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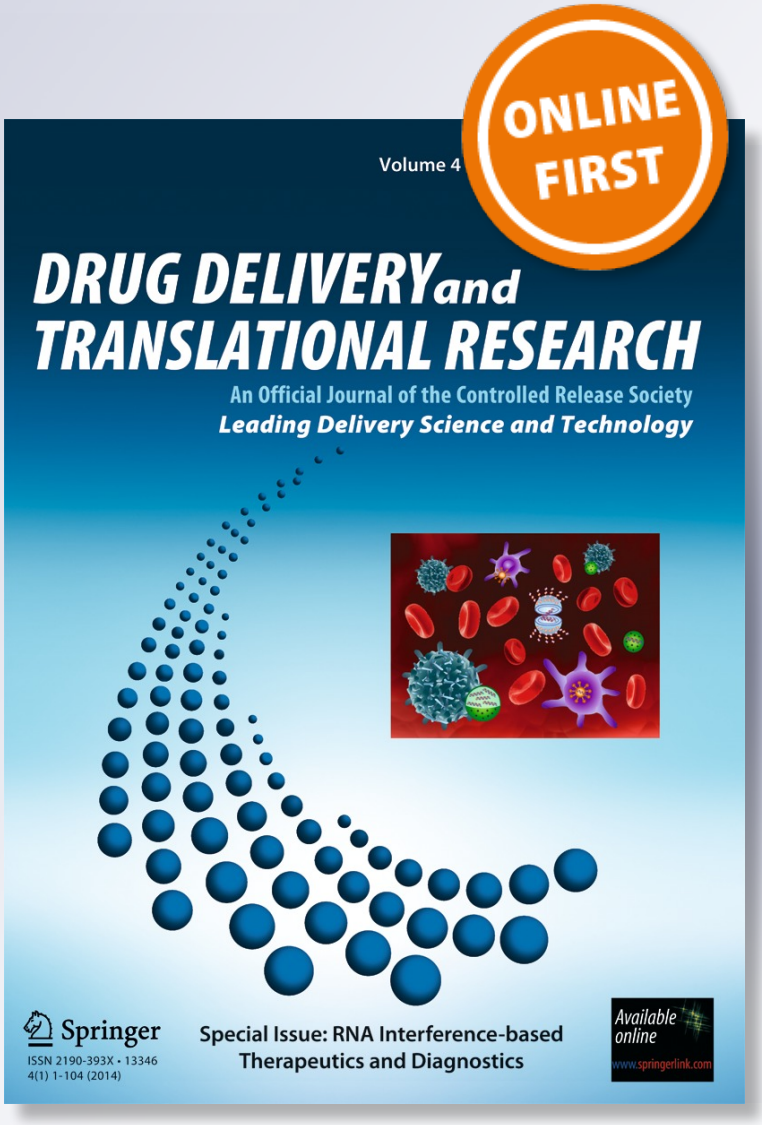

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CLINICAL TRIAL

Nanomedicine for glaucoma: sustained release latanoprost offers a new therapeutic option with substantial benefits over eyedrops

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Abstract Glaucoma is a chronic progressive optic neuropathy that is characterized by optic nerve changes and visual field loss. Elevated intraocular pressure (IOP) is the main modifiable risk factor. Chronic instillation of daily eyedrops to lower IOP is the primary treatment of choice, although it requires patient adherence and correct performance. We have developed a nanoliposome drug delivery system for the longer term delivery of latanoprost. In the present open-label, pilot study, the safety and efficacy of a single subconjunctival injection of liposomal latanoprost was evaluated in six subjects with a diagnosis of either ocular hypertension (OHT) or primary open-angle glaucoma (POAG). Subconjunctival injection of liposomal latanoprost was well tolerated by all six subjects. From a baseline IOP of 27.55±3.25 mmHg, the mean IOP decreased within 1 h to 14.52±3.31 mmHg (range 10–18 mmHg). This represented a mean decrease of 13.03±2.88 mmHg (range 9–17 mmHg), or 47.43±10.05 % (range 37–63 %). A clinically and statistically significant IOP reduction (≥20 % IOP reduction, P=0.001 to 0.049) was observed through 3 months after injection. The nanomedicine reported here is the first nanocarrier formulation that has an extended duration of action in humans, beyond a couple of weeks. The findings in this study open up a new treatment modality, which will greatly enhance patient compliance and improve treatment outcomes. The current study provides the evidence and support for further clinical studies of liposomal latanoprost in the treatment of glaucoma.

Keywords Nanomedicine · Glaucoma · Sustained release · Human subjects · Patient compliance · Liposomal latanoprost

Introduction

Glaucoma is a chronic, progressive optic neuropathy that causes irreversible blindness. It is the major cause of irreversible blindness worldwide [1]. The global burden of glaucoma is estimated to rise to affect 80 million worldwide by 2020, primarily due to an increasing ageing population in the world [2]. Elevated intraocular pressure (IOP) is the only known effective modifiable risk factor. The use of daily eyedrops to lower the IOP remains the first line treatment for glaucoma.

Glaucoma is an asymptomatic and chronic disease that often requires long-term ocular hypotensive medical therapy. Like any chronic treatment, ocular hypotensive agents, in this case in the form of eyedrops, require patient adherence. In addition, eyedrops have the added issue of correct performance, that is, patients have trouble using eyedrops and applying them correctly in the eye. A consequence to poor patient adherence is treatment failure and a poor outcome from disease progression [3, 4]. It is estimated that at least

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10 % of blindness is directly attributed to poor patient adherence to prescribed medications [5].

Since the conventional eyedrops cannot have a sustained duration of action beyond a few hours due in part to low drug bioavailability, we developed a subconjunctival injection of nanocarriers incorporating latanoprost that is capable of extended duration of IOP lowering to overcome the limitations of eyedrops. The challenges here were twofold: to develop a drug-encapsulating carrier formulation that has some level of optical clarity following injection, which rules out micron-sized carriers; and to sustain the delivery of latanoprost over at least 3 months following a single injection.

Latanoprost is a well-known prostaglandin approved worldwide for daily topical use for the lowering of IOP in patients with open-angle glaucoma and ocular hypertension [6]. Our nanoliposomal latanoprost formulation with high loading of drug and an average size of 100 nm showed a reproducible and prolonged IOP lowering in rabbits [7, 8]. More recently, an IOP lowering of 120 days was demonstrated in ocular hypertensive Macaque monkeys following a single subconjunctival injection of the liposomal latanoprost delivery system [9]. Based on the demonstration of prolonged duration of action with a persistent clinically significant IOP lowering, as well as the absence of local irritation or inflammation in animals, approval was obtained for a first-in-man trial to evaluate the safety and efficacy of liposomal latanoprost.

Materials and methods

Study design

The present study was a first-in-human evaluation of the liposomal latanoprost delivery system. We sought to determine whether the efficacy observed in Macaques could be reproduced in humans and whether the liposomal latanoprost formulation was associated with any adverse events. We present the results of the study on patients with a diagnosis of ocular hypertension (OHT) or primary open-angle glaucoma (POAG), who each received a single subconjunctival injection of liposomal latanoprost. The primary outcome was safety, and the secondary outcome was clinical efficacy in terms of IOP lowering.

This was an open-label, non-comparative study planned for six patients and conducted between January and June 2013. Enrollment was open to adult patients with a diagnosis of POAG or OHT, with unmedicated IOP≥24 mmHg at two morning visits 2–7 days apart and corrected visual acuity of +1.0 logMAR by early treatment diabetic retinopathy study (ETDRS) or better (equivalent to Snellen 20/200) in one or both eyes. Excluded from the study were patients with IOP> 36 mmHg, secondary glaucomas, previous laser iridotomy, trabeculoplasty or trabeculectomy, corneal thickness less than 460 μm or greater than 620 μm, or contraindications to latanoprost.

The study was approved by the Singapore Health Science Authority (HSA) and the local research ethics committee. All patients provided written informed consent. The study was conducted in compliance with the Good Clinical Practice guidelines and the Declaration of Helsinki. The study is registered with the Singapore HSA, trial no. 12B6420C, and in ClinicalTrials.gov, trial no. NCT01987323.

Treatment procedure and outcome assessments

Study visits

Potentially eligible subjects were assessed at a screening visit. Medical and ocular histories were recorded. Visual acuity was measured by ETDRS, and automated static visual fields (if not performed within 6 months) and slit lamp examination were performed. The IOP was measured with a calibrated Goldmann applanation tonometer. All ocular measurements were performed on both eyes. A required washout period before baseline visit included 4 weeks for β adrenergic antagonists and prostaglandin analogues, 2 weeks for adrenergic agonists and 5 days for carbonic anhydrase inhibitors [10].

Following qualification, a subconjunctival injection of liposomal latanoprost was administered in one eye after the 1600 h IOP measurement. The eye was first washed with povidone iodine and topical anaesthesia eyedrops (proxymethacaine) applied, as well as a proxymethacaine-soaked cotton bud applied onto the superior bulbar conjunctiva at the proposed injection site for 30 s. A volume of 100 μL of liposomal latanoprost (1 mg/ml, mass of latanoprost injected was 100 μg and mass of liposomes injected was 1.19 mg) was injected into the superior bulbar conjunctiva using a 30-gauge needle on an insulin syringe under slit lamp guidance. After 1 h, the eye was re-examined by slit lamp microscopy and the IOP recorded. The subject was given a 5-day course of fortified topical antibiotics.

Study visits occurred at baseline day 0, week 1, week 2, month 1, month 2 and month 3 (0900 h). In addition, at baseline, month 1 and month 3, IOP was also measured at 1200 and 1600 h. At each visit, the visual acuity was measured and slit examination performed. Adverse events were monitored and recorded by the investigators throughout the study. The safety of the procedure, which included any reported symptoms of ocular irritation or pain or signs of reduced vision, redness or discharge by the subjects, was documented.

Statistical analysis

The primary endpoint was mean change in IOP from the 0900 h baseline to each timepoint. In addition, percent change was calculated, as well as categorical decreases. IOP was summarized by a continuous measure by timepoint (as applicable) using descriptive statistics (*n*, mean, median, SD, minimum and maximum). IOP was also assessed by categorical analysis using raw and percent change from post-washout baseline (visit 1, hour 0900). The null hypothesis of no change from baseline was tested by the one-sample *t* test with a 5 %, two-sided significance level (SAS System, version 9.3, Cary, NC).

In vitro studies

Preparation of large unilamellar vesicles (LUVs) The preparation strategy was similar to the method described previously [9], and an initial drug/lipid weight ratio of 0.1 was used. A known concentration of EggPC (Lipoid, GMBH) was dissolved in PBS pH 6.7 for 3 h at room temperature to form multilamellar vesicles (MLVs). Separately, a known concentration of latanoprost (Everlight Chemical Industrial Corporation, Taiwan) was dissolved in ethanol and dried away under a stream of nitrogen gas to form a thin drug film maintained at 50 °C in a water bath. A bench top extruder (Northern Lipids Inc., Canada) was used to reduce the particle size of MLVs to large unilamellar vesicles (LUVs) of ~100 nm. The MLVs were passed 3–5 times through 3×80 nm stacked polycarbonate membranes to form LUVs. The drug film was hydrated with EggPC LUVs at room temperature for 2–3 h until no oil droplets were observed on the glass walls. Liposomal latanoprost was sterile filtered using a 0.2-μm capsule filter and stored at −20 °C until further analysis.

Stability studies

Determination of particle size The particle size was measured based on the principles of dynamic light scattering using a Malvern Zeta Sizer ZS 90. Sodium chloride (0.9 %) was pre-filtered using a 0.22-μm syringe filter. Fifty microliters of liposomal latanoprost was diluted with 1.0 ml of 0.9 % sodium chloride taken in cuvette, and three independent measurements were made using Zeta Sizer. The values of average size and average polydispersity index are summarized in Fig 2.

Determination of latanoprost amount Latanoprost amount in liposomes was determined by HPLC using a gradient method. Two mobile phases were used, water/acetic acid and acetic acid/acetonitrile.

Results

Patients

Six out of the six subjects initially screened were enrolled in the study following the washout period. All subjects completed all the study visits. The demographics of the study population are shown in Table 1. Of the six patients, the mean age± SD was 64.7±17.4 years (range 39–83), 5 were male (83 %) and 4 were Chinese (67 %). In the study eye, five patients had a diagnosis of OHT and one had a diagnosis of POAG. All

Table 1 Baseline characteristics of subjects enrolled

Analysis	Statistic	(*N*=6)
Age	*n*	6
	Mean	64.67
	SD	17.44
	Median	66
	[Min, max]	[39, 83]
Sex		
Male	*n* (%)	5 (83.33 %)
Female	*n* (%)	1 (16.67 %)
Any previous glaucoma medications?		
No	*n* (%)	0
Yes	*n* (%)	6 (100.0 %)
Latanoprost		4 (66.67 %)
Bimatoprost		1 (16.67 %)
Timolol (0.5 %)		1 (16.67 %)
Race		
Chinese	*n* (%)	4 (66.67 %)
Malay	*n* (%)	1 (16.67 %)
Indian	*n* (%)	0
Other	*n* (%)	1 (16.67 %)
Right eye diagnosis		
None	*n* (%)	1 (16.67 %)
Ocular hypertension	*n* (%)	4 (66.67 %)
Primary open-angle glaucoma	*n* (%)	1 (16.67 %)
Left eye diagnosis		
None	*n* (%)	2 (33.33 %)
Ocular hypertension	*n* (%)	3 (50.00 %)
Primary open-angle glaucoma	*n* (%)	1 (16.67 %)
Any ocular surgery/laser treatment?		
No	*n* (%)	2 (33.33 %)
Yes	*n* (%)	4 (66.67 %)
Cataract surgery		4 (66.67 %)
Past medical history		
Hypertension		4 (66.67 %)
Diabetes		3 (50.00 %)
Hypercholesterolemia		3 (50.00 %)
Asthma		1 (16.67 %)
Prostate cancer		1 (16.67 %)

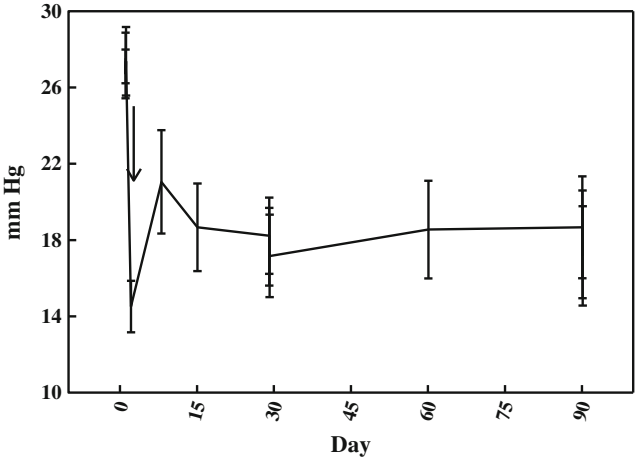

Fig. 1 Intraocular pressure (IOP) changes of all six subjects. Mean (± s.e.m.) intraocular pressure. *Arrow* indicates injection of liposomal latanoprost after 1600 h IOP measurement. The IOP was recorded at 1700 h, 1 h post injection

were using previous ocular hypotensive medications (4 latanoprost, 1 bimatoprost and 1 timolol).

Safety

All 6 (100 %) eyes remained white immediately post injection. There was no evidence of localized trauma (e.g. subconjunctival haemorrhage at the injection site). Five of subject patients reported some ocular discomfort shortly after the injection, but no pain was reported in any subjects. Subject 6, who had ocular surface disease pre-study, reported ocular discomfort and intermittent blurred vision, the symptoms of which were alleviated by the administration of ocular lubricants. Throughout the study period, the best corrected visual acuity (BCVA) in the six subjects remained unchanged from baseline.

Efficacy intraocular pressure

After washout of ocular hypotensive medication as required, day 0 mean IOP at 0900 h was 27.55±3.25 mmHg, with a slight decrease throughout the day at 10:00 and 1600 h. All subjects showed a decrease in IOP within 1 h of the injection at 1600 h (Fig. 1). Mean IOP decreased to 14.52±3.31 mmHg (range 10–18 mmHg). This represented a mean decrease of 13.03±2.88 mmHg (range 9–17 mmHg), or 47.43 %± 10.05 % (range 37–63 %). This mean decrease was statistically significant at each post-treatment visit (P=0.001 to 0.049) as shown in Supplemental Tables 1 and 2.

At the next return visit, 0900 h on day 8 (visit 2), mean IOP was 21.05±6.64 mmHg, (range 11–30 mmHg). At subsequent visits throughout the 3 months of the study, mean IOP was 17.17 to 18.67 mmHg (Fig. 1). This represented a mean decrease from baseline of 8.88 to 10.38 mmHg, or 32.98 to 38.48 %. Using a categorical analysis with arbitrary divisions, all 6 subjects had an immediate ocular hypotensive response of 9 mmHg or more (≥20 %).

Four subjects showed reduction at approximately this level throughout the 3 months of follow-up. These four subjects were previously using latanoprost eyedrops. The maximum IOP reduction at 1 h post injection was between 8.6 to 15.3 mmHg, or 35.39 to 60.47 %. At the end of the study at 3 months, the 4 previous users of latanoprost showed a decrease in mean IOP from baseline of 4.3 to 15 mmHg, or 17.7 to 60 % as shown in Table 2. One subject showed a modest response, subject 3, from 33.3 to 17.7 mmHg (15.6 mmHg reduction or 46.85 % lowering) at 1 h post injection. Subject 3 was using bimatoprost prior to enrollment and during the study period showed a mean IOP of 23.3 to 26.3 mmHg. This represented a mean decrease from baseline of 7–10 mmHg, or 21.0 to 30.0 %. Of note, subject 6, who showed an initial response of 8.7 mmHg IOP lowering or 33.85 % reduction 1 h after injection, had a somewhat less ocular hypotensive response throughout the subsequent 3 months with a maximum of 5 mmHg lowering of 19.46 % reduction observed on day 8, and gradually returning back to baseline IOP by month 3 as shown in Table 2. This subject was previously using timolol.

In vitro results

Drug loading The drug, latanoprost, was loaded into preformed EggPC large unilamellar vesicles (LUVs). This preparation technique was simple, reproducible and easy to scale up. The final sizes of the latanoprost loaded liposomes

Table 2 IOP change for the six individual subjects after injection of liposome latanoprost, mmHg (SD)

Visit	Subject					
	1	2	3	4	5	6
Day 0	26.7 (0.6)	24.7 (0.0)	33.3 (0.6)	25.3 (0.6)	28.7 (0.6)	27.3 (0.6)
Day 8	16.0 (0.6)	11.0 (0.6)	29.6 (0.6)	24.0 (0.0)	23.0 (0.0)	22.3 (0.6)
Day 14	15.0 (0.6)	11.0 (0.0)	26.3 (0.6)	19.0 (0.0)	17.3 (0.6)	23.7 (0.6)
Day 28	14.7 (0.8)	11.3 (0.6)	24.9 (1.4)	16.4 (0.2)	16.6 (2.0)	22.1 (1.7)
Month 2	13.0 (0.0)	10.7 (0.5)	26.3 (0.6)	19.3 (0.6)	17.0 (0.0)	25.0 (0.6)
Month 3	13.5 (0.7)	10.0 (0.0)	26.7 (0.7)	15.1 (1.2)	17.3 (2.3)	24.7 (2.3)

were in the nanometer size range (Z_{avg}=103.18±5.1 nm) with a narrow polydispersity index (0.13±0.06). The actual loading concentration of the drug in liposomes was found to be ~1± 0.006 mg/ml, which were ~20-fold greater than the concentration in topical Xalatan® (50 μg/ml) eyedrops. The final drug/lipid weight ratio was determined to be 0.085.

Discussion

We investigated the feasibility of using a nanoliposome-based latanoprost delivery system to lower the IOP in glaucoma. The study demonstrated that overall, a single subconjunctival injection of liposomal latanoprost formulation was well tolerated with no evident safety issues or adverse events reported. The one subject with tolerability issues of note had pre-study ocular surface disease and which was treated uneventfully with topical lubricants. Furthermore, the same subject demonstrated notably less ocular hypotensive efficacy with the liposomal latanoprost than the other five subjects. As this subject had been previously using timolol, their response to a prostaglandin analogue may not be known, and the subject may be one of the approximately 25 % of the population that do not show full response to these agents [11]. All the other subjects in the study had been previously using a prostaglandin analogue and thus presumed to be responders.

The ocular hypotensive efficacy seen in the present study, a reduction of 13 mmHg or 47 %, is at least as effective as previous reports of latanoprost ophthalmic solution [6]. The authors acknowledge that the present report is a pilot, open-label, non-comparative study and that a larger, comparative trial is required to provide a true comparison.

Given the established issue with patient adherence, performance and the impact on clinical outcomes, there have been many attempts to develop ophthalmic drug delivery systems for sustained ocular hypotensive effects [12]. Specifically, attempts have been made to extend the duration of release of lipophilic molecules from nanocarriers such as nanoliposomes, but with limited success in the laboratory or the clinic [13–15]. In the field of therapy of ophthalmic disease, currently there are no FDA-approved products that employ nanocarriers, with the exception of Visudyne®. Visudyne® is an intravenously injected liposomal formulation of verteporfin in a mixture of eggPC and DMPC lipids and is used in the photodynamic therapy of age-related macular degeneration. Visudyne® is not a sustained-delivery system, and in fact, the verteporfin is released from the liposomes (sub-0.22 μ) within minutes [16]. The lack of nanomedicinal ophthalmic systems that exhibit sustained action is primarily due to the difficulties of loading sufficient amount of lipophilic drugs such as latanoprost into liposomes without disrupting the nanoliposomal structure, as well as controlling leakage of drug from the liposomes under ambient conditions. By maximizing latanoprost interaction with the liposomes, we have been able to achieve both goals [9].

A stability study in vitro showed that drug was retained in the liposomes for 1 year when stored at −20 °C (Fig. 2a). In addition, the nanoliposome size and structure were retained during this period of storage (Fig. 2b). The enhanced stability was primarily due to favourable drug-lipid interactions as

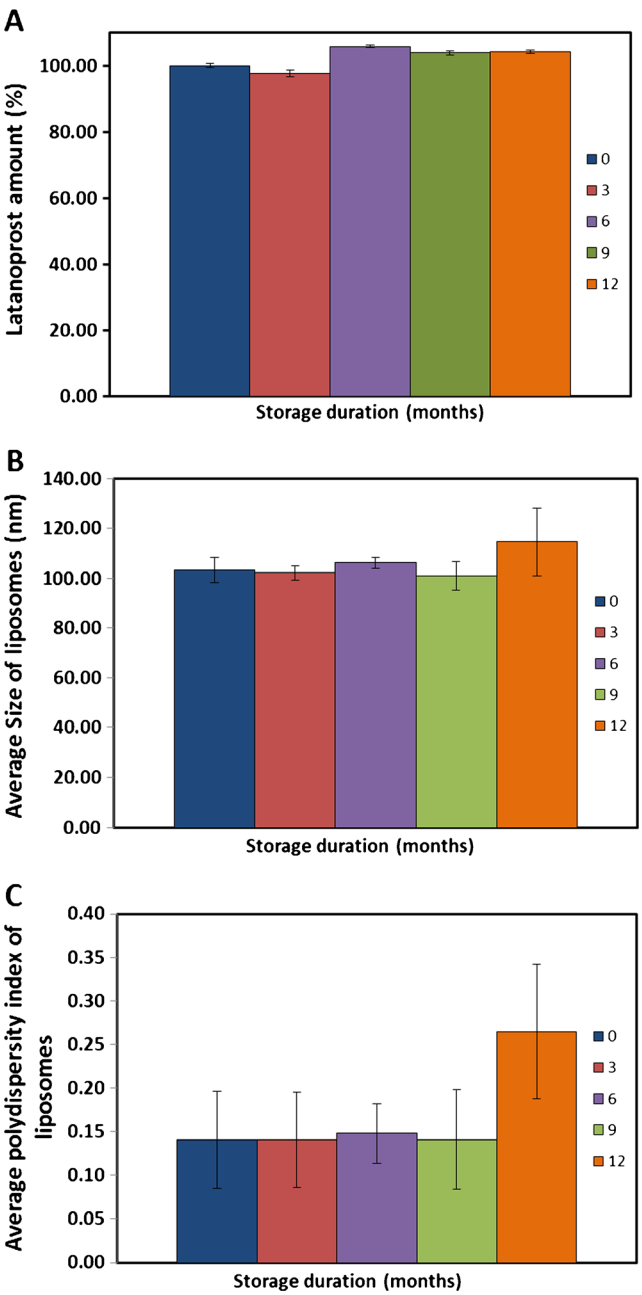

Fig. 2 Storage stability studies under frozen conditions (−20 °C) up to a year for a batch of GMP manufactured latanoprost loaded liposomes. **a** Latanoprost amount in liposomes (%) against duration (months). **b** Average particle size of liposomes (nanometers) against duration (months). **c** Average polydispersity index of liposomes against duration (months). Data shown is an average of three replicates and standard deviation are shown as *error bars*

discussed in our previous work [9]. From isothermal titration calorimetry studies, we found that specific molecular interactions between latanoprost and EggPC liposomes were the reasons behind improved stability, without affecting the size or shape of the nanocarriers. More specifically, binding of latanoprost was found to be driven by favourable enthalpy and favourable entropy changes, suggesting the importance of structural similarity between the drug and the lipid for enhanced stability. This work clearly outlines a unique strategy of loading prostaglandin drugs, latanoprost, into liposomes. Hence, our formulation of liposomal latanoprost need not be lyophilized and reconstituted prior to use, which is the case with leakier drug-liposome formulations. Such reconstitution also leads to variable drug release profiles and size changes which is particularly undesirable in sustained-release formulations.

In terms of efficacy of action, there is currently no other report of latanoprost in a drug delivery system that provided a magnitude of ocular hypotensive efficacy in the range of latanoprost ophthalmic solution, or of a duration of 3 months following a single dose application in humans. The latanoprost drug delivery system was developed by encapsulating the latanoprost drug into a liposomal nanocarrier (average size ~100 nm). Typically, drugs incorporated into nanocarriers are released within 2 or 3 days, when compared to larger carriers; achieving prolonged duration of release from nanocarriers required the optimization of the molecular interactions between the latanoprost and the liposome molecule [9]. Previously, Moon et al. [17] reported that a subconjunctival injection of liposome-bound low-molecular weight heparin (LMWH), with improved absorption rate, behaved like a depot system and remained at the site of injection up to 5 days, possibly due to the relatively large particle size of nanocarriers (~550 nm). However, in our studies, a small bleb was formed immediately upon injection which dissipated within 60 min, suggesting that nanosized carriers with or without latanoprost of size (~100 nm) may not be fully localized in the anterior chamber. Sustained IOP lowering effect in humans (beyond 3 months) may be attributed to both retention of encapsulated latanoprost within the anterior chamber and the sustained release of the drug from the nanocarrier over time; in addition, it is possible that the bound drug is not cleared away rapidly from the eye. Pharmacokinetic studies and in vivo release experiments are in progress to explain for these proposed mechanisms of action.

The acceptance of patients to such an alternate therapy to eyedrops was evaluated in a questionnaire-based survey in 151 patients with glaucoma. They were asked whether they would trade their daily anti-glaucoma eyedrops for a hypothetical new mode of treatment involving a 3-monthly subconjunctival injection of their medication [18]. Almost three quarters of individuals interviewed (74.4 %) would prefer the injection instead of continuing to use their current anti-glaucoma eyedrops. Not surprisingly, those that preferred the injection route were medicating more frequently, used more eyedrop bottles and admitted to non-adherence. In another study that reported the attitudes of glaucoma patients towards an implant for drug delivery in glaucoma, it was found that there was a willingness from patients to pay up to twice the cost of their current treatment [19]. This corroborates with another study by Jampel et al. [20] which reported that 50 and 60 % of patients would opt for a larger copayment of an eyedrop if it required less instillations down from thrice daily to twice and once daily administration, respectively.

Limitations of the study are the small sample size and that it was open label, so both patients and investigators were aware of the treated eye and intervention. This study also did not include a control group to provide comparison in efficacy against latanoprost ophthalmic solution.

In conclusion, the ocular hypotensive efficacy of liposomal latanoprost formulation, as measured by IOP, change from baseline IOP, or percent change from baseline in IOP was in the range of that clinically observed with topical latanoprost ophthalmic solution 0.005 % [18]. A clinically significant ocular hypotensive effect was maintained and achieved for at least 3 months in subjects previously using prostaglandin eyedrops. The results of this study provide evidence of a potential alternate and new method of medical treatment of glaucoma other than daily eyedrops for patients. We are currently planning additional controlled clinical studies.

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Conflict of interest Tina Wong, Jayaganesh Natarajan and Subbu Venkatraman are named on the patent for the technology reported in the manuscript.

Gary Novack has received consultancy fees in the past 3 years from AbbVie, Inc., Aciont, Inc., Actelion, Acucela, Inc., Advanstar, Aerie Pharmaceuticals, Inc., Aerpio Therapeutics, Akebia, Alcon Laboratories, Alexion, Allergan Pharmaceuticals, Altacor, Ltd., Altheos, Inc., Amakem NV, Ampio, Apple Tree, Aquesys, Astellas Pharma Global Development, Astex, Aton Pharma, Inc., Auspex Pharmaceuticals, Inc., Avedro, Axar, Axon Advisors, Balance Therapeutics, Inc., BioGeneration Venture, Brickell Biotech, Inc., Calvert, Canaan Partners, Carlsbad Biotech, Celtic, Ceregene, Charlesson LLC, Chiltern, Clearside Biomedical, Concert Pharmaceuticals, Inc., Drais, Effcon Laboratories, Inc., EGS, Eleven Biotherapeutics, Elmedtech, LLC, Elsevier, Essex Woodlands, Ethis Communications, EyeCyte, Eyetech, Inc., Fidelity Biosciences, Fish & Richardson, Forest, Fovea Pharmaceuticals, Inc., Gerson Lehman Council, Glaukos, Inc., GREG, Harbor, Hatteras Partners, High Point Pharma, InnoPharma LLC, Innovent Biologics, InSite Vision, Inc., Inspire Pharmaceuticals, Inc., Investor Growth Capital, Inc., IOP, Inc., Johnson & Johnson, LEK Consulting LLC, Lexicon, Liquidia Technologies, Inc., Lithera (formerly Lipothera), Mati Therapeutics, Inc., Merck & Co.,

Mimetogen Pharmaceuticals, Mystic Pharmaceuticals, Nanyang Technical University (NTU), Nicox, NovaBay Pharmaceuticals, Inc., Novagali Pharma, Novartis, OcuCure Therapeutics, Inc., Ocular Theraputix, Inc., Ocularis Pharma, Omeros Corp., Ono Pharma USA, Onyx, OrbiMed Advisors, Panoptica, Inc., Parion Sciences, Inc., Perrigo Pharmaceuticals, PGTi Biosciences, Inc., Pieris, AG, Principia, Procter & Gamble, Promedior, Inc., QLT PhotoTherapeutics, Inc., Retina Pharmaceuticals, Inc., Rho Ventures, Rho, Inc., Santen Inc., SARCode, Senju Pharmaceutical Co., Ltd., Shire Pharmaceuticals, Inc., Sristek, Surmodics, Sylentis, Tear Film & Ocular Surface Society, Teva Pharmaceutical Industries, Thomas McNerney & Partners, Thrombogenics, Ltd., Usher III Initiative, Valeant, Velocity, Vistakon, Wells Fargo Securities, LLC, Xoma

Ching Lin Ho and Hla Myint Htoon declare they have no conflict of interest.

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