Pharmacokinetics of topically applied tacrolimus dissolved in Marinosolv, a novel aqueous eye drop formulation

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Cortin recent and macrolide immunomodulators such as tacrolimus are effective drugs for the topical treatment of inflammatory eye diseases like allergic conjunctivitis or dry eye. However, tacrolimus is practically insoluble in aqueous solutions and is therefore currently formulated as dispersion. This leads to low bioavailability. Here, we present a novel pharmacologically acceptable, aqueous formulation of tacrolimus based on the “Marinosolv formulation platform”. Marinosolv allows the solubilization and thereby improvement of the bioavailability of many otherwise practically insoluble drugs, since dissolved drugs permeate faster into tissues, including ocular tissues. To visualize the benefits of Marinosolv in ophthalmic formulations, we investigated the permeation of a fluorescently labeled estradiol dissolved in Marinosolv compared to a formulation containing the compound as dispersion. Permeation was studied ex-vivo and in-vivo in porcine eyes. Further, we evaluated the improved permeation of topically applied tacrolimus dissolved in Marinosolv compared to a commercially available topically applied tacrolimus dispersion. The Marinosolv formulation was also compared to oral tacrolimus treatment, the standard application route for this drug in case of severe posterior uveitis. Finally, the ocular tissue levels of tacrolimus in all groups were determined using HPLC/MS. We demonstrated that tacrolimus dissolved in Marinosolv reached significantly higher levels in ocular tissues compared to the marketed topical product or after oral application and thus may be a suitable novel option for the treatment of several eye diseases, such as allergic conjunctivitis or uveitis. Thus, Marinosolv may be considered as a new vehicle for tacrolimus eye drops.

1. Introduction

Corticosteroids as well as immunomodulators are the drugs of choice for the treatment of immune-mediated inflammatory anterior eye compartment diseases, such as allergic conjunctivitis, dry eye, or anterior uveitis [1–3]. Generally, treatment using anti-inflammatory eye drops containing e.g. corticosteroids or immunomodulators, rapidly leads to a significant improvement [2,3]. However, prolonged use of corticosteroids may produce side effects such as cataract formation or steroid-induced glaucoma as well as increased susceptibility to infection [4–7]. Hence, for the treatment of anterior inflammatory ocular disorders, anti-inflammatory therapies using non-steroidal pharmaceuticals are recommended [4,8].

Tacrolimus (FK506) belongs to a group of macrolide immunomodulators, such as cyclosporine A. Although their target molecule, the calcium-dependent phosphatase calcineurin, is identical, tacrolimus is 50–100 times more potent than cyclosporine A [9]. Tacrolimus binds to tacrolimus binding protein, which together form a calcineurin–calmodulin–calcium complex that inhibits the calcium phosphatase activity [2,7,9,10]. Thus, the ability of calcineurin to dephosphorylate NFAT (nuclear factor of activated T cells) is inhibited [9,10]. However, tacrolimus is a highly lipophilic macrolide lactone and therefore shows very poor water-solubility of 1–2 µg/ml [7,11–14]. An additional problem is the susceptibility to hydrolysis, leading to very low stability in aqueous solutions [15]. Hence, tacrolimus is formulated as dispersion for ocular instillation, containing nearly 100% of

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undissolved tacrolimus. However, drugs, such as tacrolimus, should generally be dissolved to allow efficient transport or permeation and reach the target site to become therapeutically effective [16]. It is anticipated that dissolved tacrolimus would permeate faster into ocular tissues, thereby increasing bioavailability and decreasing therapeutic failure [16].

A novel IP protected technology platform, called Marinosolv, was developed which allows the solubilization of lipophilic and hardly water-soluble drugs such as tacrolimus, but also corticosteroids such as budesonide or fluticasone propionate (manuscript in preparation). The solubilization mechanism is based on the formation of micelles by the addition of saponins, such as glycyrrhizin from liquorice root and/or escin from horse chestnut in a buffered aqueous solution (pH 6.0), and the additional use up to 10% of solvents, such as propylene glycol (PG) and dexpanthenol. Osmolarity is between 200 and 340 m-osm/kg. All ingredients in this formulation, except for tacrolimus, are classified as excipients and suitable for both ocular and nasal applications; since they have either already been used as excipients in ophthalmic market products and/or have GRAS (“Generally Recognised As Safe”) status. Using Marinosolv, a solubility of up to 900 µg/ml tacrolimus in an aqueous formulation could be achieved, resulting in an approx. 300-fold increase of dissolved drugs [17].

To visualize the benefits of a dissolved drug in an ophthalmic formulation and thus the benefits of Marinosolv, we monitored the uptake of the hardly soluble, fluorescently labeled drug estradiol, formulated as dispersion compared to dissolution in Marinosolv. Furthermore, we established a porcine ex-vivo model in which different tacrolimus formulations were topically applied onto freshly prepared porcine eyes. After one-hour incubation we determined the absorbed tacrolimus content in the tissues, thereby increasing bioavailability and decreasing therapeutic failure [16].

2. Methods

2.1. Preparation of Marinosolv eye drops & dispersion

Tacrolimus (0.03, 0.05 or 0.07% w/v) was dissolved in Marinosolv containing glycyrrhizin (2% w/v), escin (0.03% w/v), dexpanthenol (5% w/v), EDTA (0.1% w/v), mannitol (2.7% w/v), iota-carrageenan (0.24% w/v), and PG (3% w/v) in a buffered solution. The formulation was prepared by dissolving all water-soluble components in WFI. Fluorescently labelled estradiol (Estradiol glow, Jena Bioscience, #PR-958) (0.001% w/v) was dissolved in Marinosolv containing glycyrrhizin (2% w/v), escin (0.03% w/v), dexpanthenol (5% w/v), EDTA (0.1% w/v), mannitol (2.7% w/v), iota-carrageenan (0.24% w/v), and PG (10% w/v) in a buffered solution. The formulation was prepared by dissolving all water-soluble components in WFI. Fluorescently labelled estradiol was pre-diluted in PG resulting in 300, 500, or 700 µg/ml tacrolimus in Marinosolv four times (at 0, 5, 10, 15 min) resulting in 60, 100, and 140 µg tacrolimus in the respective compartments, cross sections of the cornea and of the sclera-retina layer were prepared. Counter-staining was performed using DAPI (SigmaAldrich, #D9542). For mounting, ProLong Gold (Cell Signaling, #9071) was used. The extent of permeation was determined by laser scanning microscopy using a Zeiss LSM880 Airyscan (excitation wavelength 405 nm and detection wavelength 410–479 nm for DAPI; excitation wavelength 488 nm and detection wavelength 500–550 nm for fluorescein). The extent of permeation was determined by laser scanning microscopy using a Zeiss LSM880 Airyscan (excitation wavelength 405 nm and detection wavelength 410–479 nm for DAPI; excitation wavelength 488 nm and detection wavelength 500–550 nm for fluorescein).

2.2. Determination of tacrolimus content by LC-MS/MS

A Bruker Esquire HCT Ion Trap Mass Spectrometer was combined with an Agilent 1100 series HPLC, equipped with a pre-column UHPLC Guard 3PK Poroshell (2.7 µm particle size, 120 EC-C8 2.1x5 mm) and a Poroshell 120 EC-C18 reversed phase column (4 µm particle size, 2.1 x 100 mm). The isocratic chromatographic separation was performed at 50 °C with a flow rate of 0.2 ml/min of a mobile phase containing 25% H2O, 75% MeOH, and 0.1% FA. The LC system was controlled using the software package Bruker Hystar Version 3.2/SR4. The mass spectrometer scanned from 300 to 1000 m/z in a positive electrospray ionization mode. The target substance was identified as Na-adduct [M + Na]+ at 826.35 → m/z 816.2 and 808.5, and the internal standard (IS) 13C-tacrolimus at the m/z transitions 831.6 → m/z 821.4 and 813.4 at an amplifier voltage of 0.7. The MS system was controlled using the software package Bruker Daltonics esquire 6.1. Data evaluation was performed using Quantanalysis Version 1.8 by plotting peak area ratios of tacrolimus vs. IS 13C-tacrolimus. The limit of quantitation (LOQ), determined according to the signal-to-noise and linear regression method, showed similar values of 0.58 ng/ml for blood samples, 0.62 ng/ml for cornea tissue, 1.25 ng/ml for retina tissue, and 0.83 ng/ml for choroid tissue, corresponding well to published LOQ values [19–21].

2.3. Detection of fluorescently labeled estradiol by Laser Scanning Microscopy

Porcine eyes were treated ex-vivo or in-vivo, respectively, topically with fluorescently labeled estradiol either dissolved using Marinosolv or as dispersion. To control for auto-fluorescence, one eye was treated with PBS in ex-vivo studies and one eye was left untreated in in-vivo studies, respectively. At the end of the experiment, eyes were dissected and prepared for cryo-section. For detection of fluorescently labeled estradiol in the respective compartments, cross sections of the cornea and of the sclera-retina layer were prepared. Counter-staining was performed using DAPI (SigmaAldrich, #D9542). For mounting, ProLong Gold (Cell Signaling, #9071) was used. The extent of permeation was determined by laser scanning microscopy using a Zeiss LSM880 Airyscan (excitation wavelength 405 nm and detection wavelength 410–479 nm for DAPI; excitation wavelength 488 nm and detection wavelength 500–550 nm for fluorescein-glow).

2.4. Animals

Thirty-nine healthy Large White pigs weighing 30–40 kg were included in the experiment. Eye drops were instilled with a pipette onto the conjunctiva of the inferior eyelid after fixation of the pigs. Venous blood samples were taken by puncture of the jugular vein. For ex-vivo and in-vivo experiments eyes were processed immediately after euthanizing animals with an intracardiac instillation of T61 following general IV anaesthesia with 10 mg/kg ketamine and 1.3 mg/kg aza-perone. All experimental procedures were performed according to Austrian Animal Welfare Law (GZ 68.205/0131-WF/V/3b/2016, GZ 68.205/0140-WF/V/3b/2017).

2.5. Pharmacokinetic properties & permeation of tacrolimus into the inner ocular compartments

For ex-vivo experiments, treatment of prepared eyes started within 30 min (Fig. 1). Eyes were assigned to one of four groups and treated in defined intervals. Explanted eyes with surrounding eye sockets were instilled with 50 µl of 300, 500, or 700 µg/ml tacrolimus in Marinosolv four times at (0, 5, 10, 15 min) resulting in 60, 100, and 140 µg tacrolimus in the respective compartments, cross sections of the cornea and of the sclera-retina layer were prepared. Counter-staining was performed using DAPI (SigmaAldrich, #D9542). For mounting, ProLong Gold (Cell Signaling, #9071) was used. The extent of permeation was determined by laser scanning microscopy using a Zeiss LSM880 Airyscan (excitation wavelength 405 nm and detection wavelength 410–479 nm for DAPI; excitation wavelength 488 nm and detection wavelength 500–550 nm for fluorescein-glow).
resulting in 200 µg tacrolimus/eye, or with Prograf diluted to a tacrolimus concentration of 0.03% was measured. After a total of 60 min, cornea, choroidea, and retina tissues were harvested and homogenized for determination of the tacrolimus content by HPLC-MS/MS.

During the in-vivo experiments, pigs were assigned to groups of three animals each. Treatment groups receiving tacrolimus in Marinosolv, Talymus as an ophthalmic comparator, or Prograf as standard oral comparator, are shown in Table 1. In the read out group “tacrolimus tissue content”, blood samples were taken from each animal before treatment and at the end of the experiment. In the read out group “blood PK” (Table 1), blood samples were taken from each animal 1, 4, and 6 h after treatment. Tacrolimus content in blood samples as well as in cornea, choroidea, and retina tissue was determined by HPLC-MS/MS. Statistical analysis was performed using one-way ANOVA and Tukey’s test post-hoc (α = 0.05%).

2.6. Tissue dissection

Eyes were explanted undamaged, rinsed, and washed several times with PBS before dissection to obtain samples of cornea, retina, and choroidea.

3. Results

3.1. Permeation of fluorescently labeled estradiol into cornea and sclera-retina layer ex-vivo and in-vivo

Porcine eyes were treated ex-vivo within 15 min with 2 µg estradiol either as solution (dissolved in Marinosolv) or as dispersion (Fig. 2). Cornea from eyes treated with estradiol solution showed fluorescence as an indicator of estradiol Glow permeation through the epithelial cells, whereas cornea from eyes treated with estradiol Glow dispersion showed no permeation. Control samples treated with PBS also did not show any fluorescence (Fig. 2A). Additionally, permeation into the sclera-retina layer was shown only when estradiol Glow was dissolved in Marinosolv (Fig. 2B).

In in-vivo experiments pigs tolerated droplet instillation very well and showed no local irritations. In-vivo eyes were treated in analogy to the ex-vivo treatment and showed similar histological results as obtained in the ex-vivo studies (Fig. 3). Estradiol Glow dissolved in Marinosolv permeated into the cornea, whereas treatment with estradiol in a dispersion showed fluorescence exclusively at the surface area of the cornea (Fig. 3A). Furthermore, in sections of the sclera-retina-layer, estradiol could be detected only when treated with estradiol dissolved in Marinosolv (Fig. 3B).

2.7. Ex-vivo stimulation of whole blood samples

Blood samples obtained from the read out group “blood PK” (Table 1) were examined for signs of systemic immunosuppression after topical treatment with tacrolimus. For this purpose, anti-coagulated whole blood was diluted 1:1 with PBS and aliquots were transferred into 96-wells. T-cells were stimulated with PMA (phorbol 12-myristate 13-acetate, 20 ng/ml, Sigma) and ionomycin calcium salt (1 µg/ml, Sigma) (PMA-Ionomycin) in duplicates. At each time point, an unstimulated control was included. For the blood drawn four hours after treatment, an additional tacrolimus (positive) control (100 ng/ml) was included to demonstrate the immunosuppressive effect towards PMA-Ionomycin in each individual. The tacrolimus was spiked to the blood one hour before PMA-Ionomycin stimulation. Controls were also prepared in duplicates. Total incubation time in a cell culture incubator after seeding in assay plates was 24 h. Then, samples were centrifuged and the supernatant was frozen for cytokine analysis of IL-4. Supernatants were analyzed at a 1:10 dilution in a bead-based microsphere immunoassay [22]. IL-4 was determined using the standard CSC1283, part 55.128.10 (TF) and CSC1283, part SS.128.09 (TF) as capture antibody as well as ASC0849 (TF) as detection antibody.

3.2. Tacrolimus content in eye tissue compartments

In ex-vivo experiments different concentrations of tacrolimus

<table>
<thead>
<tr>
<th>Read out</th>
<th>Days of treatment</th>
<th>Route of application/formulation</th>
<th>content of tacrolimus [µg/ml]</th>
<th>Times per day</th>
<th>Daily dosage of tacrolimus/eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>tacrolimus tissue content</td>
<td>1</td>
<td>topical Marinosolv</td>
<td>500</td>
<td>2</td>
<td>50 µg/day/eye</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>700</td>
<td>4</td>
<td>100 µg/day/eye</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>topical Talymus</td>
<td>1000</td>
<td>4</td>
<td>200 µg/day/eye</td>
</tr>
<tr>
<td></td>
<td></td>
<td>topical Marinosolv</td>
<td>700</td>
<td>2</td>
<td>70 µg/day/eye</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oral Prograf</td>
<td>0.2 mg/kg BW</td>
<td>1</td>
<td>200 µg/day/kg BW</td>
</tr>
<tr>
<td>blood PK</td>
<td>1</td>
<td>topical Marinosolv</td>
<td>700</td>
<td>1</td>
<td>35 µg/day/eye</td>
</tr>
<tr>
<td></td>
<td></td>
<td>topical Talymus</td>
<td>1000</td>
<td>1</td>
<td>50 µg/day/eye</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oral Prograf</td>
<td>0.2 mg/kg BW</td>
<td>1</td>
<td>200 µg/day/kg BW</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vehicle (n = 1)</td>
<td>0</td>
<td>1</td>
<td>0 µg/day/eye</td>
</tr>
</tbody>
</table>

BW: body weight
dissolved with Marinosolv (300 µg/ml, 500 µg/ml, and 700 µg/ml corresponding to 60, 100, and 140 µg tacrolimus / eye) were compared to the marketed product Talymus (1000 µg/ml tacrolimus corresponding to 200 µg tacrolimus / eye). The lowest concentration of tacrolimus in Marinosolv, 300 µg/ml, resulted in comparable levels of the drug as those obtained with Talymus in all tissues evaluated (Fig. 4). In eyes treated with 500 and 700 µg/ml of tacrolimus dissolved in Marinosolv, 8-fold and 19-fold, respectively, higher concentrations were found in the cornea, compared to the cornea of Talymus treated eyes. Additionally, compared to Talymus treated eyes, 4-fold and 3-fold higher concentrations of tacrolimus were found in the choroidea and retina, respectively, of Marinosolv formulation treated eyes. Comparison of topically applied Prograf diluted to a concentration of 0.03% tacrolimus with a Marinosolv formulation containing the same tacrolimus.

Fig. 2. (A) Laser Scanning Microscopy images of cornea and (B) sclera-retina-layer after ex-vivo ocular treatment with fluorescently labeled estradiol. Sections were fixed, counterstained with DAPI, and mounted with ProLong Gold.

Fig. 3. (A) Laser Scanning Microscopy images of cornea and (B) sclera-retina-layer after ocular treatment in-vivo with fluorescently labeled estradiol. Sections were fixed, counter stained with DAPI, and mounted with ProLong Gold.
concentration showed approx. 0.6-fold lower concentration of tacrolimus in the cornea.

The in-vivo study included a single day experiment with repeated applications of two concentrations of tacrolimus dissolved in Marinosolv (500 µg/ml and 700 µg/ml, 2 and 4 times a day) and Talymus as comparator (1000 µg/ml, 4 times a day), as well as a 4-day experiment with repeated applications (2 and 4 times a day) of only one concentration of tacrolimus dissolved in Marinosolv (700 µg/ml) and Prograf 0.2 mg/kg BW/day given orally as comparator. Tissue concentrations of tacrolimus were determined by HPLC-MS/MS.

The results of the single day experiment showed, independent from the total dose given, a concentration of 3–4 µg/g tacrolimus in corneal tissue when treated with the Marinosolv formulation (Fig. 5). Higher corneal tissue concentrations of 13 µg/g tacrolimus were found after multiple dosing (4 times) over 4 days (Fig. 6). In contrast, the tacrolimus applied as dispersion from the marketed product Talymus revealed only a low tissue concentration of 180 ng/g in corneal tissue after 1-day application (Fig. 5). Additionally, Prograf given orally for 4 days lead to a level of 20 ng/g in the cornea, which is even 10-times lower than topically applied Talymus and more than 100-times lower than the lowest dose of a topically applied Marinosolv formulation (Fig. 6).

In the choroidea, tissue levels of 50–100 ng/g were found independently from given dosages and formulation, when applied for one day (Fig. 5). When treated over 4 days with Marinosolv formulation, tissue levels of 145 ng/g tacrolimus with a 2-times daily application and concentration showed approx. 0.6-fold lower concentration of tacrolimus in the cornea.

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272 ng/g tacrolimus with a 4-times daily application were found (Fig. 6). When given orally (Prograf), tacrolimus tissue levels reached 35 ng/g (Fig. 6).

Independent of given dosages and formulations, as well as independent of the route of application, the tacrolimus tissue concentrations in the retina were between 31 and 141 ng/g with a high inter-individual variation.

3.3. Pharmacokinetics & cytokine levels of tacrolimus in porcine blood samples

In all PK blood samples obtained from animals treated topically in-vivo, the concentration of tacrolimus was lower than the LOQ of 0.586 ng/ml in HPLC-MS/MS. In blood samples obtained from pigs treated orally, one (out of three) animals showed levels slightly above the LOQ, with a peak of 4 ng/ml 2h after treatment. Four hours after treatment the level remained at 1.8 ng/ml, and 6h after treatment blood concentration decreased to the level of LOQ.

T-cells in blood samples from all time points were further stimulated ex-vivo with PMA/Ionomycin for testing potential systemic immunosuppressive effects by the applied tacrolimus. Unstimulated controls of the whole blood ex-vivo stimulation assay were largely below the respective cytokine detection levels. Tacrolimus added in-vitro to the samples (positive controls) strongly inhibited the production of cytokines upon PMA/Ionomycin stimulation (data not shown). Treatment of the animals with Prograf (blood taken at 1h, 4h, and 6h after application) resulted in reduced levels of IL-4 expression in-vitro stimulated whole blood by approx. 2.3-fold to 3.0-fold compared to the groups where tacrolimus was applied topically (Fig. 7). However, the data were statistically not significant at each time point in a one-way-ANOVA.

4. Discussion

The bioavailability of highly potent and hydrophobic drugs, such as tacrolimus, is generally limited by two principal factors: the low solubility and the low stability in aqueous systems [23]. Due to the low solubility, such substances are generally formulated as dispersions or dissolved in solvents, which are not miscible with aqueous solutions. However, dispersed drugs hardly permeate through ocular tissues. They are also further irritating the already affected eye, leading to increased tear fluid production and consequently even faster washout of the drug. Dissolved drugs permeate faster and in higher concentrations into the different ocular compartments [16]. They are less likely washed out before they reach therapeutic levels, leading to higher efficacy and an earlier onset of action at a comparable dose. The low stability of drugs like tacrolimus, which exists in aqueous solutions as equilibrium of three forms (tacrolimus and two tautomers), is based on temperature- and water-sensitive degradation forming several impurities [24]. Drug degradation decreases concentration of the active substance and the therapeutic efficacy. Furthermore, for drug products the strict assessment of impurities is part of the pharmaceutical development. Therefore, it is beneficial for the treatment of ocular conditions to develop a tacrolimus formulation with increased solubility and stability.

In order to demonstrate the benefits of using the novel aqueous formulation Marinosolv for an ophthalmic tacrolimus-containing product, fluorescently labeled estradiol as model substance was applied onto porcine eyes. The histological examinations showed improved permeation into the cornea as well as into the retina-sclera layer, compared to porcine eyes treated with estradiol formulated as dispersion. Hence, Marinosolv does not only increase the solubility of hardly soluble substances such as tacrolimus or estradiol, it also increases the tissue availability of hydrophobic substances. Those findings were shown in both ex-vivo, comparing topically applied Prograf and the Marinosolv at a tacrolimus concentration of 0.03%, and in-vivo experiments, after topical application as well as oral treatment.

In this study, the novel aqueous formulation Marinosolv was used to completely solubilize therapeutically effective doses of tacrolimus for ocular delivery after topical instillation. The novel formulation was able to dissolve tacrolimus to concentrations that are up to 200-fold higher compared to the solubility in water [11–14]. Furthermore, the formulation can be stored several months at 2–8 °C and at least four weeks at room temperature with a recovery of at least 90%, (data not shown) resulting in a much higher long-term storage stability than reported recently [25,26].

During the in-vivo experiments, a commercially available
ophthalmic dispersion, Talymus, as well as Prograf, a marketed tacrolimus-based oral or injection treatment indicated in the prevention of transplant rejection, were used as comparators. When tacrolimus dissolved in Marinosolv was instilled in porcine eyes in-vivo, we found at least 15-fold increase in concentration of tacrolimus in the cornea compared to eyes that were treated with Talymus. Thus, a higher tissue concentration by a factor of 10 to around 30 was achieved by the help of Marinosolv than reported previously[10]. Based on detected tissue levels of tacrolimus, oral treatment was even less effective in this study, since the concentration of tacrolimus in the cornea was up to 645-times lower when compared to tacrolimus formulations applied via the topical route. This confirms the hypothesis that the permeation of the active ingredient, such as tacrolimus, formulated as solution in Marinosolv is more efficient than an ophthalmic dispersion or tissue accumulation of tacrolimus by oral treatments. Thus, Marinosolv formulated eye drops could be more suitable for the treatment of a corneal disease or any anterior eye disease, such as allergic conjunctivitis or dry eye syndrome. Furthermore, tacrolimus dissolved in Marinosolv seems to permeate in higher concentrations into posterior ocular compartments such as the choroida, indicating that the new formulation could also be effective in posterior eye diseases.

In order to evaluate the inhibitory systemic effect due to systemically available tacrolimus, we determined the inhibition of IL-4 production in ex-vivo PMA/Ionomycin stimulated whole blood derived from the PK samples (1 h, 4 h, and 6 h). Tacrolimus is a potent inhibitor of stimulated T-cells as exemplified by different cytokines and therefore we chose IL-4 as responsive representative [27]. Levels of IL-4 after stimulation of whole blood with PMA/Ionomycin were comparable in all topically treated groups (Marinosolv dissolved tacrolimus as well as Talymus at all time points) but decreased significantly and were 3-times lower in whole blood derived from animals treated orally at all time points. Thus, we could demonstrate that topical ocular application of tacrolimus does not result in relevant systemic exposure to the drug while orally given tacrolimus results in systemic drug levels sufficient to reduce ex-vivo induced cytokine production by (and thus activity of) T-cells. Similar results were recently reported after topical application of 0.1% ocular preparation, and the authors concluded that 0.1% tacrolimus ophthalmic suspension is safe and effective and makes it therefore an important option for the treatment of allergic conjunctivitis [2,28].

In summary, we investigated the drug distribution in compartments of the porcine eye after topical application of tacrolimus dissolved in the novel formulation Marinosolv compared to the commercial dispersion Talymus as well as to an oral treatment with Prograf. Our results demonstrate that the permeation into ocular tissue is improved with the Marinosolv formulation also at reduced doses compared to marketed products. No negative local or systemic impacts have been detected and additionally, non-GLP local tolerance studies in rabbits for 14 days, with a dosage of 25 µg (4x 50 µl of a 500 µg/ml formulation) four times daily, has shown no histopathological macroscopical or microscopical findings and a Fluoro-Jade stain did not reveal any degenerative cellular alteration. In conclusion, tacrolimus dissolved in Marinosolv could prove a suitable non-steroidal treatment option for anterior and posterior eye diseases.

Declaration of interest

Competing Interests: The authors have read the journal's policy and declare that the authors Cornelia Siegl, Marielle König-Schuster, Sabine Nakowitsch, Christiane Koller, Philipp Graf, Nicole Unger-Manhart, Yvonne Schindlegger, Norman Kirchoff, and Eva Prieschl-Grassauer are or have been employed by Marinomed Biotech AG. The author Eva Prieschl-Grassauer is co-founder of Marinomed Biotech AG. Cornelia Siegl, Sabine Nakowitsch and Eva Prieschl-Grassauer are inventors of the patent #WO2017009480A1, “Method for improving aqueous solubility of water-insoluble or slightly water-soluble drugs”. The patent is held by Marinomed Biotech AG and is related to the content of the manuscript. A product containing tacrolimus (related to the content of the manuscript) is under development at Marinomed Biotech AG. There are no marketed products to declare. This does not alter the authors’ adherence to all the European Journal of Pharmaceutics and Biopharmaceutics policies on sharing data and materials.

Fig. 7. Full blood analysis after ex-vivo stimulation with PMA/Iono. Data are mean ± SD.
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References


