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Formulation of an ophthalmic lipid emulsion containing an anti-inflammatory steroidal drug, difluprednate

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Abstract

Preparation of oil-in-water (o/w) type lipid emulsion is one of the approaches to formulate drugs that are poorly water-soluble but can be dissolved in the oil phase of the emulsions. A synthetic glucocorticoid medicine, difluprednate (DFBA), is a water-insoluble compound. We formulated DFBA (0.05%, w/v) ophthalmic lipid emulsion containing 5.0% (w/v) caster oil and 4.0% (w/v) polysorbate 80. The appearance of the emulsion was blue and translucent lipid emulsion, and the median particle size of the lipid emulsion was 104.4 nm. Neither separation nor change in particle size was observed after 6 months at 40 °C. Furthermore, when compared with DFBA (0.05%, w/v) ophthalmic suspension, the lipid emulsion showed 5.7-fold higher concentration of DFB that was an active metabolite of DFBA in aqueous humor at 1 h after instillation. Ophthalmic lipid emulsion enhances the intraocular penetration of drugs, and it is useful as a delivery system for the ophthalmic preparations of lipophilic drugs.

Keywords: Lipid emulsion; Polysorbate 80; Difluprednate; Intraocular penetration

1. Introduction

Formulation studies of ophthalmic preparations have often been confronted with a problem for formulation technologies of water-insoluble or poorly soluble drugs. The formulation approaches for drugs that are poorly soluble in water have been addressed by solubilizing the drugs using detergents, organic solvents and solutions with a pH outside the physiological range (Alvarez Nunez and Yalkowsky, 1998; Chien, 1984; Loftsson and Petersen, 1998; Rajagopalan et al., 1988; Sweetana and Akers, 1996). However, these approaches may cause eye irritation, which would be major problems in clinical practice.

The most direct approach is to formulate an ophthalmic suspension. Dose uniformity of ophthalmic suspensions critically depends upon their homogeneity for their precise administration. Many dosing errors

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occur because of flocculation and caking, and also poor re-dispersibility of the suspensions (Deicke and Suverkrup, 1999; Diestelhorst et al., 1998; Kwon et al., 1996). Although ophthalmic preparations require an aseptic product, it is difficult to sterilize the ophthalmic suspensions, because a sterile-filtration technique cannot be applied to the suspensions.

Preparation of oil-in-water (o/w) type lipid emulsion is one of the approaches to formulate drugs that are poorly water-soluble but can be dissolved in the oil phase of the emulsions. The lipid emulsion, primarily intended for parenteral applications, has been investigated as a vehicle of a drug delivery system. The lipid emulsion has no uniformity problems and can apply the sterile-filtration technique. Additionally, the potential of lipid emulsions in ophthalmology is improvement of the ocular bioavailability. The disadvantage of topical application of current ophthalmic preparations is poor bioavailability due to precorneal processes such as the rapid removal of drug from the absorption site and the existence of a cornea that restricts the passage of the drug molecules (Lee, 1990; Lee and Robinson, 1986). A lipid emulsion containing cycrosporine A 0.05% (Restasis^{TM}, Allergan, Irvine, USA) is now exploited commercially as a vehicle to improve the ocular bioavailability (Lallemand et al., 2003).

High concentration of surfactants may lead to ocular toxicity (Vandamme, 2002), although surfactants are needed to manufacture lipid emulsions. The ionic surfactants are generally too toxic to be used for the preparation of lipid emulsions; therefore, nonionic surfactants such as the poloxamars, polysorbates, polyethylene glycol and tyloxapol are preferred (Attwood, 1994; Vandamme, 2002). Polysorbate 80 is widely applied to pharmaceutical preparations including ophthalmic preparations due to its history of usefulness and safety, and it is listed in the United States Pharmacopeia-National Formulary, the European Pharmacopoeia and the Japanese Pharmacopoeia. Selecting appropriate oils and their concentrations is another critical point to manufacture lipid emulsions. Thermodynamic stability of the lipid emulsions changes with the combination of drug, surfactant and oil characteristics (Koycha et al., 1988; Washington et al., 1990). High concentration of oils may cause blurred vision, and oil concentration is limited to 5% (w/v) or less to prevent potential blurred vision (Tamilvanan and Benita, 2004).

A synthetic glucocorticoid medicine, difluprednate (DFBA; 6α,9-difluoro-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione 17-butyrate 21-acetate), is used for the treatment of inflammation (see Fig. 1) (Gardi et al., 1972). DFBA is easily hydrolyzed to DFB (6α,9-difluoro-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione 17-butyrate), a deacetylated metabolite of DFBA, in aqueous humor after instillation (Fig. 1) (Yasueda et al., 2003). DFB also shows the similar activity to that of DFBA (Fujino et al., 1985).

An ophthalmic preparation is desired to bring out the potential of DFBA under clinical practice. However, DFBA is water-insoluble compound. Therefore,

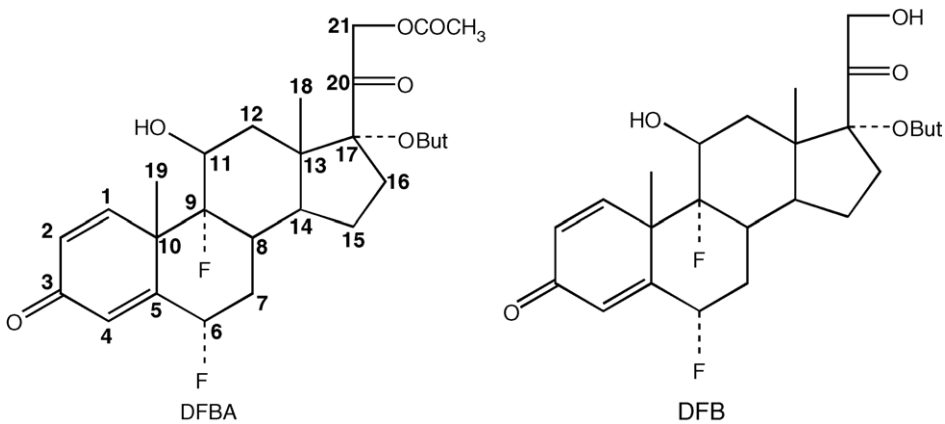

Fig. 1. Difluprednate and its metabolites. Difluprednate (DFBA): 6α,9-difluoro-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione 17-butyrate 21-acetate; DFB: 6α,9-difluoro-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione 17-butyrate.

we formulated a favorable ophthalmic lipid emulsion of DFBA that showed good intraocular penetration.

2. Materials and methods

2.1. Materials

DFBA and DFB were supplied by Mitsubishi Pharma Corporation (Chuo-ku, Osaka, Japan). Castor oil was purchased from Sioe Pharmaceutical (Chuo-ku, Osaka, Japan). Medium chain fatty acid triglyceride was obtained from Mituba Boeki (Shinjyuku-ku, Tokyo, Japan). Cottonseed oil, oleic acid, olive oil, peanut oil and soybean oil were purchased from Nacalai Tesque (Nakagyo-ku, Kyoto, Japan). Polysorbate 80 was purchased from Nikko Chemicals (Chuo-ku, Tokyo, Japan). Water was purified with an auto still (Model WG220, Yamato, Chuo-ku, Tokyo, Japan). Acetonitrile (HPLC grade) was purchased from Kanto Chemical (Chuo-ku, Tokyo, Japan). Other reagents were HPLC grade or the highest grade commercially available. Male Nippon albino rabbits (about 2 kg) from Fukusaki Laboratory Animals Inc. (Fukusaki, Hyogo, Japan) were used in the present study.

2.2. High-performance liquid chromatography (HPLC)

An HPLC system (LC-10A, Shimadzu, Nakagyo-ku, Kyoto, Japan) was composed of an autosampler (SIL-10ADvp), a pump (LC-10ADvp), a column oven (CTO-10ASvp), a UV detector (SPD-10AVvp) and data processing software (CLASS-VP). An octadecylsilica column (TSK-gel ODS-80Ts, 150-mm, 4.6-mm i.d., Tosoh) was also used. Analysis of DFBA and DFB was carried out using a mixture of 10 mM phosphate buffer (pH 7.0) and acetonitrile (55:45, v/v) as mobile phase at a flow-rate of 1.3 ml/min at 40 °C. Detection was performed at 240 nm. Standard solutions of DFBA and DFB were prepared by dissolving each of them (2.0 mg) in acetonitrile (10 ml). A portion of each solution was mixed and diluted with 50% (v/v) acetonitrile.

2.3. Solubility of DFBA in oil

DFBA (0.2 g) was suspended in caster oil, cottonseed oil, medium chain fatty acid triglyceride, oleic acid, olive oil, peanut oil and soybean oil (5 g), then stirred for 3 h at room temperature. Undissolved DFBA suspending particles were removed by membrane filter (pore size 0.45 μm). The filtrate was diluted by tetrahydrofrane to measure concentration of DFBA by HPLC. The injection volume was 20 μl.

2.4. Preparation of DFBA ophthalmic lipid emulsion

Oil-in-water emulsions were prepared containing DFBA (0.05%, w/v), caster oil (5%, w/v) as an oil phase and varying concentrations of polysorbate 80 as an emulsifying agent (0.5–4.0%, w/v). Preparation of emulsion was performed in two steps. As the initial step, 450 ml of water was placed in 1000-ml glass beaker. Polysorbate 80 (2.5, 5.0, 10.0, 15.0 or 20.0 g) and glycerin (11.0 g) were then added to the water and mixed. Sodium acetate (0.25 g) and boric acid (0.5 g) as buffering agents were then dissolved in the solution. DFBA (0.25 g) was added to caster oil (25 g) and dissolved at 70 °C. The caster oil containing DFBA was then added to the solution previously heated at 70 °C and emulsified by a homogenizer (Robomics, Tokusyukikaikogyo, Fukusima-ku, Osaka, Japan) at 8000 rpm for 1 h. The mixture was then cooled at room temperature, and then adjusted at pH 5.5 by hydrochloric acid (1N). The coarse-emulsion was obtained after making it up to a fixed volume by water (500 ml). At the second step, the coarse-emulsion was treated by a high-pressure emulsifier (Microfluidizer M-110EH, Microfluidics Corporation, Newton, MA, USA, or DeBee 2000, BEE International, South Easton, MA, USA). The inlet pressure was set at 1.47×10^5 kPa for Microfluidizer M-110EH and 2.76×10^5 kPa for DeBee 2000. The individual batches were processed through Microfluidizer M-110EH and DeBee 2000 for 20 and 3 discrete volume cycles, respectively, and collected into glass beakers. To cool the emulsion, running water, the temperature of which was controlled at 40 °C around the metal coil, dissipated the heat produced during the microfluidization process. After the treatment, the emulsion was cooled at room temperature (25 °C).

2.5. Particle size analysis

The diameter of the dispersed phase oil droplets in the emulsions was analyzed using a dynamic

light scattering particle size analyzer (HPPS, Malvern Instruments, Worcestershire, UK) without dilution at 25 °C.

2.6. Physical and chemical stability

DFBA ophthalmic lipid emulsion was filtered through a 0.22 μm filter (GVWP, Millipore, Shinagawa-ku, Tokyo, Japan), then filled in 5-ml polypropylene ophthalmic containers. The emulsion was stored for an appropriate period at 25, 40 and 50 °C and assayed for physical and chemical stability. The particle size and pH were used as indicators of physical stability. DFBA content was monitored by HPLC during the stability program.

2.7. Preparation of DFBA ophthalmic suspension

For the DFBA (0.05%, w/v) ophthalmic suspension, hydroxypropylmethyl cellulose (0.2 g), sodium acetate (0.1 g) and sodium chloride (0.8 g) were dissolved in about 80 ml of water in a 100-ml glass beaker. DFBA (0.05 g) was added in the solution, and the suspension was mixed by a magnetic stirrer. The suspension was adjusted at pH 5.5 by hydrochloric acid (1N), and water was used to make it up to a fixed volume (100 ml). The median particle size of DFBA ophthalmic suspension was 4.0 μm.

2.8. In vivo ocular penetration

Unanesthetized rabbits were kept in a prone position on a stainless plate. Ophthalmic emulsion or ophthalmic suspension of DFBA (50 μl) was instilled directly onto the rabbit eye. A rabbit was sacrificed by intravenous overdose administration of sodium pentobarbital solution at 0.5, 1.0, 3.0 and 5.0 h after the instillation, and the aqueous humor (about 200 μl) was collected with a syringe after the eye was washed with saline. The whole eye was enucleated, and then the cornea was excised. The excised cornea was minced in methanol (1 ml) to extract DFBA and the metabolite (DFB). The supernatant was obtained after centrifugation. A portion (50 μl) of the sample was analyzed by HPLC. All experimental procedures were approved by the Institutional Committee for the Care and Use of Laboratory Animals.

2.9. Statistical analysis

Statistical comparisons were done using a student's *t*-test. A *p*-value of 0.05 was retained for significance. The calculation was performed with PRISM (Version 4.0) for Windows (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1. Solubility of DFBA in oils

DFBA is a poorly water-soluble drug and its solubility in water is under 1 μg/ml. DFBA solubility in various oils was examined to formulate a lipid emulsion. The results are summarized in Table 1.

DFBA showed high solubility at 15.78 mg/g in caster oil. Soybean oil is often used for parenteral applications (DeLuca and Boylan, 1984); however, DFBA showed low solubility at 0.57 mg/g in soybean oil. DFBA solubility in medium chain fatty acid triglyceride was 2.12 mg/g, and DFBA solubility in the other oils was under 0.64 mg/g. From these results, we selected caster oil as lipid phase for DFBA ophthalmic lipid emulsion.

3.2. Appearance of DFBA ophthalmic lipid emulsion

The appearance of DFBA ophthalmic lipid emulsion was observed. We selected polysorbate 80, which is a non-ionic surfactant, as emulsifying agent of the lipid emulsion due to its history of usefulness and safety in ophthalmic area. The lipid emulsion containing 5.0% (w/v) caster oil and various concentrations of polysorbate 80 was prepared using a high-pressure emulsifier.

Table 1
Solubility of DFBA in oil

Oil	Solubility (mg/g)
Caster oil	15.78
Medium chain fatty acid triglyceride	2.12
Cottonseed oil	0.64
Olive oil	0.58
Soybean oil	0.57
Peanut oil	0.52
Oleic acid	0.45

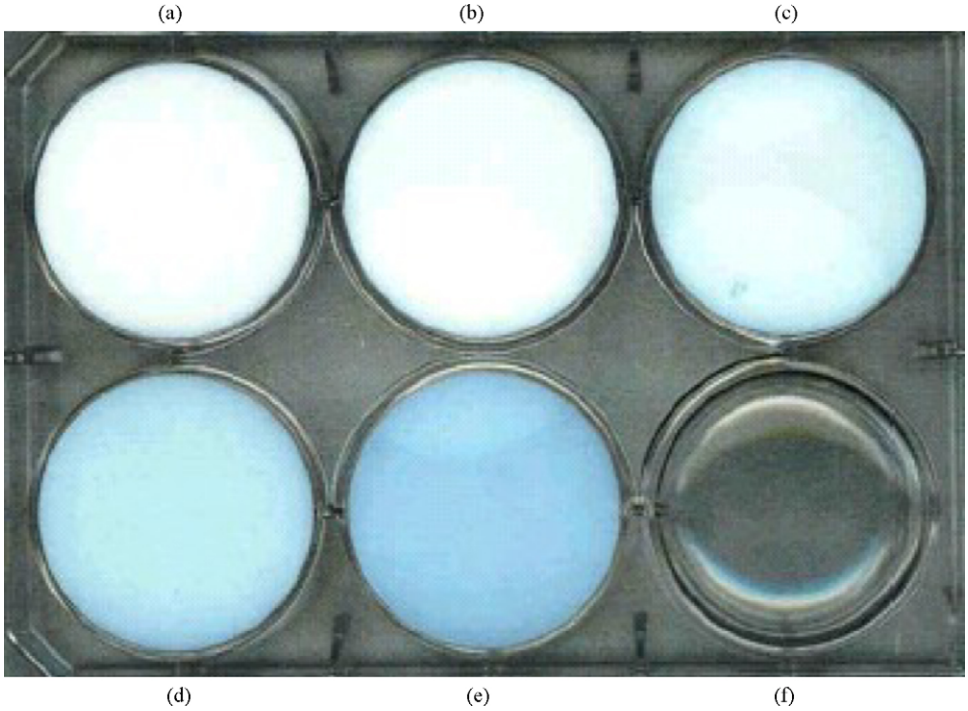

Fig. 2. Appearance of DFBA lipid emulsion. Polysorbate 80 concentrations are: (a) 0.5% (w/v), (b) 1.0% (w/v), (c) 2.0% (w/v), (d) 3.0% (w/v) and (e) 4.0% (w/v). Control is water (f).

Fig. 2 shows a picture of the lipid emulsions in petri dishes.

White lipid emulsions were observed at 0.5, 1.0 and 2.0% (w/v) polysorbate 80 (Fig. 2(a–c)). A slightly blue lipid emulsion was observed at 3.0% (w/v) polysorbate 80 (Fig. 2(d)). Interestingly, the lipid emulsion was translucent blue when it contained 4.0% (w/v) polysorbate 80 (Fig. 2(e)). The translucent emulsion should avoid blurred vision after instillation in eyes. The change of appearance was probably due to the particle size of the lipid emulsion.

3.3. Particle size analysis

Fig. 3(A) shows particle size distributions of the coarse-emulsion and the emulsion treated by the high-pressure emulsifier. A broad distribution from 30 to 900 nm was observed for the lipid emulsion containing 4.0% (w/v) polysorbate 80 before treatment by the high-pressure emulsifier (Fig. 3(A, a)). After treatment by the high-pressure emulsifier, a sharp distribution from 40 to 400 nm was observed (Fig. 3(A, b)). The treatment by the high-pressure emulsifier was an effective approach to align the particle size of the lipid emulsion.

Fig. 3(B) shows the particle size distributions of the lipid emulsions containing various concentrations of polysorbate 80. The particle size distributions were gradually sharpened and shifted to lower particle sizes according to increasing the polysorbate 80 concentrations. The median particle sizes were also decreased according to the increasing of polysorbate 80 concentrations, and they were 204.3, 166.1, 139.8, 121.9 and 104.4 nm at 0.5, 1.0, 2.0, 3.0 and 4.0% (w/v) polysorbate 80, respectively (Fig. 3(C)). The decrease of the median particle sizes was in correlation to the appearance of translucent blue. We did not investigate polysorbate 80 concentration over 4.0% (w/v), because it is preferable to reduce the quantity of surfactants to under 5% in order to avoid the risk of ocular intolerance (Vandamme, 2002). We confirmed previously that no sign of irritation was also observed at less than 4.0% (w/v) polysorbate 80 by ocular toxicity studies in rabbits (data not shown). From these results and for these

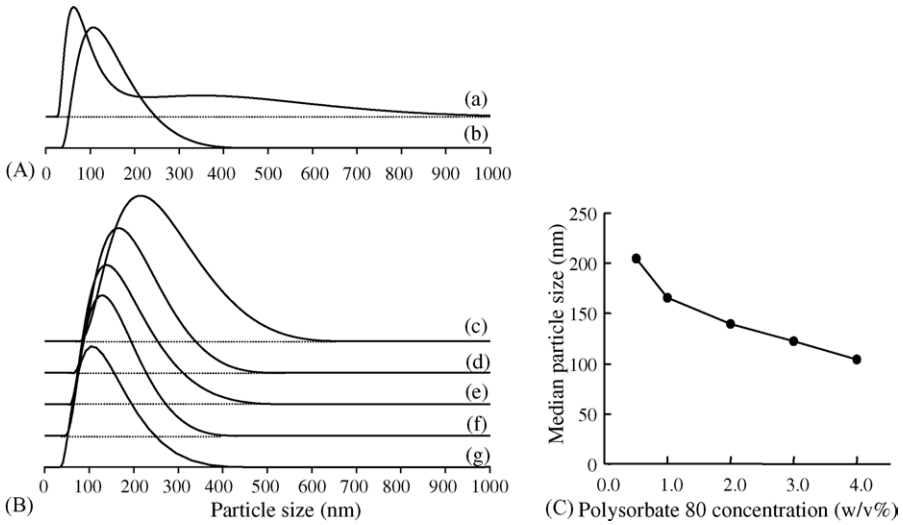

Fig. 3. Particle size distributions of the DFBA lipid emulsion. (A) Effect of treatment by a high-pressure emulsifier: (a) coarse-emulsion and (b) treated-emulsion by a high-pressure emulsifier. The emulsion contained 4.0% (w/v) polysorbate 80. (B) Effect of polysorbate 80 concentrations: (c) 0.5% (w/v), (d) 1.0% (w/v), (e) 2.0% (w/v), (f) 3.0% (w/v) and (g) 4.0% (w/v). The emulsion was treated by a high-pressure emulsifier. (C) Effect of polysorbate 80 concentration on median particle size of DFBA ophthalmic lipid emulsions.

reasons, DFBA ophthalmic lipid emulsion containing 5.0% (w/v) caster oil and 4.0% (w/v) polysorbate 80 was adopted.

3.4. Stability of DFBA ophthalmic lipid emulsion

Stability of the optimized lipid emulsion as described above was investigated after filter-sterilization followed by filling in polypropylene ophthalmic containers. The results of stability are summarized in Table 2.

DFBA content was slightly decreased to 97.2, 96.6 and 98.2 at 25 and 40 °C for 6 months and at 50 °C for 4 weeks, respectively. This was probably due to hydrolysis of DFBA dissolved in water phase of the lipid emulsion, although DFBA solubility in the water phase was low (under 1 μg/ml). DFBA was supplied continuously from oil phase to water phase, when DFBA in water phase was hydrolyzed to DFB. In fact, DFB content of the emulsion was increased with storage time (data not shown). Neither separation nor change in particle size under experimental condition at 25 and 40 °C for 6 months was observed, but the particle size was slightly increased from 108.4 to 115.0 nm at 50 °C for 4 weeks. No change in pH and polydispersity index was observed in all conditions.

Many controversial data were reported on the stability of lipid emulsions. Lipid emulsions are stabilized by a combination of forces such as electrokinetic, hydration and steric repulsive forces, that is, those of the Derijafuin–Landau–Verway–Overbeek (DLVO) theory (Martin et al., 1993). Stability of emulsion

Table 2
Stability of DFBA ophthalmic lipid emulsion

Storage condition	DFBA content (mg/ml)	pH	Particle size (nm)
Initial	0.502 ± 0.004[a] (100.0%)[b]	5.54	108.4 (0.13)[c]
25 °C			
3 months	0.496 ± 0.001 (98.8%)	5.54	106.8 (0.17)
6 months	0.488 ± 0.003 (97.2%)	5.51	108.9 (0.15)
40 °C			
3 months	0.489 ± 0.003 (97.5%)	5.52	107.9 (0.13)
6 months	0.485 ± 0.004 (96.6%)	5.50	109.2 (0.13)
50 °C			
2 weeks	0.490 ± 0.004 (97.6%)	5.50	111.4 (0.12)
4 weeks	0.493 ± 0.001 (98.2%)	5.49	115.0 (0.12)

[a] Mean ± S.D.
[b] Residual percentage.
[c] Polydispersity index.

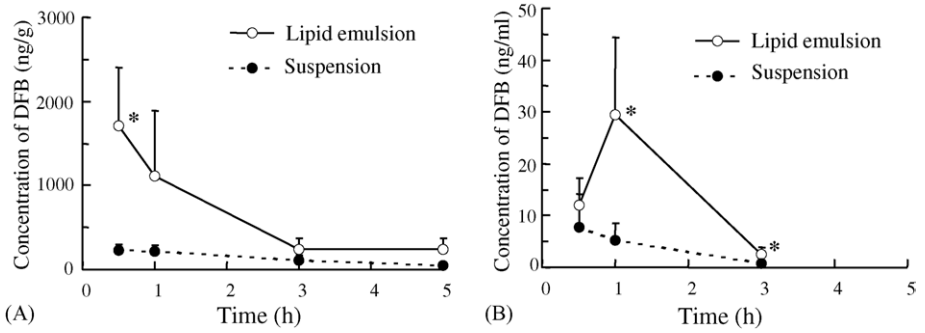

Fig. 4. Concentration–time profile of DFB in the cornea (A) and the aqueous humor (B) after instillation of DFBA ophthalmic lipid emulsion (◯) and DFBA ophthalmic suspension (●) in rabbits. Each value represents the mean ± S.D. ($n = 3$–4). Significant difference: $^{*}p < 0.05$ (Student's *t*-test).

depended on the amount of ζ potential by increasing the electrostatic repulsive force between oil droplets (Chansiri et al., 1999; Hosokawa et al., 2002; Yamaguchi et al., 1995). On the other hand, the stability of lipid emulsions containing non-ionic surfactant did not depend on ζ potential (Elworthy et al., 1971). The ζ potential of DFBA ophthalmic lipid emulsion was almost natural (−2.6 mV), because neutral detergent of polysorbate 80 was used. Our results supported DFBA ophthalmic lipid emulsion containing non-ionic surfactant being very stable against thermal stress, although the ζ potential was natural.

3.5. In vivo ocular penetration

Fig. 4 shows the concentration–time profiles of DFB in cornea and aqueous humor after instillation of ophthalmic lipid emulsion and ophthalmic suspension containing 0.05% (w/v) DFBA. Since DFBA was quickly hydrolyzed to an active metabolite of DFB (Yasueda et al., 2003), it was not detected in cornea and aqueous humor. DFB is a major metabolite in each eye tissue (data not shown). Hydrolysis of DFBA to DFB is probably due to the action by esterase in the eye. The lipid emulsion showed 7.4-fold higher concentration of DFB than the suspension in cornea at 30 min after instillation ($p < 0.05$; Fig. 4(A)). The lipid emulsion also showed a higher concentration of DFB than the suspension in cornea from 1 to 5 h after instillation, but this was not significant. In addition, the lipid emulsion showed 5.7- and 3.1-fold higher concentrations of DFB than the suspensions in aqueous humor at 1 and 3 h after instillation, respectively ($p < 0.05$; Fig. 4(B)).

Table 3
AUCs of DFB in cornea and aqueous humor after instillation of lipid emulsion and suspension in rabbits

Preparation	Cornea $AUC_{0\to5}$ (ng h/g)	Aqueous humor $AUC_{0\to3}$ (ng h/ml)
Lipid emulsion	2954.5 (4.6)[a]	45.3 (4.1)[a]
Suspension	641.6	11.2

[a] Ratio to suspension.

DFB in aqueous humor at 5 h after instillation could not be detected because it was under the detection limit.

The area under curves (AUC) of lipid emulsion and suspension is summarized in Table 3. AUCs of lipid emulsion in cornea and in aqueous humor were 4.6- and 4.1-fold higher than that of suspension, respectively.

In the present study, formulation of an ophthalmic lipid emulsion of DFBA and its advantage was demonstrated. The translucent blue emulsion was observed when a concentration of polysorbate 80 was optimized. DFBA showed good physical and chemical stability in the lipid emulsion. The lipid emulsion had improved intraocular penetration and AUC of DFB, the active metabolite of DFBA, compared to the suspension. A lipid emulsion is a useful choice as a delivery system for the ophthalmic preparations of lipophilic drugs.

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