NANOPARTICLES IN GLAUCOMA TREATMENT

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S u m m a r y. Glaucoma is characterized by the progressive death of the retinal ganglion cells (RGCs), followed by the excavation of the optic nerve disc and gradual loss of the visual eld. Different risk factors are associated with the development of glaucoma, the major of which are increased intraocular pressure (IOP), advanced age, black race and positive rst-degree family history. Female gender increases the risk of primary angle-closure glaucoma. A new approach to drug delivery could overcome the limitations of the current glaucoma therapies. Recent studies have shown that nanoparticles could be used for modi cations of the drugs. Nanoparticles are nanostructured materials of unique properties. As investigations advance to clinical trials, it will be crucial to evaluate the possible risks and adverse effects of nanoparticles. Further detailed studies into nanoparticles will determine their future potential clinical application. Hopefully, the advancement of nanotechnology will overcome limitations of current treatments for this debilitating disease.
K e y w o r d s: glaucoma, nanoparticles.

INTRODUCTION

Glaucoma is a major cause of irreversible blindness worldwide. It is estimated that approximately 70 million people across the world suffer from this debilitating disease [1]. Glaucoma is characterized by the progressive death of the retinal ganglion cells (RGCs), followed by the excavation of the optic nerve disc and gradual loss of the visual eld.

Different risk factors are associated with the development of glaucoma, the major of which are increased intraocular pressure (IOP), advanced age, black race and positive rst-degree family history. Female gender increases the risk of primary angle-closure glaucoma [2]. Despite the wide range of potential risk factors, high intraocular pressure is believed to play main role in developing glaucoma [3]. Therefore, most of current treatments of glaucoma are focused on lowering IOP.

First-line glaucoma treatment is represented by topical eye drops. There are two main groups of medicines used for lowering intraocular pressure. First group focuses on suppressing the production of the aqueous humor at the ciliary body. It is represented by inhibitors of carbonic anhydrase, beta-blockers and alpha-agonists. On the other hand, drops containing prostaglandins and cholinergic receptor agonists, which belong to the second group, increase the outflow of aqueous humor through the trabecular meshwork [4].

Despite the effectiveness of modern anti-glaucoma pharmacological therapies, they have several limitations. One of them is the occurrence of side effects; red eyes, irritation or allergies are amongst the most common. Prostaglandins, in addition to eyelashes extension, which is usually more desired than unwanted effect, may also cause keratitis and increased pigmentation of iris. Cholinergic receptor agonists, on the other hand, cause pupil contraction, as opposed to dilation effect of alpha1-agonists. Both contraction and dilation of the pupil impede visual acuity. Additionally, systemic absorption of drugs may cause other side-effects as shown in Table 1 [5].

Table 1. Main systemic side effects of anti-glaucoma eye drops [5].

Beta-blockers	Bradycardia, heart blocks, bronchial contraction, decrease of libido
Alpha1-agonists	Hypertension, tachycardia, headache
Alpha2-agonists	Hypotension, dry mouth, fatigue, insomnia
Cholinergic-agonists	Salivation, hyperacidity
Carbonic anhydrase inhibitors	Bitter taste in mouth
Prostaglandins	Pain of muscles and joints, headache, flu-like symptoms

Another limitation of eye drops is their poor adhesion to the surface of the cornea. A precorneal tear film constituted by deep mucous layer and superficial aqueous layer is constantly cleared by blinking. Therefore, half-life time of a drop in the precornea is approximately 1 minute [6]. During this time drug has to cross the cornea to access the aqueous humor. As a result, bioavailability of a drug administrated on the eye is low, less than 10% of the drug is absorbed into to the eye and approximately 1% reaches the aqueous humor [7, 8]. Even when extending the exposure time by using for example gels or inserts, there are other factors that limit the absorption of the drug. One of them is the corneal epithelium, which slows down the perfusion of the drug, and for particles over 500Da is completely impermeable [9]. Bigger particles, however, can penetrate through the conjunctiva and the underlying sclera, which are more permeable than the cornea. Additionally, surface of the conjunctiva is larger than surface of the cornea. Nevertheless, 80% of the drug is absorbed to the systemic blood vessels through conjunctiva, vasculature, lowering the amount of drug in desired tissues and increasing the risk of systemic side effects described above [10]. Particles of medicine that pass into the anterior chamber stay there only for about 2 hours before being filtered through the trabecular meshwork, which is another disadvantage of current eye drops [11].

The main disadvantage of therapies based on lowering IOP is that neuroprotection offered by them is indirect; none of currently used topical drops targets the retina. Another path of delivering drugs to target tissue is intravitreal injection [12]. This procedure bypasses most barriers and delivers the drug directly to the vitreous body offering higher concentration in target tissue. It is crucial for therapies focused directly on protection of the retinal ganglion cells rather than reducing intraocular pressure. The injections, however, introduce other wide range of risks [13], including infection or even endophthalmitis.

Administration frequency is another issue. Current drugs have to be administrated very often, 1-3 times a day in most cases. That is one of the reasons why therapeutic compliance is decreased. Patients often forget to use prescribed drug. Moreover, the technique of administration is poor in most cases. One study found that 9 out of 10 patients did not apply their drops to the eye properly [14].

A new approach to drug delivery could overcome the limitations of the current glaucoma therapies. Recent studies have shown that nanoparticles could be used for modifications of the drugs. Nanoparticles are nanostructured materials of unique properties. They may be constructed from various materials like metals, polymers or lipids. Due to their small size, which is their key feature, they present different physical properties from those typical of classic medicaments alone, because, unlike currently used medications, interactions of nanoparticles are best described by the quantum mechanisms. Another feature is their higher surface-to-volume ratio, so they offer more sites for chemical reactions [15]. By combining nanoparticles with drug particles, the latter can be protected from degeneration while delivered to the target tissue. Moreover, nanoparticles covering the drug can regulate its release [16].

Considering the advantages of nanoparticles many studies attempt to evaluate their potential application in glaucoma therapies. Rui et al. [17] in their study used solid lipid nanoparticles (SLNs) to deliver methazolamid (MTA) into the eye. Unfortunately, this antiglaucoma drug administered systemically penetrates poorly into the aqueous humour. Therefore, a drug has to be administered frequently and in high concentration, which causes many systemic side effects including vomiting, renal failure, depression and anorexia [18]. Topical administration of MTA, on the other hand, is limited by its low solubility in water and the impermeability of the cornea [19]. However, the authors of the study combined MTA with SLNs, formulating the modified drug as ocular eye drops. The advantage of SLNs is that they penetrate into deeper layers of the eye, including the aqueous humour, with more ease. As the authors indicate, therapeutic efficiency of MTA-SLNs was higher, the maximum concentration occurred later, and its effect lasted longer compared to drug solution or commercial product [20].

Another study [21] showed that liposomes could offer an efficient method of drug delivery

to target sites. Natarajan et al. fabricated latanoprost –loaded egg-phosphatidylcholine (EggPC) liposomes. Latanoprost is an antiglaucoma lipophilic drug, very effective in reducing intraocular pressure [22]. However, its active form, latanoprost acid, is more hydrophilic and has lower bioavailability due to higher penetration resistance through the layers of the cornea. Liposomes, because of their physical structure, are able to bind both hydrophilic and lipophilic forms of latanoprost [23]. Liposomal encapsulation increases stability of the drug by protecting it from hydrolase-dependent decay in the eye tissues. Unfortunately, studies showed that the penetration into the eye of liposomes administered topically is poor [24]. However, liposomes could prolong the effects of subconjunctival injections, since other studies demonstrated the limited sustainability of current drugs delivered that way [25, 26]. The researchers from Singapore monitored rabbit eyes after subconjunctival injection of the EggPC liposomes loaded with latanoprost. The results were promising. During *in vitro* phase 60% of latanoprost was released during 14 days in a slow and sustained manner. *In vivo* results were even better; a study demonstrated that a single injection lowered intraocular pressure for up to 90 days, and IOP decrease was signi cantly greater compared to daily topical administration of latanoprost [27].

Similarly, Kadam and colleagues performed subconjunctival injections with poli-L-lakcid microparticles. Microparticles have a lower surface-to-volume ratio that provides a slower release of drug and allows for a bigger reservoir of the medicine. The researchers found, poli-L-lakcid microparticles released triamcinolone for at least 2 months after the injection. This method was used to deliver triamcinolone to the eyes with choroidal neovascularization with promising results [28]. Another *in vitro* study demonstrated that antiglaucoma therapeutic, timolol, exhibited relevant concentration level in the anterior chamber for over 3 months after subconjunctival injection of microparticles containing the drug [29].

Nanoparticles made of other materials can provide similar bene ts. Bhagav et al. [30] used mucoadhesive Eudragit polymer to encapsulate brimonidine tartare (BRT), and studied the release time of the drug. Eudragit-BRT nanoparticles were prepared as topical drops in phosphate-buffered saline, and compared to a market drug formulation in glaucomatous rabbits. The results showed that the nanoparticles decreased the level of IOP for 72 hours, compared to 6 hours obtained by conventional drugs. Additionally, the solution was well tolerated, with no signs of irritation or toxicity. Likewise, Wadhwa et al. [31] combined timolol maleate with chitosan, a mucoadhesive and biodegradable polymer. Similarly, an *in vivo* study was performed in rabbits Chitosan-timolol topical formulation lowered IOP more effectively than timolol eye drops available on the market. Thereafter, the effect was prolonged by further combing the particle with hyaluronic acid. Hyaluronic acid strengthened mucoadhesion of chitosan, which improved pharmacodynamics of the released drug and resulted in longer IOP decrease.

Another promising direction is using nanoparticles in gene therapy. In glaucoma models, adeno-associated viruses (AAV) were effective in the transduction of neuroprotective genes to the ganglion cells [32] and the trabecular meshwork cells [33]. The limitation of AAVs, however, is their high production cost, the size of transducted genes and safety concerns. Nanoparticles can be a safer and cheaper alternative. DNA can be delivered directly to target cells by cationic polymers similarly as done by bacteriophages. DNA packed inside nanoparticles can be released to target cells of the host after reaching the target tissue. When using these nanoparticles, researchers observed better releasing ef cacy and higher expression of genes compared to bacteriophageal transduction [34]. Another study by Farjo et al. evaluated the ef cacy of gene transduction in mice by subconjunctival injection of glycol-substituted lysine peptide nanoparticles complexed with plasmid DNA. The results showed that nanoparticles were capable of transfecting almost every type of cell in the eye and the transfected cells exhibited solid dose-dependent levels of gene expression. The examined nanoparticles did not provoke any immune responses. Moreover, the plasmid size delivered that way is theoretically unlimited, which provides the whole spectrum of new opportunities. However, as the authors highlighted, the most impressive fact was that transfection succeeded in nearly all of the photoreceptor population, and it exhibited expression levels almost as high as that of rodopsin, the retina's highest expressed gene. Additionally, it is possible to further modify the nanoparticles by adding ligands speci c to trabecular meshwork and thus expanding the options for glaucoma treatment [35].

CONCLUSIONS

Nanotechnology is a new approach to glaucoma treatment and, as most researchers highlight, needs further investigation. It is important to note, that the procedures involving nanoparticles have not been studied in humans, so the outcomes of the studies are limited. However, the current results are encouraging. It is proven that antiglaucoma drugs modified by nanoparticles have better bioavailability and longer half-lives in target tissues. Moreover, nanoparticles are able to release the drug in the course of months. These factors will hopefully reduce the number of applications, which is crucial for patient's compliance, as well as can lower the side effects of therapies. By functionalizing nanoparticles with specific ligands it is possible to achieve more targeted therapy, directing it to desired tissues like the trabeculum or to the retinal cells. Using nanoparticles as gene vectors can be a milestone in glaucoma treatment, as modification of genes responsible for the aqueous humour outflow could even result in a permanent IOP decrease. As investigations advance to clinical trials, it will be crucial to evaluate the possible risks and adverse effects of nanoparticles. Further detailed studies into nanoparticles will determine their future potential clinical application. Hopefully, the advancement of nanotechnology will overcome limitations of current treatments for this debilitating disease.

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