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DEVELOPMENT OF PG MODIFIED LIPID NANO-VESICLES OF TACROLIMUS TO ENHANCE THE OCULAR ABSORPTION

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Abstract

Ophthalmic medication delivery applications using liposomal formulations have received much research during the last decade. This study set out to determine whether nano-vesicles modified with propylene glycol (PG) and tacrolimus (0.01%) applied topically may help alleviate symptoms of experimental dry eye syndrome (DES) in rabbits. PNVs treatment and tacrolimus solution 0.01% were compared to untreated and healthy patients. The clinical results for PNV-treated animals improved, with higher tear production and a decreased TBUT. Most metrics were within normal range, and the PNV therapy was shown to be more successful than the TAC solution. Therefore, tacrolimus PNV formulation (0.01%) may provide a promising therapeutic option for dry eye condition.

Keywords: Tacrolimus Dry eye syndrome Ocular Proglycosomes Nanovesicles

INTRODUCTION

Due to the eye's inherent defensive systems, attaining an optimal medication concentration during ocular drug administration is difficult. To design drug delivery systems with therapeutic concentration in the target area, it is crucial to understand the static and dynamic barriers of the eye. The front and back of the eye are the two main divisions that may be made. The vitreous humor is behind the anterior part of the eye, which consists of the cornea, pupil, aqueous fluid, iris, lens, and ciliary body. The posterior segment, which includes the retina, vitreous humor, macula, and optical nerve, is located in the rear two-thirds of the eye. Topical, systemic, periocular, and intravitreal medication delivery are the most prevalent methods for treating eye problems. Patient compliance is higher with topical administration, and it's also less intrusive, thus it's the method of choice. Drugs injected topically may be absorbed in one of two ways: either through the cornea or around it. The three main layers of the cornea are the epithelium, stroma, and endothelium; each has its own polarity and may act as a rate-limiting structure for medication absorption.

The eyes feel dry, gritty, irritated, or as if a foreign body is embedded in them, soreness, redness, hyperemia, and keratoconjunctival epithelial diseases are all symptoms of DES. Discomfort without discoloration characterizes mild DES symptoms, whereas irritability without dryness characterizes severe symptomatology, with persistent dry eye pain representing the severity of the condition. 3−6 Hyperosmolar tears, caused by ocular surface inflammation, cause increased evaporation and reduced generation of tears. When the immune system is triggered, T lymphocytes play a role in the release of inflammatory mediators. Pathogenesis of DES is mediated by ocular surface inflammation that continues to spread, regardless of what initially set it off. As a result, preventing inflammation is now key to treating DES effectively. Reduced mucin production, caused by the release of inflammatory mediators, exacerbates tear film instability. Meibum, a lipid-rich fluid, accumulates in the meibomian glands as inflammation worsens, providing a fertile breeding ground for bacterial growth. The production of lipase by these colonies modifies the meibum. The solidification of this meibum contributes to the vicious inflammatory cycle that leads to increased tear loss.

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Newer nonsteroidal immunomodulator tacrolimus has a lower risk of side effects. The research by Moscovici et al. evaluating topical tacrolimus 0.03% for the treatment of dry eye showed encouraging findings. Since tacrolimus is so highly lipophilic and hardly dissolves in water, making an eye drop out of it is difficult. The primary issue of extended drug administration has been addressed by outstanding nanotechnology-based solutions in the realm of medicine delivery, notwithstanding penetration limitations for topically administered pharmaceuticals. Liposomes have risen to the top of the list of cutting-edge drug delivery methods for ocular use because they are extremely biocompatible, biodegradable, and non-toxic. Yet, there is a problem with stability and the escape of drugs. Nevertheless, the ocular surface is severely damaged by the addition of surfactant, alcohol, or bile salts to liposomes, which improves their solubility and stability. Proglycosomes nano vesicles (PNVs) are lipid vesicles that have been treated with propylene glycol (PG) for effective topical administration of tacrolimus. PNVs had more corneal penetration, longer precorneal retention, and sustained TAC release. We also found that the PNVs (0.1%) were helpful in reducing the severity of rabbits with uveitis brought on by an experimental procedure. The aim of this research was to determine whether topical tacrolimus PNVs were effective in treating rabbits with experimental dry eye syndrome.

LITERATURE REVIEW

Qianni Wu, et al. (2019), Tacrolimus has been used extensively to treat post-transplant organ rejection. Unfortunately, the hydrophobicity and limited corneal penetrability of the typical pharmaceutical formulation of tacrolimus restrict its applicability in ocular treatment. We identified these shortcomings and worked to enhance tacrolimus-loaded methoxy poly nanoparticles for corneal transplant rejection by using a nanotechnology-based technique. Drug loading was 8.01 0.23%, and encapsulation efficiency was 80.10 2.33% in the TAC-NPs that were produced. The pharmacokinetics of TAC-NPs after a single dosage were also studied, this time in New Zealand rabbits with the use of high-performance liquid chromatography tandem mass spectrometry. TACNPs dispersion drops significantly increased the effective suppression of IL-2, IL-17, and VEGF production in rats having allogenic penetrating keratoplasty as compared to the standard 0.1% tacrolimus drops. To learn more about the sustained release property of TAC-NPs, researchers looked at two different topical application strategies. Overall, small TAC-NPs improved trans-corneal permeation and absorption of TAC and more effectively inhibited corneal allograft rejection, demonstrating the promising potential of a biodegradable polymeric nanomaterials-based drug delivery system for increasing the therapeutic efficacy of hydrophobic drugs in clinical practice.

S.S. Shoughy (2017), Immune-mediated inflammatory disorders of the anterior segment present a wide range of symptoms and are treated with high levels of immunosuppression. Serious ocular adverse effects are possible with topical steroid treatment. Immunomodulatory medicines are increasingly employed as a means of avoiding the potentially blinding side effects of topical steroids. Reduced ocular inflammation and inhibition of T cell activity may be induced by the calcineurin inhibitor tacrolimus. Research on the effectiveness of tacrolimus in treating inflammatory diseases of the anterior segment is very new. Here, we'll take a look at how topical tacrolimus may be used to treat T-cell-mediated anterior segment inflammation while minimizing the need for steroids.

Pople P.V. and Singh K.K. (2011), To combat tacrolimus's low in vivo bioavailability due to its poor solubility in carrier matrices, researchers developed a unique method: the construction of modified nanolipid carriers (MNLC). The purpose of this research was to create MNLC for topical distribution that increased drug solubility in a carrier lipid matrix by employing lipophilic solubilizers. Particle size, morphology, and rheology were characterized in depth for tacrolimus-loaded MNLC (T-MNLC). By altering the lipids, the crystals formed were less flawless, creating room for the dissolved medication, and increasing the entrapment efficiency to 96.66 percent. DSC, FT-IR, and (1)H NMR were used to

analyze the compatibility and mixing behavior of the carrier components. The capability of decreasing the overall lipid content in the carrier may account for the high degree of stability shown by T-MNLC. Studies on skin irritation found that T-MNLC was much less irritating than the standard. Results from this work demonstrate that by incorporating lipophilic solubilizers into colloidal lipid carriers, the encapsulation efficiency of the resulting T-MNLC was increased, resulting in improved performance in terms of stability and skin localization.

Ahmed, S., Amin, M.M. & Sayed, S. (2019), The eye's intricate anatomy and physiology make targeted drug administration challenging. Scientists have been interested in developing efficient topical administration methods for many decades. Their challenging job is to increase medication residence duration and ensure adequate penetration into the eye. The eye's multiple layers, particularly the precorneal, corneal, and blood-corneal ones, provide significant obstacles to drug delivery. The vast majority of commercially available items are liquids. Ointments and gels are examples of semi-solid items, whereas powder, inserts, and lenses are examples of solid ones (in situ gel). The potential of nanotechnology-based carriers to entrap hydrophilic and lipophilic medicines, increase ocular permeability, maintain residence time, improve drug stability, and increase bioavailability has led to their increased use in recent years. Several in vitro, ex vivo, and in vivo characterisation methodologies may be used to anticipate the outcomes of the constructed nanocarriers. This article is an attempt to demystify ocular anatomy, ocular disorders, and ocular delivery issues. In addition, it investigates the pros and cons of various dosage forms and methods of administration for use in the eye. Several nanostructured platforms and methods for characterizing them are also included in this overview. Methods to improve ocular bioavailability are discussed as well. Developments in ocular delivery are discussed at length.

Chen X, et al. (2019), Dry eye is often treated with ophthalmic formulations like eye drops. However, due to the cornea's impermeable barrier and the short ocular retention period, the ocular bioavailability of eye drops is low. In this study, we sought to improve the bioavailability of eye drops by creating a cationic liposome eye drop for the treatment of dry eye. Liposomes of tacrolimus have a +30 mV surface charge and a diameter of around 300 nm. It is possible that cationic liposomes might interact with the anionic ocular surface, increasing the quantity of tacrolimus absorbed by the cornea and lengthening the ocular retention duration. The ocular retention time of eye drops was dramatically increased by the addition of cationic liposomes, which increased the tacrolimus concentration at the ocular surface. Dry eye is often treated with ophthalmic formulations like eye drops. However, the retention duration in the eye is quite brief due to the cornea's restricted permeability. Because of their increased ocular retention time and biocompatibility, drug-loaded cationic liposomes have promising applications in the treatment of ocular diseases; further research is needed in this area.

Materials and Methods

Jubilant Organosys Ltd. was kind enough to send along a sample of Tacrolimus (Biocon Ltd., India) (Noida, India). Lipoid GmbH (Ludwigshafen, Germany) generously provided a free sample of their phospholipid product (Phospholipon 90H), and SD Fine Chemicals supplied the phosphoglyceride (PG) (Mumbai, India). Strips of lissamine green, fluorescein, and Schirmer were acquired from Akriti Oculoplasty Logistics (Hyderabad, India). We used Bausch & Lomb's atropine sulphate eye drop and CHD Fine Chemicals of India's benzalkonium chloride. All of the analytical solvents came from Merck Ltd.

Preparation of Tacrolimus PNVs

Our team used the methods previously published to manufacture proglycosomes containing tacrolimus.

Twenty-one lipid films were made by evaporating a solution containing tacrolimus, 80 mg of phospholipid, and 20 mg of PG that had been stored under vacuum for one night. The necessary PNVs were found in the resulting lipid film after redydration with a 10% aqueous PG solution for 1 hour. Five minutes of ultrasonication at 80 amplitude, the size of PNVs was further decreased. Centrifugation and high-performance liquid chromatography were used to examine the drug loading and release profile of the entrapped medicines, respectively. Lyophilized PNVs containing 1.14 mg of tacrolimus were reconstituted with 1.0 ml of sterile water to provide a final concentration of 0.01% w/v (PNV formulation). While making a 0.01% tacrolimus solution (TAC sol), 1.0 mg of tacrolimus was dissolved in 10 ml of light mineral oil and then vortexed to ensure complete dissolution.

Development of Experimental Des in Rabbit

Each group had eight eyes (n = 8), and the total number of eyes in all sixteen animals was 96. Twelve mice were given a combination of 1.0% atropine sulphate and 0.1% BKC to apply topically to their eyes, causing them to develop experimental DES in both eyes (DES group) (DE solution). For two weeks, three times a week, a 50 cc DE solution was administered topically. Rabbits that had DES injected into their bloodstreams were split into three groups and given either no treatment, 25 milliliters of a 0.01% tacrolimus solution once a day, or 25 milliliters of a 0.01% PNV formulation once a day. Day 7, one hour after DE solution administration, treatment began and lasted for 7 days. A healthy control group (n = 8) was given topical saline eye drops for two weeks.

Results

Lipid and PG were used in the preparation of tacrolimus PNVs. Figure 1 shows a typical PNV and describes some of its characteristics.

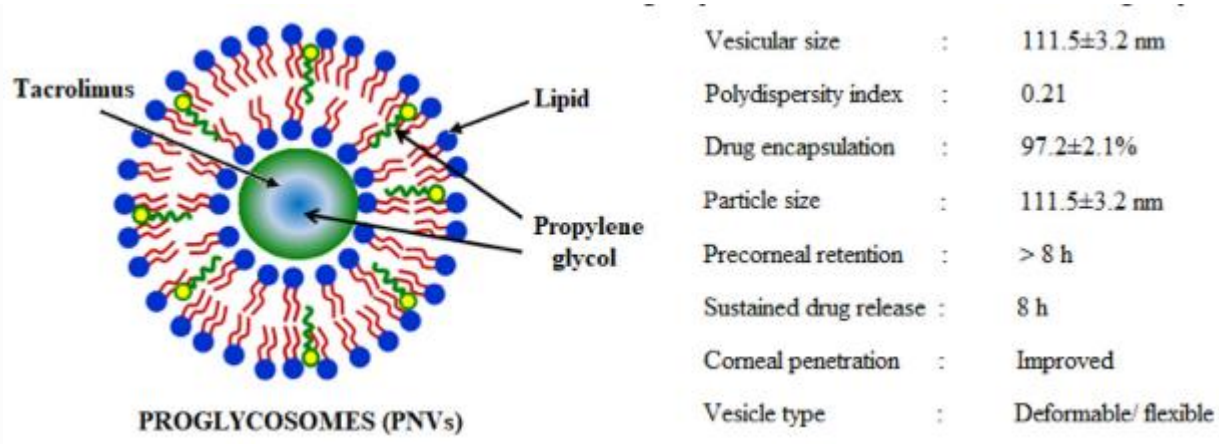

Figure 1. Image and characteristics typical of tacrolimus PNVs

Application of Nanotechnology in Ocular Drug Delivery

The traditional method of dosing medication is evolving as a result of nanotechnology. The capacity to use science and technology to control structures and characteristics at the nanoscale range has the potential to completely transform the process by which novel medicines are produced and old ones are improved. Evidence suggests that nanocarriers may help alleviate some of the problems associated with conventional treatment methods. Particles used in a medication delivery system that fall within the nanometer range (1-1000 nm) are referred to as nanoparticles. Nanoparticle technology has several applications in the pharmaceutical industry, drug preservation against physical, chemical, and biological breakdown, solubilization of hydrophilic and weakly water-soluble drugs, and enhancement of

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bioavailability and pharmacokinetic features. Moreover, these systems' ability to move and penetrate natural ocular barriers facilitates accurate medication administration to the intended spot. Drug maintenance at specified concentrations is a crucial part of the therapy for several chronic inflammatory illnesses, including as age-related macular degeneration (AMD) and uveitis. Major obstacles in the development of dispersed systems for ocular delivery include particle size, particle size dispersion, and stability. Ocular therapy using nanoscale drug delivery systems administered via eye drops is safe because of their small dimensions, uptake into corneal cells is possible, and targeting toward affected tissues may reduce potential side effects and required doses. In Table 1, we can see the pros and cons of using nanoparticle systems based on lipids.

Table 1. Advantages and disadvantages of lipid-based nanoparticulate systems in ocular delivery

Advantages	Disadvantages/Limitations
High encapsulation efficiency High ocular permeation Appropriated pharmacokinetic properties Sustained and controlled release Enhancing drug pre-corneal retention time and drug corneal permeability Increase ocular bioavailability and distribution Prevent ocular toxicity Good stability and biocompatibility	Initial burst release from SLNs Low drug loading capacity Lack of recent extended clinical trials since most of the studies are just in vivo assessment The toxicity of lipid nanoparticles on retinal cells is not entirely studied

Injecting a liquid colloidal medication delivery device is a simple process. Microparticles, liposomes, and nanoparticles may all be part of a colloidal system.

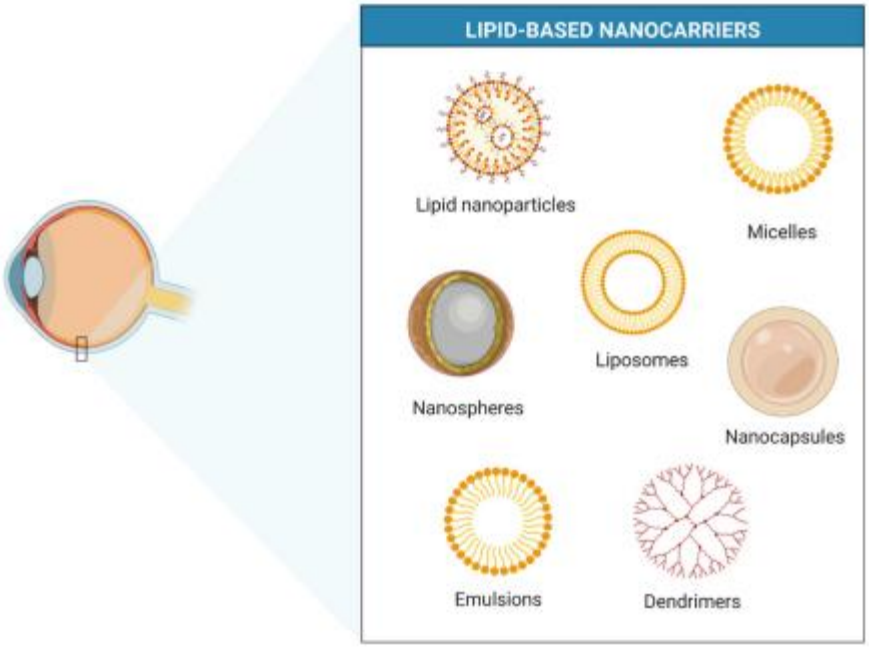

Figure 2. Nanostructured lipid carriers for topical administration of drugs

Ocular Irritation Test

As surfactant is used in making FK506 liposomes, it was important to assess in vivo ocular irritation.

The ocular irritation test was performed to investigate the irritant properties of FK506 liposomes. After 72 hours of monitoring, no discomfort was detected when FK506 liposomes and PBS were implanted (Figure 3C). The cornea, conjunctiva, and eyelids all looked normal, with no edema, redness, or discharges. Therefore, it was concluded that the usage of FK506 liposomes did not result in any pain to the eyes.

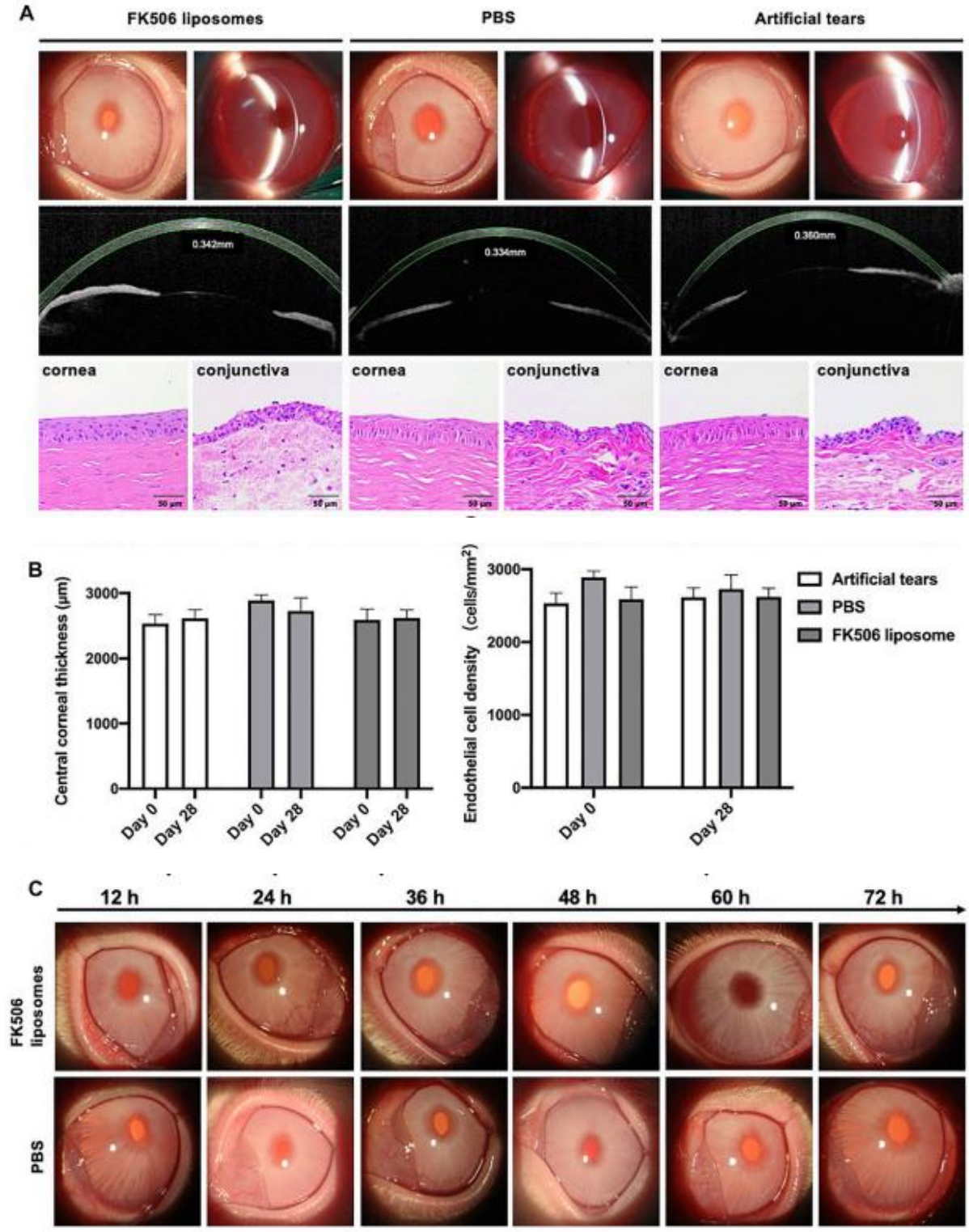

FIGURE 3 Evaluation of FK506 liposomes for biocompatibility and ocular irritation in rabbits in

vivo.

Tear Production and Stability

Table 2 shows that both treatment groups increased tear production as a consequence of the Schirmer tear test. When compared to the untreated group, the STT was much greater in the TAC and PNV treatment groups. Results from the STT confirm that treatment with TAC or PNV raises TBUT over those in the untreated group. Table 2 shows that the PNV group's TBUT was longer at 18.7 0.52 seconds than the TAC group's (14.3 1.03 seconds), but there was no difference between the PNV and healthy groups in terms of TBUT ($p > 0.05$).

Table 2 Clinical Parameters of DES for Control and Treatment Groups

Clinical Parameter	Groups			
	Healthy	Untreated	TAC	PNV
Tear volume (mm)	16.7 ± 1.21	6.3 ± 0.52	11.7 ± 0.82	15.8 ± 1.33
TBUT (s)	18.3 ± 1.20	3.5 ± 0.55	14.3 ± 1.03	18.7 ± 0.52
MMP-9	0/8	8/8	4/8	0/8
OST (°C)	32.4 ± 0.55	34.4 ± 0.58	34.6 ± 0.58	32.3 ± 0.34
OSS score	0.0	8.3 ± 0.52	5.7 ± 0.52	0.7 ± 0.52
Globlet cell density (cells/mm^2)	64.2 ± 8.54	8.3 ± 3.20	32.2 ± 4.40	53.3 ± 4.80

Eight of eight eyes in the untreated group showed signs of MMP-9. Four of the eight eyes tested positive for MMP-9 after TAC therapy. In the PNV group, MMP-9 was not detected in any of the eyes. IR thermography results showed that OST was lower in the PNV-treated group (32.3± 0.34) compared to the untreated group (34.4 ±0.58) by a statistically significant amount (p 0.05). Similar OSTs were seen in the PNV and healthy groups. Interestingly, there was no statistically significant difference ($p > 0.05$) between the OST for TAC group and the control group (34.6± 0.58). The RTD estimates yielded the same kind of outcomes (Fig 4, table 2). After PNV therapy, RTD readings were in line with the healthy group's, but after TAC treatment, they were higher and in line with the untreated group's.

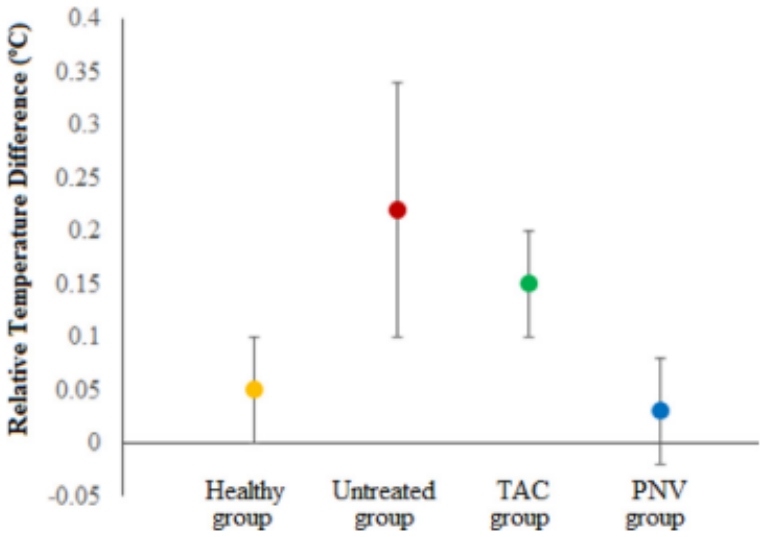

Figure 4. Comparison of control and treatment groups' mean relative temperature differences.

The OSS scores of the untreated group were significantly higher than those of the healthy controls (0.0). While the OSS was reduced after TAC and PNV therapy, the PNV group's decrease was statistically significantly larger, and their values were closer to those of the healthy group. Additionally, globlet cell density was considerably greater in the TAC and PNV treated groups compared to the untreated group. Although there was a significant difference between the two treatments (p 0.05), it was clear that PNV had a greater beneficial impact on goblet cell density.

Discussion

For the treatment of immune-mediated inflammatory ophthalmic disorders, tacrolimus is quickly becoming a popular alternative to cyclosporine. Tacrolimus is also difficult to administer topically, like cyclosporine.

To the cornea and conjunctiva, we recently shown that lipid- and PG-modified PNVs may be able to effectively deliver therapeutic doses of tacrolimus. As a result, we test the effectiveness of tacrolimus PNVs (0.01%) in a rabbit model of experimental DES to see whether they may alleviate symptoms of dry eye syndrome. Atropine and benzalkonium chloride (DE solution) generated experimental DES in the rabbit eye, which is similar to human DES. Atropine reduces tear formation and alters tear functioning, both of which contribute to stress. Benzalkonium chloride, on the other hand, is an irritant. Ocular surface inflammation and damage are the result of the immune system's production of inflammatory mediators in response to stress and irritation. On day 7, the DES group demonstrated substantially decreased tear production and tear stability (TBUT), increased ocular surface inflammation, and ocular and epithelial damage, which is consistent with previous research. These clinical alterations, seen 7 days after topical treatment with DE solution, confirmed the formation of experimental DES in rabbit eye. When STT measurements were compared between the DES group and the untreated group in Table 2, we found that although TBUT and globlet cell density were significantly reduced, the OSS score and OST were significantly enhanced. It seems from the data that after another week of using DE solution, all clinical measures except tear production worsened.

The PNV formulation efficiently reduces ocular surface inflammation, as shown by the absence of MMP-9 in the PNV group of animals and by the reduction in OST and RTD temperatures as determined by IR thermography. There is no evidence of a correlation between PNV and healthy populations. The results show that inflammation of the ocular surface is drastically reduced once PNV formulation is applied. Nevertheless, TAC sol was not successful in reducing inflammation. The modest dosage, limited corneal penetration, and quick evacuation from the eye surface caused by tear turnover and blinking may all be to blame. Furthermore, as tear film works as a heat distributor and aids in reducing heat across the ocular surface, RTD estimate over the surface of the eye might be a determining factor for tear stability as well.

Damage to the epithelial cells and ocular surface is caused by tear film irregularities in dry eye. The OSS score is based on the staining of unprotected epithelial cells with lissamine green to determine the level of damage. The OSS score for the treated group was significantly lower after 1 week with the PNV formulation compared to the untreated and TAC sol treated groups. Goblet cells, which are found in the conjunctiva and fornix, are used to anticipate the extent of damage to the ocular surface. The density of goblet cells is a key indicator of eye health since decreased secretion from these cells results in a weaker tear film and an exposed ocular surface. The density of goblet cells was considerably greater in the PNV formulation group compared to the untreated and TAC sol treated groups, which is promising. The lipid and PG that make up PNVs are a benefit since they have been used therapeutically in the treatment of dry eye condition.

CONCLUSION

This research lends credence to the idea that tacrolimus PNV (0.01%) formulation may be useful in the treatment of rabbits with experimental dry eye syndrome. Because of their altered composition, PNV interacted with the tear film and cornea. Because of their size, they tend to cluster in the conjunctival folds, where they might increase corneal penetration, extend bioavailability, and improve effectiveness.

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