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Article

Safety and tolerability of topical ophthalmic triamcinolone acetonide-loaded liposomes formulation and evaluation of its biologic activity in patients with diabetic macular edema

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Abstract: Intravitreal injections (IVTs) of corticosteroids as triamcinolone acetonide (TA) are frequently used for the treatment of many vitreous and retinal disorders. However, IVTs are related to severe ocular complications. Lately, a topical ophthalmic TA loaded liposomes formulation (TALF) was designed to transport TA into the posterior segment of the eye when instilled in the ocular surface. To evaluate the safety, tolerability, and biologic activity of TALF, an animal study and a phase I clinical assay was performed. Moreover, four patients with diabetic macular edema (DME) were treated with TALF in order to explore the biologic activity of the formulation. No inflammation, lens opacity, swelling or intraocular pressure rising were recorded after the instillation of TALF in any of the animal or clinical study. Mainly, mild and transient adverse events such as dry eye (30%) and burning (30%) were reported. TALF improves significantly visual acuity and diminishes central foveal thickness in patients with DME. The current data demonstrate the safety, tolerability, and biologic activity of TALF. It seems that TALF can be used topically to treat vitreous and retinal diseases that respond to TA such as DME, avoiding the use of corticosteroids IVTs and its associated hazards.

Keywords: ocular drug delivery system; topical liposomes; posterior segment of the eye; safety and tolerability; biologic activity; diabetic macular edema

1. Introduction

Intravitreal injections of corticosteroids are commonly used for the treatment of many vitreous and retinal disorders as retinal vein occlusions [1], uveitis [2] and diabetic macular edema (DME) [3]. Among the different synthetic corticosteroids, triamcinolone acetonide (TA) is extensively used in intravitreal injections (IVTs). However, despite its low cost, the risk of severe potential complications, intrinsic to intravitreal injection of TA, such as infectious and noninfectious endophthalmitis, retinal detachment, cataract formation and vitreous hemorrhage [4-7], as well as the risk of severe and intractable intraocular pressure (IOP) elevation [8-10], represent constant concerns about intraocular TA administration. In addition, the discomfort caused to the patient by IVTs itself, could cause poor adherence to the treatment with intraocular corticosteroids [11-13].

Although oral or topical corticosteroids routes of administration could be safer than IVTs, these have limited effectiveness for vitreoretinal disorders. Due to the blood–retinal barriers, these routes scarcely reach the posterior segment of the eye [14]. Therefore, to diminish the ocular risks associated with intravitreal injections, different topical ap-

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proaches have been developed to deliver therapeutic concentration of TA into the vitreous cavity [15-18]. Among these, topical liposomes (LPs) are one of the most promising strategies [19]. In fact, LPs constitute the only topical system to deliver TA into the vitreous with clinical evidence of its effectiveness [20-22]. LPs are biocompatible vesicles composed of a phospholipid-bilayer with structural resemblance to cell membrane, which form small spheroids that are able to carry both hydrophilic and lipophilic drugs. LPs represent a potential alternative for ocular drug delivery based on their various advantages: an increased residence time for drug absorption, protection of the encapsulated drug from the external environment, prolonged half-lives in vitreous body with low toxicity, increase efficacy and therapeutic drug index, and the potential to improve penetration to ocular tissues [19].

Recently, a topical ophthalmic triamcinolone acetonide-loaded liposome formulation (TALF) was designed for our group to transport TA into the posterior segment of the eye. The efficiency of this drug delivery nanosystem was successfully tested in vitro and in vivo models [18]. The in vitro diffusion assay of TALF was performed using diffusion chambers with rabbit corneas as membranes, whereas the in vivo assay was carried out in New Zealand white rabbits. As exposed in the previous report [18], TALF was able to cross the cornea and to deliver TA to the vitreous and retina, reaching the highest peak at 12 hours (32.6 ± 10.27 ng/g and 252.1 ± 90.00 ng/g respectively). Additionally, in the same report, TALF underwent a physicochemical characterization, as well as a cell toxicity assay using primary human corneal fibroblasts cultures. We found that TALF have a pH of 5.8, viscosity of 70 cP and osmolarity of 334 mOsm/L. Therefore, TALF was found to be highly viscous, near to the physiologic pH of tears (6.5 to 7.6) [23] and non-irritating to the eye according to the criteria of the Pharmacopoeia of Estados Unidos Mexicanos, where one formulation is considered suitable for ophthalmic use when its osmolarity is between 205 and 684 mOsmol/l. Besides, cell viability was unaffected by TALF. Finally, the microscopic characterization of TALF using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM), revealed that TALF is capable to solubilize large TA crystals into nanoparticles and encapsulate them at the same time [22].

After an extensive physicochemical, microscopic, and pharmacokinetic characterization of TALF, the present study has the purpose to effectively evaluate its ocular safety and tolerability in animals, as well as evaluate its safety and biologic activity in patients with diabetic macular edema.

2. Materials and Methods

Preparation of TALF

OPKO Health, Inc. (Guadalajara, Mexico) provided the TALF. The preparation of TALF was performed in Good Manufacturing Practice (GMP) facilities and was carried out as previously described [18]. Composition of TALF is provided in **Table 1**. TALF was extruded through a size-controlled 0.22µm pore size polycarbonate membrane for 10 cycles under nitrogen pressure (<=200 psi) to obtain lipid vesicles with a unimodal size distribution. Final TA concentration was 2mg/mL (0.2%) in the resultant dispersion.

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Table 1. Triamcinolone acetonide-loaded liposomes formulation composition.

Reagent	Volume
Triamcinolone acetonide	2.0 mg
Kolliphor HS 15	50 mg
PEG-12 glyceryl dimyristate	100 mg
Ethyl alcohol	14 µL
Citric acid anhydrous	0.8 mg
Sodium citrate dihydrate	4.675 mg
Benzalkonium chloride	0.1 mg
Grade 2 purified water	Q.S.1.0ml

Evaluation of safety and tolerability of TALF in animals

Thirty-two male New Zealand white rabbits, weighing 2-2.5 kg each and free of any sign of ocular inflammation or gross abnormality were used. The animals were housed individually in standard cages with controlled conditions, exposed to a 12 hours dark/ 12 hours light cycle, with continuously ventilated rooms at constant, identical and defined room temperature (18ºC ± 3°C) and humidity (45%-75% relative humidity). The rabbits received a standard dry pellet diet and water *ad libitum*.

Animals received 1 drop (50 µl) of TALF every two hours 6 times daily in right eyes, also called study eyes (only in the 12-hour of light period), carrying out an eye clinical evaluation under anesthesia at 10, 30 and 60 minutes, 6, 12 and 24 hours, and 7 and 14 days after treatment. Anesthesia was achieved using an intramuscular injection of ketamine hydrochloride 30 mg/kg and chlorpromazine hydrochloride 15 mg/kg. The control eyes (left eyes) received one drop (50 µl) of placebo solution (saline balanced solution) in the same frequency as TALF was applied. TALF and placebo solution were stored at controlled room temperature (18ºC ± 3°C) in the interim. Four rabbits were euthanized posterior to each clinical evaluation to obtain ocular tissues and fluids that were placed under freezing conditions at -70 ºC, to its storage and subsequent shipment to the Research and Pharmaceutical Research and Development Laboratory facilities (PRDL) to determinate the TA concentration in them. TA levels were analyzed by high performance liquid chromatography (HPLC) at 30ºC. The pharmacokinetic findings were yet described in a previous report [18].

The potential ocular irritancy and/or damaging effects of the formulation (TALF) were evaluated under anesthesia at each established time according to a modified Draize

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test [24]. A slit lamp (CSO Elite, Stagnacci, Firenze, Italy) was used for ophthalmic evaluation. We analyzed and measured the congestion, swelling and discharge of the conjunctiva and they were graded on scales from 0 to 3, 0 to 4, and 0 to 3, respectively (0 was considered normal). Iris hyperemia and lens opacity were also graded on a scale from 0 to 4 (0 means normal). Ophthalmic evaluation of the rabbits also included fluorescein (AK-Fluor® Akorn, US) and lissamine green (Rose Stone Enterprises, Alta Loma, CA, US) stains, for evaluation of the cornea and conjunctiva respectively. For lissamine green (LG) staining, we used 1.5mg impregnated strips of 1% of the reagent, these were moistened with about 10-20ul of saline and applied to the lower fornix. The time for evaluating conjunctival staining was between two and four minutes after the instillation to avoid instant viewing of the staining pattern that could result in misinterpretation due to any pooled dye which has not dissipated. We reported the number of conjunctival spots (if any) stained with LG. For fluorescein staining of the cornea, we used sodic fluorescein solution at 2% six minutes following the instillation (and clinical examination) of LG. We reported the number of corneal spots (if any) stained with fluorescein.

Ocular irritability test was also evaluated according to the Pharmacopeia of Estados Unidos Mexicanos. A positive irritant reaction is considered when more than one animal show iris or conjunctival inflammation and dilatation of conjunctival vessels particularly around the cornea, corneal ulceration and/or corneal opacity.

Furthermore, we also measured intraocular pressure (IOP) (Icare® TA01i tonometer, Belleville, MI, US), and performed a fundus evaluation with binocular indirect ophthalmoscope (Killer Vantage Plus LED, Malvern, PA).

Evaluation of safety and tolerability in healthy volunteers

A phase I clinical assay was designed to evaluate the safety and local tolerability of TALF upon repeated-dose topical application to one eye in healthy male and women volunteers between 18 and 60 years old. Healthy subjects were defined as the absence of medical and surgical history (except cataract surgery) in their medical records. Healthy eyes were defined as best corrected visual acuity (BCVA) > 80 letters in the Early Treatment Diabetic Retinopathy Study (ETDRS) chart, tear film rupture time > 9 seconds, unanesthetized Schirmer test ≥ 10 mm and central foveal thickness (CFT) < 300 μm, [measured by optical coherence tomography (Cirrus OCT Carl Zeiss, Meditech, Dublin, CA)].

Subjects were excluded from participating if clinically significant slit-lamp findings or abnormal lid anatomy was observed at screening, including dry eye syndrome, previous trauma, Stevens-Johnson syndrome, active blepharitis, meibomian gland dysfunction, or lid margin inflammation. They were also excluded if they had history of any intraocular surgery (except cataract surgery at least 3 months before baseline visit) or ocular laser surgery. Active ocular allergies, the use of contact lenses within 3 months before screening or ongoing ocular or systemic infection were also part of exclusion criteria. Eyes were eligible for the study if they met all inclusion criteria and none of the exclusion criteria. Finally, subjects were also excluded in case of tear film break-up time less than 9 seconds with OSDI score corresponding to mild to severe dry eye symptoms in either eye at the screening or unanesthetized Schirmer scores <10 mm in either eye at the screening.

Demographic and baseline clinical exams were collected for every volunteer 1-5 days before the use of TALF. The study eye was randomly selected. Subjects who met all eligibility requirements began the study with a TALF instillation regimen. All the healthy volunteers were instructed to apply one drop of TALF every 2 hours in the study eye, while awake (6 times a day), for 2 weeks (14 days). Follow up was extended for another week to monitor local and systemic tolerability (week 3), for a total of 21 days of follow up. Final TA concentration in the used formulation (TALF) was 2 mg/mL (0.2%).

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This dose was based on the preclinical data from a pharmacokinetic study on rabbits [18].

General safety was assessed through the monitoring of vital signs according to COFEPRIS guidelines. Tolerability was assessed through the collection and summary of ocular and non-ocular AEs, serious AEs (SAEs), ocular assessments and vital signs, whether volunteered by the enrolled patients, discovered by study site personnel during questioning, or other means. Subjects were withdrawn if they presented any evidence of poor tolerability or any AE, such as corneal ulcers, corneal opacities, epithelial defects, anterior chamber inflammation (cell/flare), and conjunctival and/or episcleral infection related to the use of this topical formulation. The investigator determined severity and relationship to the drug. AEs were assigned standard codes for the event based upon the MedDRA Coding dictionary, version 18.1. In case any AEs were detected, the subject was instructed to stop the research drug and was excluded from the study.

The safety and ocular tolerability assessments of the Farmacopea de los Estados Unidos Mexicanos 2018 (FEUM) were also included. Ocular AEs were reported in obedience to the NOM-220-SSA1-2016 that stablishes the Mexican regulatory guidelines for instillation and handling of research and commercial drugs and its adverse events. In accordance with this guideline, ocular AEs must be reported using establishing degrees: mild (no treatment is needed), moderate (specific treatment may be needed, study drug is not suspended) and severe (specific treatment is needed, study drug is suspended).

Other ocular examinations included, BCVA assessed at 4 m using standardized procedures based on the ETDRS protocol, contrast sensitivity (CS) evaluated by the Pelli-Robson contrast sensitivity test, intraocular pressure (IOP) measurement using a Goldmann Applanation Tonometer, corneal endothelial cell density (cECD) determined by specular microscopy (Perseus endothelial microscope, Costruzione Strumenti Oftalmici, Firenze, Italy), retinal thickness and structural changes of the retina evaluated by OCT before and after treatment.

Evaluation of the biologic activity of TALF in patients with diabetic macular edema

A single center, single arm, open-label, prospective, nonrandomized study was performed to evaluate biologic activity in patients with DME. The inclusion criteria were as follows: glycated hemoglobin (HbA1c) less than 6.5% diagnosis of DME as defined by ETDRS [25], BCVA equal or worse than 20/40 Snellen equivalent, and CFT equal or more than 250 μm measured by OCT (Cirrus OCT Carl Zeiss, Meditech, Dublin, CA). The exclusion criteria included proliferative diabetic retinopathy, ocular hypertension or glaucoma, previous vitreo-retinal surgery, laser photocoagulation, and/or IVT in the study eye within 3 months previous the enrollment.

Demographic and baseline clinical exams were collected for selected subjects 1 to 14 days before intervention. Patients were instructed to apply one drop of TALF every 2 hours in the study eye, while awake (6 times a day), for 6 months.

Follow-up visits were scheduled monthly. Each clinical evaluation included BCVA estimation using the ETDRS chart at 4 m, IOP measurement, anterior segment and retina observation with slit-lamp and CFT measured by OCT. The AEs were recorded as previously stated.

Rescue therapy with 0.5 mg of intravitreal ranibizumab (Lucentis®, Novartis Farmacéutica, S.A. de C.V., Ciudad de Mexico, Mexico) was considered if there was registered a diminution in visual acuity (five letters or more in the ETDRS chart) compared to

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previous visit, or if it was recorded an increase in the CFT in 50 μm or more, measured by OCT, compared to previous visit.

Ethical considerations

The animals assay accomplished the guidelines of the Association for Research in Vision and Ophthalmology (ARVO) and the guidelines from the 2010/63/UE European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. The clinical studies were conducted at a private-based retina specialty center in Guadalajara, Mexico (Centro de Retina Medica y Quirurgica S.C.). Institutional Review Board (IRB)/Ethics Committee approval was obtained before enrollment of patients (COFEPRIS 173300410A0035/2017). The studies were implemented in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients before enrollment.

Statistical Analysis

Quantitative variables are presented as means ± standard deviations of the mean. Qualitative variables are described using frequencies and percentages. For the analysis of differences, a Fisher exact test or a Friedman test of repeated measures were performed. Significance was defined as a P value less than 0.05. To analyze the data, we used the SPSS 22.0 software (IBM SPSS Statistics for Macintosh, Version 22.0 (IBM Corp, Armonk, NY, USA).

3. Results

TALF is well tolerated in the preclinical model

Examination of the ocular surface (cornea and conjunctiva), as well as the inner parts of the eye (iris, lens and retina) of rabbits was performed in order to characterize the potential side effects that may result from the high number of daily instillations of TALF. Ocular examination did not show any major findings or adverse events through the follow up. **Figure 1** shows the Mean Draize test score for the study and control eyes at different points of the follow up.

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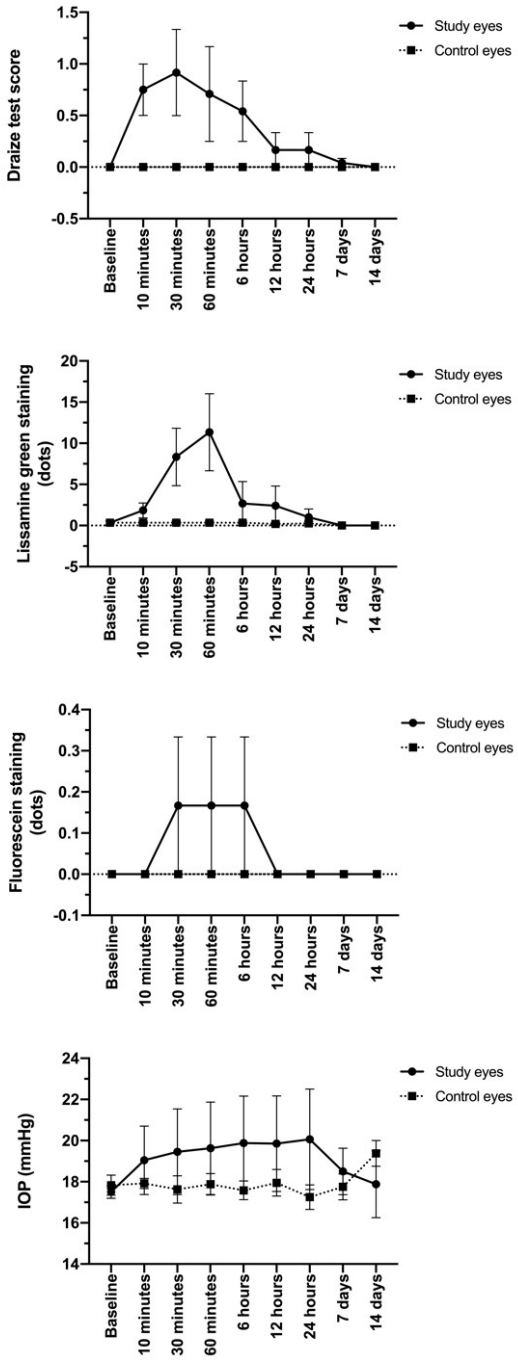

Figure 1. Mean Draize test score, staining score and intraocular pressure in study and control eyes. Non-significant differences were observed between study treated with triamcinolone acetonide-loaded liposomes formulation and control eyes receiving an instillation of saline balanced solution during the follow up.

To quantify the ocular irritation potential of TALF, the parameters of congestion, swelling and discharge of conjunctiva, iris, hyperemia and corneal opacity were evaluated in the rabbits. Since the first instillation of TALF and until the end of the study, there was no evidence of inflammation, tissue alteration and/or discomfort in rabbit eyes. Zero score (0) was achieved in all eyes for inflammation. For swelling and conjunctival con-

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gestion zero score was also registered at the end of the follow up in all groups. Iris hyperemia and corneal opacity scores were also equal to zero at all observations. We did not observe changes in the lens along the follow up of the animal groups. Representative images of study and control eyes that show no inflammation, swelling or discharge after the instillation of TALF or placebo are presented in **Figure 2.**

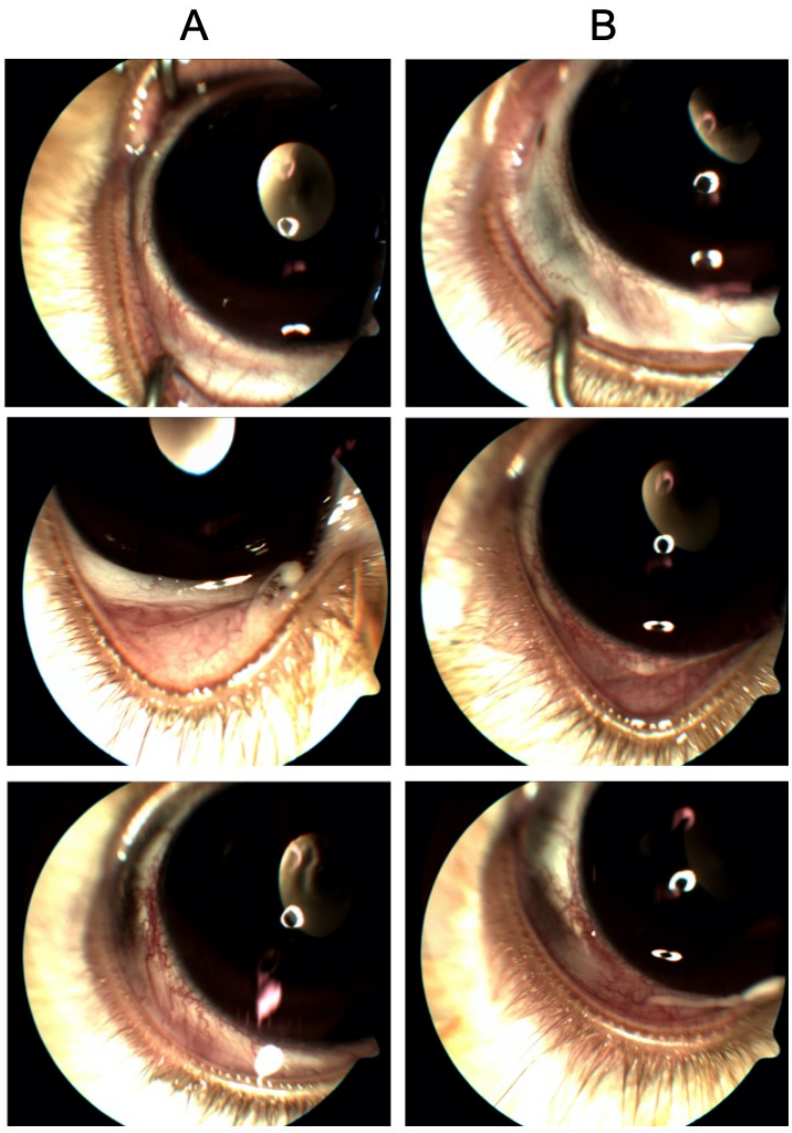

Figure 2. Representative images of study and control eyes. (A) Study eyes and (B) control eyes pictures show no inflammation, swelling or discharge after the instillation of Triamcinolone acetonide-loaded liposomes formulation (TALF) or placebo solutions, respectively.

Staining with fluorescein and lissamine green showed superficial epithelium punctate keratitis in the first 6 hours after instillation of the formulation. This condition was resolved in all cases in the examination at 12 hours after the administration of the formulation. Staining score of ocular surface and are presented in **Figure 1.**

Finally, no increase in intraocular pressure was observed in any of the study animals (normal intraocular pressure in this species is 12-28 mmHg). The behavior of IOP is presented in **Figure 1**.

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TALF is safe for healthy volunteers.

Twenty healthy volunteers were enrolled in the study. The mean age was 37 ± 14.81 years. Thirteen (65%) subjects were women and 7 (35%) were men. Fifteen of the 20 study eyes were right and 5 were left. All subject demographics and characteristics are summarized in **Table 2.**

Table 2. Demographics and Clinical Characteristics of healthy subjects treated with triamcinolone acetonide-loaded liposomes formulation.

				Baseline				14th day			
Patient	**Gender**	**Study eye**	**Age (years)**	**BCVA (ETDRS letters)**	**Contrast (l/contrast)**	**IOP (mmHg)**	**CFT (✾m)**	**BCVA (ETDRS letters)**	**Contrast (l/contrast)**	**IOP (mmHg)**	**CFT (✾m)**
1	F	OD	25	85	1.65	10	250	85	1.65	12	248
2	F	OS	25	85	1.65	11	264	85	1.5	13	269
3	F	OD	26	85	1.35	10	256	85	1.35	16	260
4	F	OS	26	85	1.35	10	259	85	1.35	16	261
5	M	OD	25	83	1.35	13	242	84	1.5	14	243
6	M	OS	25	84	1.35	12	252	85	1.5	12	254
7	F	OD	24	84	1.35	11	243	85	1.5	8	247
8	F	OS	24	85	1.35	11	247	85	1.5	7	241
9	F	OD	24	85	1.5	16	255	85	1.5	11	258
10	F	OS	24	85	1.35	17	257	85	1.65	9	256
11	M	OD	56	85	1.5	16	257	85	1.65	15	256
12	M	OD	35	85	1.65	17	263	85	1.65	14	265
13	F	OD	47	85	1.65	13	246	85	1.65	15	245
14	F	OD	35	85	1.65	18	245	85	1.65	16	247
15	F	OD	63	83	1.35	12	258	85	1.65	14	260
16	M	OD	56	85	1.65	15	260	85	1.65	14	262
17	F	OD	40	85	1.65	16	262	84	1.65	14	259
18	F	OD	38	84	1.5	12	252	85	1.5	11	255
19	M	OD	63	84	1.5	16	249	84	1.5	13	248
20	M	OD	59	85	1.35	15	263	85	1.65	16	260
X ± s			37 ± 14.81	84.60 ± 0.66	1.48 ± 0.13	13.55 ± 2.68	254 ± 7.01	84.85 ± 0.36 ‡	1.56 ± 0.10 †	13 ± 2.67 ‡	254.7 ± 7.77 ‡
i_n	F= 13 M= 7	OD= 15 OS= 5									
P								0.096	0.014	0.4853	0.2825

in; frequency, F; female, M; male, OD; right eye, BCVA; best corrected visual acuity, CFT; central foveal thickness, ETDRS; Early Treatment Diabetic Retinopathy Study, IOP; intraocular pressure, TALF; triamcinolone acetonide-loaded liposomes formulation, †; statistically significant differences from baseline values (P< 0.05), ‡; no statistically significant differences from baseline values (P> 0.05).

Related to safety and tolerability outcomes, we observed that the TALF was well tolerated during the study period. No systemic AEs were reported. None of the 20 patients showed significant changes in IOP, BCVA, contrast sensitivity and CFT (Table 2). After using the study formulation, none of the patients required treatment with IOP-lowering drugs. No serious AEs were reported (**Table 3**) after the end of study drug application (day 14) and at the end of the study follow up (day 21).

Tabla 3. Adverse events reported in healthy subjects treated with triamcinolone acetonide-loaded liposomes formulation.

		Dry eyes n (%)	Burning n (%)	Discharge n (%)	Tearing n (%)	Blurred vision n (%)	None n (%)
Frequency	Not presented	14 (70)	14 (70)	18 (90)	17 (85)	18(90)	8 (40)
	Rare	6 (30)	4 (20)				
	Ocationally		2 (10)		3 (15)		
	Most of the time			2 (10)		2 (10)	
	All the time						
Severity	Not presented	14 (70)	14 (70)	18 (90)	17 (85)	18 (90)	8 (40)
	Mild	6 (30)	6 (30)		3 (15)	2(10)	
	Moderate			2 (10)			
	Severe						

TALF; triamcinolone acetonide-loaded liposomes formulation.

Ocular AEs were reported according to the NOM-220-SSA1-2016 as COFEPRIS request. Most AEs were mild and two of them moderate in severity. Six subjects (30%) reported occasional mild dryness in one occasion and others six (30%) reported mild burning sensation (one or two times) during the instillation. Two volunteers (10%) reported moderate secretion that disappeared after the first week of the study and 3 (15%) reported tearing at the moment of application. A relationship with the drug could not be excluded. There was no pain or discomfort reported. Neither eyelid redness, conjunctival hyperemia nor edema were observed across the follow up. No subconjunctival hemorrhages were reported in this study. Ocular surface staining, which was graded as 0 (no changes) or 1 (mild changes) in most cases was transient and considered clinically non-significant by the investigator. No pathological changes of the anterior eye chamber or lens were reported. No vitreous cells nor flares were observed. Retina structures appeared normal prior to and after dosing. Endothelial cell density and retina thickness were unaffected (2976.2 ± 414.21 cells/mm^2 vs 3036.7 ± 377.25 cells/mm^2) (**Figure 3**). No clinically relevant changes in vital signs parameters were observed. TALF eye drops did not affect blood pressure or pulse rate. No local or systemic findings required TALF to be stopped. A summary of reported ocular AEs are presented in Table 3.

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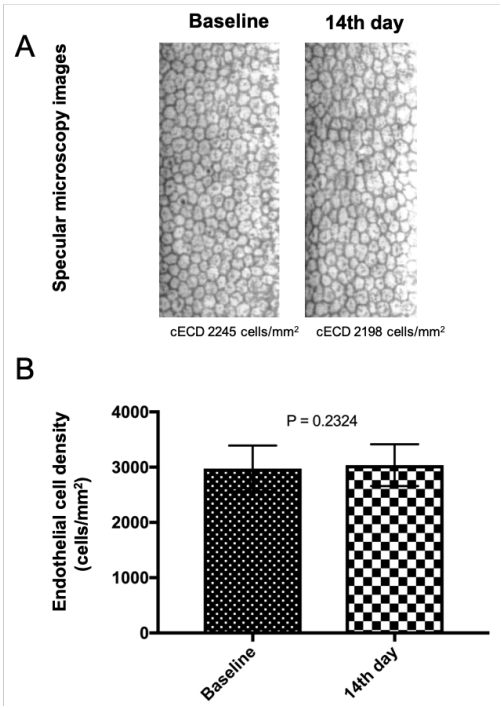

Figure 3. Corneal endothelial cell density analysis in healthy subjects treated with triamcinolone acetonide-loaded liposomes formulation. (A) Images of specular microscopy of a representative case at baseline and after 14 days of TALF instillation are presented. (B) Column bar graph from cECD analysis is presented. Non-significant difference on cECD values was established between baseline and after 14 days of TALF instillation. cECD; corneal endothelial cell density, TALF; triamcinolone acetonide-loaded liposomes formulation.

TALF improves BCVA and reduces CFT from patients with DME.

Four patients with DME were enrolled in the study. The mean age was 62 ± 7.87 years. Two patients (50%) subjects were women and other 2 (50%) were men. All study eyes were left.

Concerning to safety and tolerability outcomes, we observed that the TALF was well tolerated during the study period. No systemic or severe AEs were reported. None of the patients showed significant changes in IOP. After using TALF by 6 months, none of the patients showed intraocular hypertension.

On the other hand, BCVA and CFT improve significantly across the time (**Figure 4**). ETDRS letters increased from 41.5 ± 23.5 at baseline to 58 ± 17.79 letters after 6 months of TALF therapy (P= 0.001), whereas CFT measured by OCT reduces from 483 ± 55.39 at baseline to 268 ± 27.26 μm at the same point (P= 0.025). OCT images from all cases are provided in **figure 5.** Additionally, none patient required rescue therapy.

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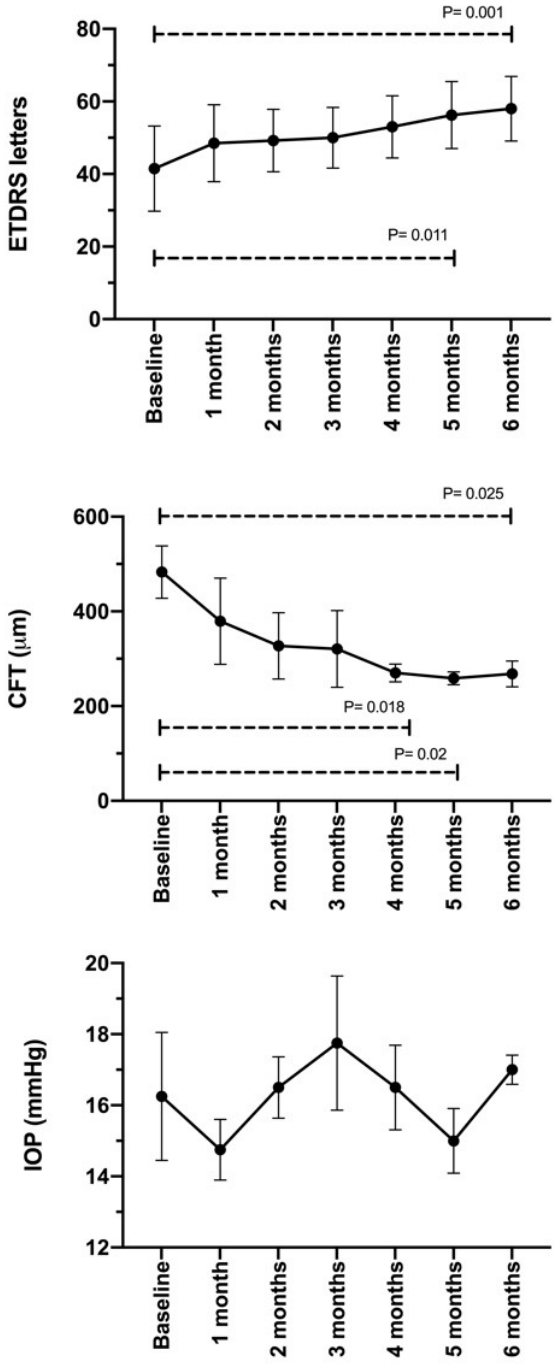

Figure 4. Best corrected visual acuity, central foveal thickness, and intraocular pressure follow-up in patients with diabetic macular edema treated with triamcinolone acetonide-loaded liposomes formulation. Best corrected visual acuity, measured by ETDRS test, improved trough the follow-up, reaching statistical significance at month 5. CFT, measured by OCT, contracted through the follow-up, accomplishing statistical significance at month 4. Non-significant variation in IOP was recorded during the study. ETDRS; Early Treatment Diabetic Retinopathy Study, CFT; central foveal thickness, OCT; optical coherence tomography.

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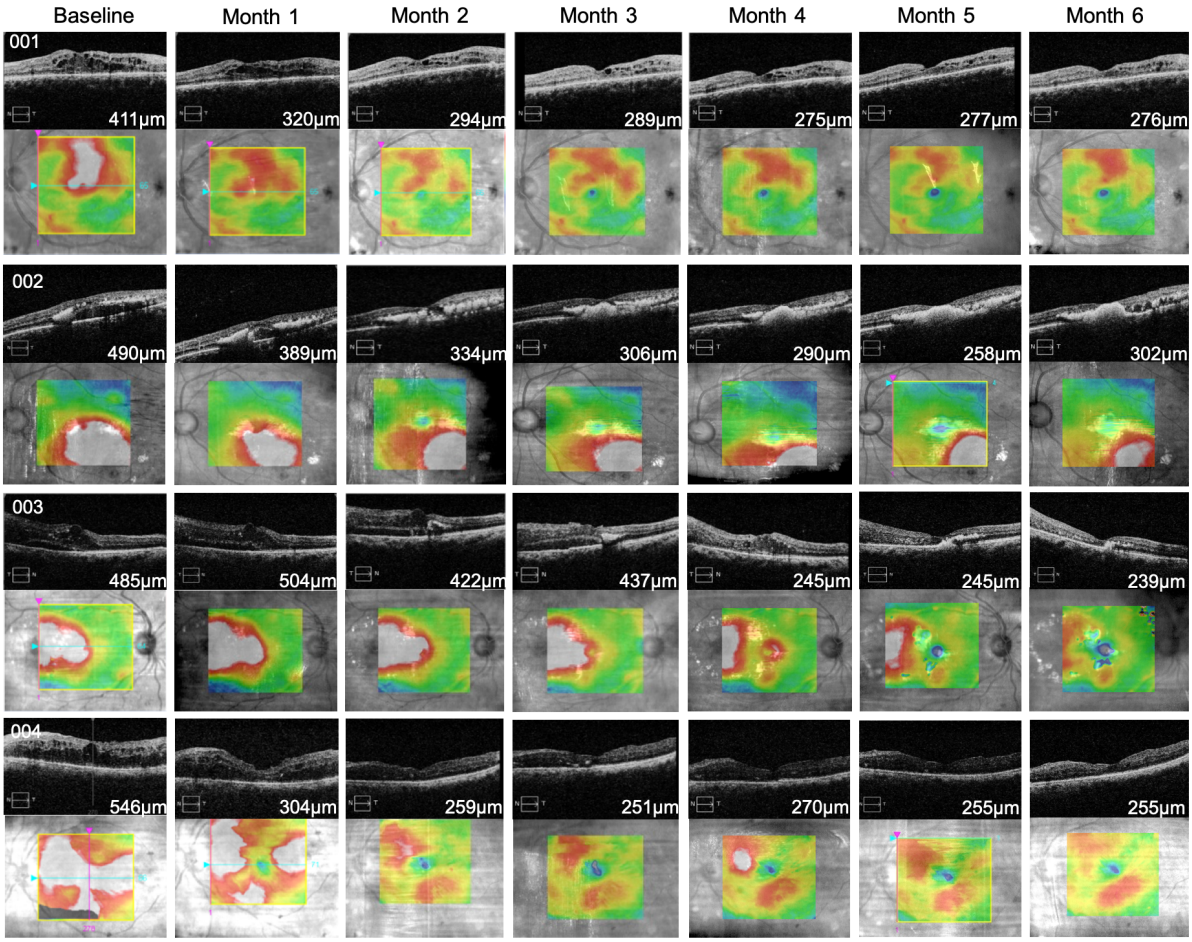

Figure 5. Optical coherence tomography images from patients with diabetic macular edema treated with triamcinolone acetonide-loaded liposomes formulation. A significant reduction in CFT was revealed through OCT analysis in patients receiving TALF. The CFT reduction was maintaining trough the follow-up in almost all cases. CFT; central foveal thickness, OCT; optical coherence tomography, TALF; triamcinolone acetonide-loaded liposomes formulation.

4. Discussion

Over the last decade of research, dramatic changes have been observed in the field of drug delivery. Developing a novel drug delivery system to target a particular human tissue has become a major goal for researchers in the field [26,27]. Although there has been great interest in the development of new topical ocular delivery systems, ocular drug delivery has been a major challenge to scientists due to its unique anatomy and physiology. Topical ophthalmic drugs molecules have to cross several layers of eye tissue and to trespass several dynamic and metabolic barriers before reaching the vitreous and retina [27,28], and very low concentrations, with almost no clinical effect, are usually obtained [27,28]. That is why conventional topical administration of drugs has not been as effective as intravitreal delivery in the treatment of retinal diseases [27].

Nowadays, IVTs are the most used pathway to deliver drugs for the posterior segment of the eye and it has become the most common intraocular procedure worldwide with increasing numbers every year [29]. IVTs are the standard drug delivery method for the treatment of retinal diseases that cause non-reversible vision impairment [30,28]. The advantage of the intravitreal method is to circumvent the ocular barriers which keep most drugs out of the eye and the ability to minimize loss of vision and to improve it over a period of years is an untold clinical benefit [31]. Due to the widespread

use of ITV, drugs such as anti-vascular endothelial growth factor (VEGF), VEGF trap-eye, and triamcinolone can now maintain or improve vision in patients who did not have treatment before [13].

However, IVTs are not exempt of potential problems. The need of multiple consecutive injections increases the incidence of adverse events (AEs) such as endophthalmitis, lens injury and retinal detachment over the months and years of use [11,12,32,33,13]. In addition, it may be a burden for patients, physicians and health systems with poor compliance in many cases [11-13]. Also, IVT requires highly specialized human resources and special infrastructure to apply it, resulting in a costly option in developing countries [13]. Research initiatives continue at a rapid race by scientists, organizations and pharmaceutical companies worldwide to find a safer, more accessible and effective ocular drug delivery method for ocular use.

In order to increase patient compliance, potentially decrease side effects from repeated monthly IVT, and to improve outcomes, novel therapeutic strategies have been pursued in the field of ocular drug delivery, including the development of drugs with longer treatment interval [34,35], sustained drug delivery system [36,37], implantable drug delivery systems [38-42], and the development of novel ocular drug delivery systems to enhance ocular bioavailability such as microemulsions, nanosuspensions, nanoparticles and liposome formulations [43-45]. In a previous work, we showed that TALF reaches significant concentrations in posterior segment of intraocular tissues and is non-toxic for human keratocytes culture [18], but the *in vivo* safety and tolerability of this formulation was still a concern, and the biologic and therapeutic effect in a chronic retinal disorder such as diabetic macular edema has not been explored previously. Thus, this report summarizes the findings about biologic activity, safety and tolerability of TALF.

With respect to the biologic activity, we found that TALF was able to reduce the thickness of the fovea (measured by OCT) in patients with adequate glycemic control but presence of DME. This finding is not surprising because it is well known the benefits of synthetic corticosteroids for the therapy of DME due its numerous targets, but the remarkable finding is the administration route. TALF is the first liposomes formulation carrying TA that showed therapeutic activity clinically. Although a complete understanding of the mechanism of action of corticosteroids has not been fully elucidated, it has been demonstrated that corticosteroids, like the TA contained in TALF, interfere with regulatory components of gene expression inhibiting the synthesis of vascular endothelial growth factor (VEGF) and multiple pro-inflammatory genes such as tumor necrosis factor α and interleukin 6, while inducing anti-inflammatory factors such as pigment epithelium-derived growth factor (PEDF) [46-48]. Besides, steroids also inhibit the phospholipase A2 pathway, diminishing the production and release of inflammatory cell mediators and chemokines. Moreover, TA specifically seems to reduce the expression of matrix metalloproteinases (MMPs) and downregulates intercellular adhesion molecule 1 in choroidal endothelial cells [49].

On the other hand, ocular tolerability is a major goal for all topical drugs. Many eye conditions are treated with ophthalmic topical formulations with good results, but tolerability is still a worry for many patients. In our animal study, we did not observe superficial irritation signs such as conjunctival hyperemia in any of the study eyes. Even though, we observed superficial epithelium punctate keratitis in rabbits during the first hours with fluorescein sodium and lissamine green staining. However, it was not visible after 6 hours of the first instillation of TALF. According to Pharmacopeia of Estados Unidos Mexicanos, ocular irritability test was satisfactory, and TALF is considered non-irritant. Additionally, in the phase I clinical assay, healthy volunteers reported predom-

inantly minor and transient adverse events with TALF instillation, that support the well tolerability of the liposomal formulation.

Although further evaluation is needed to completely define the tolerability profile of this formulation, the preliminary results of our experimental study suggest that TALF is well tolerated and is safe in the eyes of New Zealand white rabbits and may have the potential to be used as a novel delivery system to reach the intraocular tissues of the posterior segment of the eyeball. These results can be considered useful for other topical liposome formulations comprising different steroid or other molecules. It is worth mentioning that different studies have established the effectiveness and safety of liposomal formulations delivery by intravitreal injection [50-53]. However, this is a technology that analyze topical liposomes use for delivering TA into the posterior ocular segment as an alternative to IVT.

Finally, ocular safety must be a major goal for research as part of the design of noninvasive sustained drug delivery systems. Exploring the feasibility of topical routes to deliver drugs to the posterior segment may drastically improve drug delivery in the years to come. Recently, Li, J and colleagues [54] have shown a liposomal delivery platform for TA potentially more effective than our system, since they showed higher concentrations of TA in posterior segment tissues. However, increased intraocular corticosteroids concentrations such as TA are effective but are associated with increased risks of posterior subcapsular cataract development and elevation of intraocular pressure, in fact, severe and intractable elevation of IOP constitutes the major impediment to widespread steroid use [4-6,55,56]. In our study we did not observe changes or opacities in the lens of any eyes in the study groups and IOP also did not rise significantly in study eyes. This background guided us to prove that TALF was safe and may have the potential to be a new administration route for drug delivery in the posterior segment.

5. Conclusions

In conclusion, as we have shown, liberation of TA into vitreous cavity and retina using topical loaded liposomes is feasible, safe, and well tolerated. Hence, the use of topical formulations of liposomes, instead of intravitreal injections for drug delivery in ocular diseases of the posterior segment, could result, in the near future, as an accessible and safer therapy, since no special infrastructure, nor trained professionals are required for its administration. Moreover, complications related to intravitreal injections may be avoided, as well as adverse events related to the use of steroids. However, future larger experimental and clinical TALF studies to evaluate longer-term safety and therapeutic profile are necessary.

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