding to α -adrenergic receptors, and reasons that atropine may do so as well.
- There is debate about how the tissue barriers between the retina and sclera would slow and dilute the concentration of atropine, if it were acting at a non-retinal site. From animal experiments using intravitreal drug delivery, does the high dose required for atropine's effect imply low-affinity binding of non-muscarinic retinal/RPE receptors, or high-affinity binding in choroid/sclera? And, in children, what are considered high concentrations for topical application, and how should effective anti-muscarinic doses be viewed with regard to potential non-muscarinic receptor interactions?

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