MECHANISM OF ACTION OF ATROPINE IN THE TREATMENT OF MYOPIA IN CHILDREN

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Summary
Atropine drops are one of the most effective approaches towards inhibiting progression of children myopia. However, the mechanism of atropine action on myopia remains a mystery. This article reviews the more than 50-year history of the attempts to reveal the secret of the mechanism of action of atropine and outlines possible locations for its receptors. MEDLINE database have been used as the source of scientific literature using English language keywords. A total of 14 full-text articles in English were selected, the main topic of which was relevant to the aim of the review. Supplementary articles with partial relevance to the main topic were used to provide context. The literature review reveals that the atropine’s mechanism of action towards inhibiting progression of children myopia remains unclear, but the topic remains relevant for further study.

Introduction
Myopia is a refractive error of the eye that is steadily increasing in prevalence. [1]. In 2020, there will be over 2 billion myopes (≤ -0.5 D) worldwide. [1,2]. By 2050, the number of myopes in the world is projected to reach 5 billion - more than half of the total population will be short-sighted [2]. In addition to the financial burden of several hundred billion US dollars worldwide [3], myopia, especially high myopia, is also a risk factor for diseases such as glaucoma, cataracts and retinal detachment [4], and various measures are being sought to slow down the progression of myopia. Of the currently available medical and optical methods, atropine eye drops are considered to be the most effective [5].

"Deadly nightshade" (Atropa belladonna), a natural source of atropine, has been known since ancient Indian and Egyptian civilisations. Atropine-containing preparations have been used as poisons, asthma relievers and aesthetic treatments to dilate women’s pupils [6]. The purification of atropine crystals was achieved in 1831 and synthetic atropine in 1901 [6]. Systemic atropine is administered intravenously, intrareally, subcutaneously or orally for the treatment of bradycardia, salivary hypersecretion, for the relief of smooth muscle spasm, in case of poisoning by organophosphorus compounds, and for the preparation of the patient before general anaesthesia [7]. In today’s ophthalmic practice, atropine eye drops (also available in ointment form in other countries) are used as long-acting cycloplegics and/or mydriatics prior to fundus or refractive examinations, eye surgery, or in the treatment of uveitis or amblyopia [8].

Long-term follow-up studies on the effect of atropine on slowing down the progression of myopia have been ongoing since 1964 [9], when Bedrossian and Gostin presented the results of a clinical trial at the 1st International Myopia Conference [10]. The ATOM1 clinical trial from 1999 to 2004 demonstrated the effectiveness of 1% atropine eye drops in slowing the progression of myopia, but with significant side effects. [11]. In the ATOM2 clinical trial, the best therapeutic index (risk-benefit ratio) was 0.01 % atropine when different lower atropine concentrations were tested [12]. In the 2018 LAMP study, the investigators reported a better effect with 0.05 % atropine [13], but a similar study by Shifei Wei’s et al. team at the same time argued more in favour of using 0.01 % atropine. [14]. The limitation of the above studies is that they are limited to the Asian population. In a smaller clinical study in Europids, 0.01 % atropine drops slowed down the progression of myopia to a statistically significant degree compared to a control group. [15]. Despite the success of the trials, atropine drops are not prescribed according to the manufacturer’s instructions (ang. off-label) to slow the...
progression of myopia. In 2022, just over 50 years after the start of the trials, with the knowledge of the best therapeutic index concentration of atropine drops for long-term progression inhibition, the exact mechanism of action of atropine is still unknown [16,17].

**Purpose of the article** – to review scientific articles published on the internet on the mechanism of action of atropine in the treatment of myopia in children.

**Method and Methodology**

The research method is a review of the scientific literature. The MEDLINE database from 1979 to July 2022 was used to collect the data. From the 26 articles found by keyword searches, 14 full-text articles in English were selected for the review, the main topic of which was relevant to the aim of the review. Supplementary articles with partial relevance to the main topic were used to provide context.

**Results**

**Mechanism of action of atropine via muscarinic acetylcholine receptors in ciliary muscle.** The first studies on atropine inhibition of myopia progression in children were based on the theory of the accommodative mechanism [18]. When the ciliary body is contracted, the Zinn ligaments are relaxed, the natural elasticity of the lens causes it to collapse and its refractive power is increased. In that way the eye adapts to close range viewing. However, the tightened ciliary body also increases the tension of the choroid and, through it, the sclera. A nerve signal from the sclera is transmitted to control centres in the cerebral cortex and the subcortex, thus feedback stimulates the elongation of the eye axis. [19]. Therefore, as a non-selective antagonist of muscarinic acetylcholine receptors (mAChRs), atropine was thought to inhibit the progression of myopia by blocking accommodation through the inhibition of M3 receptors, which are abundant in ciliary muscle. [20].

The theory was challenged by studies with selective mAChR antagonists: Stone et al. (1991) found that only the M1 receptor subtype (in 2012, Arumugam et al. added the M4 subtype), and not the M3 receptor, was associated with slowing down myopia progression [21,22]. It was also found that the effect of atropine on myopia progression is maintained independently of ciliary muscle contraction during accommodation. In a 1993 pre-clinical study by McBrien et al., atropine was administered to chickens in which the ciliary muscle is striated and activated via nicotinic rather than muscarinic acetylcholine receptors. Accommodation or pupillary constriction was induced by additional administration of the cholinomimetic carbachol or a light stimulus. According to the investigators, the effect of atropine on the inhibition of myopia progression occurred by a different, non-accommodative mechanism [23].

**In retina.** In 1985, Raviola and Wiesel hypothesized that the growth of the eye is controlled by the retina, which responds to light stimulation and releases regulatory molecules in response to it. [24]. According to McBrien et al. (1993), the control of myopia progression is mediated through the effect of atropine on retinal neurotransmission [23] by atropine binding to M1 receptors on retinal cholinergic amacrine cells [25]. However, the involvement of cholinergic amacrine cells in the growth mechanism of the eye was ruled out by Fischer et al. in 1998, when they removed amacrine cells. The chick eyes still became myopic under direct occlusion and were prevented from developing myopia by concomitant administration of atropine. According to the researchers, the mechanism of action of atropine is not through the mAChRs of the cholinergic amacrine cells, but through the mAChRs located in the choroid, sclera or retinal pigment epithelium (RPE) [26].

According to the „termination“ theory, related to retinal neurotransmission, atropine slows down the progression of myopia through the short-term cessation of the growth stimulus to the eye [27]. The theory was formulated by Schwahn et al. in 1999. The authors linked three findings: 1) not only atropine but also apomorphine (a non-specific dopamine agonist) was found to inhibit the progression of myopia via D2 receptors [28]; 2) an injection of atropine into the vitreous for an hour increases the amount of dopamine secreted by the retinal cells [27]; and 3) myopia induced by direct occlusion was counteracted by the presence of a minimum of half an hour of normal vision per day in a chick [29]. According to Schwahn et al. by an unknown mechanism, atropine induces spreading depression potentials in the retina, which depolarise retinal neurons and release their neurotransmitter stores, which contain dopamine [27]. In 1998, Fischer et al. found that M4 receptors are located close to dopaminergic amacrine cells, to which atropine could bind and regulate dopamine synthesis [30].

**In retinal pigment epithelium.** mAChRs are also found in the retinal pigment epithelium [31]. The RPE is an important intermediate between the retina, choroid and sclera, with homeostatic, protective barrier and other functions. The RPE is also a source of cytokines and growth factors possibly involved in the growth of the eye [32]. After observing that atropine dripping induced spreading depression potentials not only in the retina but also in the RPE, Schwan et al. (2000) suggested that the RPE is also a potential site for the initiation of ocular axis elongation [27]. However, according to Zhang et al. (2015), further evidence is needed [32].

**In sclera.** Lind et al. present an alternative theory explai-
ning the mechanism of action of atropine in slowing down the progression of myopia through its effect on the sclera [33]. Following the finding that the posterior segment of the eye increases in size as a result of hyperplasia of scleral chondrocytes and increased synthesis of extracellular matrix [34], and that there are cells, such as astroglia [35] whose proliferation is stimulated by the induction of the mACrRs, Lind et al. (1998) hypothesized in 1998 that the ocular axis is lengthened by the binding of acetylcholine to the mACrRs in the sclera [33]. The presence of mACrRs in the sclera was verified by Stone et al. in 1991, when atropine was injected under the conjunctiva to reach the sclera, but limiting the contact of atropine with the retina. The development of myopia induced by direct occlusion was statistically significantly inhibited by atropine and the M1 receptor inhibitor pirenzepine [21]. However, Lind et al. do not exclude the importance of the retina in the development of myopia with this discovery and argue that it is important to establish the correlation between the retinal and scleral pathways [33].

In 2019, Hsiao et al. extended the discovery of the effects of atropine on scleral fibroblasts by applying next-generation sequencing (NGS) technology and bioinformatics analysis. Of the 389 genes associated with ocular growth, 14 canonical pathways were generated, two of which were statistically significantly associated (p < 0.01) with the inhibition of fibroblast remodelling through the inhibition of melatonin degradation [37]. Two melatonin receptors, MT1 and MT2, are also found in the sclera [38]. Melatonin together with dopamine is involved in the coordination of the circadian rhythm of the eye, which is essential for the regulation of eye growth [39,40]. Constant light or short periods of illumination at night can disrupt the normal growth of the eye and lead to refractive errors [38]. Light at night, according to Hamm et al, sharply reduces melatonin production and release [41]. Based on bioinformatics analysis and the fact that atropine is administered at bedtime, Hsiao et al. proposed that atropine, by acting on scleral fibroblasts, inhibits the degradation of melatonin during the night, thus preventing fibroblast remodelling and ocular axis elongation [37].

In choroid. Based on the anatomical localisation of the choroid between the retina and the sclera, the choroid has also been investigated as a potential site that could initiate the growth of the ocular axis. Nickla et al. (2010) observed that the choroid thins with the development of myopia and thickens with atropine administration [42]. The choroid, like the retina or sclera, contains all mACrR subtypes [36]. In 2013, Nickla et al. conducted studies with specific mACrR agonists and antagonists and found that the mechanisms are different: the choroid thins when acetylcholine is applied to the extravascular smooth muscle via the mACrR, but thic-

kens, possibly via the nitricergic or dopaminergic system [42].

Regardless of mACrR localisation

Based on studies suggesting that natural daylight reduces the risk of developing myopia [44] and the fact that nitric oxide (NO), one of the signalling molecules, is involved in the light reactions of photoreceptors [45], Carr and Stell hypothesized in 2016 that NO is involved in the development of myopia [46]. To test the relationship between atropine and NO, atropine, nitroprusside and stereospecific NO precursors (D, L-arginine) and NO synthase inhibitors (NG-(1-iminoethyl)-L-ornithine [L-NIO], L,D-NG-monomethyl arginine [D,L-NMMA]) were used for injection in various combinations. NO has been shown to be essential for the therapeutic effects of atropine. The authors propose two possible mechanisms of action of atropine via the mACrR:

1) by a direct pathway, atropine binds to M2/M4 receptors, thereby excitatory and depolarising the cell, increasing intracellular calcium concentrations, which stimulate the activity of NO synthases; 2) by an indirect pathway, atropine binds to M1/M3/M5 receptors, disrupting the effect of inhibitory neurotransmitters on NO synthases [46].

Mechanism of action of atropine not via muscarinic acetylcholine receptors

In addition to the theories discussed above, there is growing evidence that atropine may slow down the progression of myopia through a mechanism other than mACrR. Following the finding that retinal dopaminergic neurons also contain α2A-adrenoceptors (α2A-ADRs) in addition to the mACrR, which are also stimulated by dopamine release when inhibited by ADR antagonists, Mathis et al. hypothesized in 2020 that atropine may slow down myopia progression via α2A-ADRs [47]. The hypothesis is strengthened by the demonstration by Carr et al. (2018) that pirenzepine (a selective M1 antagonist) and muscarinic toxin 3 (a selective M4 antagonist), like atropine, bind to the α2A-ADR [48]. Lochner et al. also observed a link between atropine and serotoninergic receptors in 2016 [49]. Radioimmunoassays have localised 5-HT receptors in retinal amacrine cell bodies. These cells have been observed to be more abundant in myopic eyes, suggesting that atropine may inhibit the progression of myopia through inhibition of serotonin release [50].

Conclusion

There is no consensus on the mechanism of action of atropine in slowing down the progression of myopia. The theories of the mechanism of action can be divided into two groups: 1) via the mACrR and 2) not via the mACrR. The first group of theories explains the mechanism of action through the different muscarinic acetylcholine receptors (M1-M5) and their localisation in the eyeball (ciliary muscle,
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