International Journal of Pharmaceutics 412 (2011) 115–122

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Novel NSAIDs ophthalmic formulation: Flurbiprofen axetil emulsion with low irritancy and improved anti-inflammation effect

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ARTICLE INFO

Article history:
Received 21 November 2010
Received in revised form 15 March 2011
Accepted 17 March 2011
Available online 2 April 2011

Keywords:
Flurbiprofen axetil
Emulsion
Aqueous humor pharmacokinetics
Ocular irritation
Anti-inflammation
Uveitis

ABSTRACT

The aim of this study was to design and formulate a novel low-irritant NSAIDs ophthalmic emulsion of flurbiprofen axetil (FBA), the prodrug of flurbiprofen (FB). FBA ophthalmic emulsion (FBA-EM) was prepared by high-pressure homogenization with caster oil as oil phase and tween 80 as emulsifying agent. Results from the stability evaluation suggested the protect effect of oil droplets on the stability of FBA. Compared with FBA-oil solution, the $AUC_{0\rightarrow 10\,h}$ of FB in aqueous humor administered in FBA-EMs exhibited 6.7-fold (F2), 4.5-fold (F3) and 4.6-fold (F4) increase. With the increment of oil content, the *MRT* also prolonged, which of FBA-EM F2–F4 were 5.14 ± 2.23, 5.73 ± 3.35 and 8.71 ± 0.94 h, respectively. No significant difference was found between the ocular bioavailability of FBA-EM F2 and 0.03% FB-Na eye drops. Ocular irritation evaluation revealed that FBA-EM F2 had better ocular biocompatibility than 0.03% FB-Na eye drops, even though the FBA concentration was up to 0.1%. Intraocular anti-inflammation effect evaluation showed that FBA-EM F2 had a quite good anti-inflammation effect. In conclusion, FBA-EM F2 with elevated FBA concentration to be 0.1% might represent a promising NSAIDs ophthalmic emulsion with low irritancy and improved anti-inflammation effect.

1. Introduction

Over the past 2 decades, the use of NSAIDs for treating ocular inflammation has increased, although steroidal agents have been the standard treatment. The main advantage of using topical NSAIDs is the avoidance of undesirable effects of steroidal agents, which include the decreased immunological response to infection, cataract formation, steroid-induced increases in IOP, and inhibition of re-epithelialization following epithelial denudation. Being acidic, NSAIDs are inherently irritating. Reducing the pH of formulation further increases their irritation potential (Schalnus, 2003), as well as decreasing their aqueous solubility. Frequently reported adverse reactions of NSAIDs include transient burning, stinging, and minor signs of ocular irritation and instillation (Joseph, 1996). Hypersensitivity reactions with itching, reddening, photosensitivity, and keratitis punctata may occur.

Flurbiprofen (FB), 2-(2-fluorobiphenyl-4-yl) propionic acid, is one of the NSAIDs, the sodium salt of which (0.03% flurbiprofen sodium (FB-Na) ophthalmic solution, Ocufen®, Allergan) is employed to inhibit intraoperative miosis during cataract surgery, control postoperative inflammation, prevent cystoid macular edema and reduce ocular pain (Araújo et al., 2009; Vega et al., 2006, 2008). Nevertheless, transient burning, stinging upon instillation and other minor symptoms of ocular irritation, including fibrosis, miosis and mydriasis, have been reported with the use of flurbiprofen sodium ophthalmic solution. Flurbiprofen solutions of concentration greater than 0.2% (w/v) are irritating (Ahujia et al., 2008). Thus, the inherent drawback of ocular irritation limited the usage of elevated FB-Na dosage (only 0.03% in Ocufen®), which eventually leads to unsatisfied anti-inflammation effect. Therefore, it is of great importance to formulate NSAID formulations that are comfortable when applied topically to the eye.

Flurbiprofen axetil (2-fluoro-α-methyl-4-biphenylacetic acid 1-acetoxy ethyl ester, FBA) as an ethereal salt of FB would have better ocular biocompatibility through esterification of the carboxy-group in the molecule. As there are adequate esterases in the cornea (Mannermaa et al., 2006), FBA would quickly converse to its active parent drug FB to play anti-inflammation effect. The schematic picture of FBA metabolism after instillation was illustrated in Fig. 1. The purpose of this study is to formulate a novel NSAIDs ophthalmic

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doi:10.1016/j.ijpharm.2011.03.041

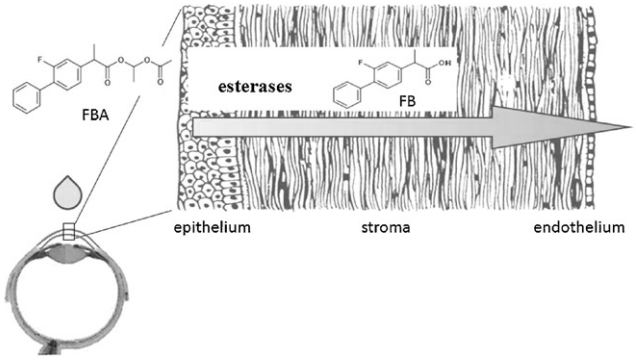

Fig. 1. Schema picture of the metabolism of FBA after administration topically.

emulsion of flurbiprofen axetil, the prodrug of FB, to overcome its drawbacks of ocular irritancy.

However, FBA is presented as yellowish oil with poor water solubility, which is unable to be formulated as aqueous eye drops. In the last decade, oil-in-water (o/w) type lipid emulsions have been investigated and are now exploited commercially as a vehicle to improve the ocular bioavailability of lipophilic drugs (Tamilvanan et al., 2002; Vandamme, 2002; Yamaguchi et al., 2005; Liu et al., 2009). The natural biodegradability, nanometer droplet size range, sterilizability and substantial drug solubilization either at the innermost oil phase or at the o/w interface, and improved ocular bioavailability are thus making the lipid emulsion a promising ocular delivery vehicle (Lv et al., 2005; Sakai et al., 2005; Tamilvanan and Benita, 2004).

Taking into consideration of this study, the idea of encapsulation of FBA into oil droplets might bring about not only the reduced ocular irritation, but also the improved ocular bioavailability of lipophilic FBA and consequently improved anti-inflammation effect.

In conclusion, the aim of this study was to design and formulate a novel low-irritant NSAIDs ophthalmic emulsion of the prodrug FBA. The effects of oil content (0.1–2.5%) on the physical and chemical stability and the in vivo aqueous humor pharmacokinetic behavior were investigated. Furthermore, the ocular irritation and anti-inflammation effect was evaluated taking 0.03% flurbiprofen sodium ophthalmic solution as reference.

2. Materials and methods

2.1. Materials

Flurbiprofen axetil and flurbiprofen were purchased from Shanghai Sanwei Pharmaceutical Co. Ltd. (Shanghai, China). Castor oil was bought from Hunan Er-kang Pharmaceutical Co. Ltd. (Hunan, China). Tween 80 was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Glycerol was purchased from Shanghai Minshi Chemical Company (Shanghai, China). Pentobarbital sodium was supplied by Shanghai Westang Biotechnology Co. Ltd. (Shanghai, China). Carbopol 974 was kindly donated by Lubrizol Specialty Chemicals Manufacturing Co. Ltd. (Shanghai, China). Deionized water was made using Milli-Q (Gradient). Other reagents were of analytical grade.

Male New Zealand albino rabbits weighing 2–3 kg were provided by the Animal Experimental Center of Shanghai Institute of Materia Medica. The animals were housed in standard cages in a light-controlled room at 19 ± 1 °C and 50 ± 5% RH and were fed a standard pellet diet and water ad libitum. All studies were approved by the Department of Laboratory Animal Research at Shanghai Institute of Materia Medica. Procedures involving animals were reviewed and approved by the Animal Ethics Committee at Shanghai Institute of Materia Medica.

Table 1
Formulation composition of FBA-EM.

Formulation	Content (g/100 ml)				
	FBA	Castor oil	Tween 80	Glycerol	Carbopol 974
F1	0.1	0.1	0.08	2.2	0.125
F2	0.1	0.5	0.4	2.2	0.125
F3	0.1	1.0	0.8	2.2	0.125
F4	0.1	2.5	4.0	2.2	0.125

2.2. Preparation of FBA ophthalmic emulsion (FBA-EM)

Oil-in-water emulsions were prepared containing FBA (0.1%, w/v), caster oil (0.1–2.5%, w/v) as an oil phase and tween 80 as an emulsifying agent (0.08–4%, w/v). The detailed formulations of FBA-EM were listed in Table 1. Preparation of emulsion was performed in two steps (Yamaguchi et al., 2005). As the initial step, 400 ml of water was placed in 1000 ml glass beaker. Tween 80 (0.4–20 g) and glycerin (11.0 g) were added to the water and mixed. Sodium acetate (0.25 g) and boric acid (0.5 g) as buffering agents, and sorbic acid (0.36 g) as preservative were then dissolved in the solution. The oil phase composed of FBA (0.5 g) and caster oil (0.5–12.5 g) was dissolved at 70 °C. The oil phase was added into the water phase previously heated at 70 °C slowly while emulsified by a high-shear dispersing emulsifier (T25 basic, IKA Guangzhou, China) at 12,000 rpm for 10 min. The mixture was cooled at room temperature, adjusted at pH 5.50 by NaOH (1 M) and then adding water to 450 ml to get the coarse-emulsion. At the second step, the coarse-emulsion was treated by a high-pressure homogenizer (AH-100D, ATS, Shanghai, China). The inlet pressure was set at 100 bar for 2 discrete volume cycles and 1200 bar of the outlet pressure for 6 cycles, respectively, the temperature of the whole homogenize process was controlled at 40 °C approximately by running water. After homogenization, the emulsion was cooled at room temperature (25 °C) and 1.25% carbopol 974 water solution (0.625 g in 50 ml) was added to meet the final volume of 500 ml to obtain FBA-EM.

Flurbiprofen sodium eye drops (FB-Na, 0.03%, w/v) were prepared taking the formulation of Ocufen® as reference (Vandamme, 2002).

2.3. Particle size, polydispersion index and encapsulation efficiency analysis

The particle size distribution of the oil droplets in FBA-EM formulations (F1–F4) were analyzed using a dynamic light scattering analyzer (Nicomp388/Zeta PALS, Particle Sizing System, USA) at 25 °C after dilution by deionized water (1:50).

The encapsulation efficiency was determined by ultrafiltration. A 500-μl aliquot of FBA-EM was transferred to the upper chamber of a centrifuge tube fitted with an ultrafilter (Vivaspin500, Sartorius, MWCO 10 kDa), which was then centrifuged at 4000 rpm for 30 min. The amount of FBA loaded in the oil droplet was calculated as the difference between the total amount used in preparation of the emulsions and the amount in the filtrate, as determined by HPLC. The drug encapsulation efficiency was calculated according to formula (1):

$$Q_W = \frac{W_{total} - W_{free}}{W_{total}} \times 100\% \approx \frac{C_{total} - C_{free}}{C_{total}} \times 100\% \quad (1)$$

where Q_W is the drug encapsulation efficiency; W_{total} is the total amount of drug in the emulsions; W_{free} is the amount of drug in the filtrate; C_{total} is the concentration of drug in the emulsions; C_{free} is the concentration of drug in the filtrate (Gan et al., 2009).

2.4. Physical and chemical stability

The storage stabilities of FBA-EM were determined as follows (Hu et al., 2006). Briefly, FBA-EM (5 ml as a unit volume) were filled into polypropylene ophthalmic containers, stored at 4 °C, 25 °C, 40 °C and 50 °C for 4 weeks, respectively. At intervals aliquots of samples were withdrawn for pH and particle size assay. The hydrolytic extent (HE%) of FBA in FBA-EM formulations was monitored during the storage time, and calculated according to formula (2):

$$HE\% = \frac{W_{FB}}{W_{total}} \times 100 \approx \frac{C_{FB}}{C_{total}} \times 100 \quad (2)$$

where W_{total} was the total amount of FBA and FB in the sample, W_{FB} was the amount of FB in the sample, C_{total} was the total concentration of FBA and FB in the sample, and C_{FB} was the concentration of FB in the sample.

The HPLC system (Agilent 1100 series) used comprised an autosampler (G1313A ALS), a pump (G1311A Quat-pump), a column oven (G1316A Column), a UV detector (G1314A VWD) and data processing software (HP Chemstation Rev.A.10.01). XDB-C_{18} column (4.6 mm × 250 mm, 5 μm) was used for FBA and FB analysis with acetonitrile:water (58:42, adjusted to pH 2.90 by glacial acetic acid solution) as mobile phase at a flow rate of 1.0 ml/min at 40 °C. Detection was performed at 247 nm. The HPLC detection method was also applied to the in vivo examinations.

2.5. Aqueous humor pharmacokinetic studies

Studies were performed on the fully awake male New Zealand albino rabbits. Rabbits were randomly divided into 5 groups, with 24 ones in each group. The following formulations were tested: FBA-EM (F2–F4), FB-Na eye drops and FBA-oil solution (0.1% w/v FBA in castor oil). At time intervals of 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0 and 10.0 h after instillation of 50 μl formulations, the rabbits were anaesthetized with an intravenous injection of pentobarbital sodium (30 mg/kg), and approximately 150 μl of aqueous humor was withdrawn by anterior chamber paracentesis. The samples were stored immediately at −20 °C before HPLC analysis.

2.6. Safety evaluation based on histological examination

Male New Zealand albino rabbits were healthy and free of any ocular damage. To examine the influence of different formulations on the cornea structure and integrity, fresh eyes were excised immediately after the rabbits were sacrificed by intravenous air injection into the marginal ear vein. The eyes were rinsed for 1 min with 0.9% (w/v) NaCl, and then incubated at 37 °C for 30 min in the FBA-EM (F2) and FB-Na eye drops. PBS and sodium dodecylsulfate (SDS) solution in PBS (0.1%, w/v) were taken as references (Baydoun et al., 2004). After incubation, the eyes were washed with PBS, and immediately fixed with a formalin solution 8% (v/v). The specimens were dehydrated with an alcohol form. Cross sections (<5 μm) were cut, stained with hematoxyline and eosine (H and E), blinded and microscopically observed for histological modifications.

2.7. Assessment for intraocular anti-inflammation effect

The intraocular anti-inflammation effect of FBA formulations were evaluated based on rabbit model of endotoxin-induced uveitis (EIU). A 1 mg/ml stock solution of endotoxin (Lipopolysaccharide Escherichia coli O111:B4, Chemicon International Inc., Canada) was diluted immediately before injection into isotonic saline under sterile conditions. A total of 9 (3 for each treatment group) rabbit eyes were injected using a 30 gauge needle inserted 2 mm posterior to the limbus in the superotemporal quadrant (Baranano et al., 2009). For each treatment group, eyes were injected with 50 μl of saline containing 10 ng of endotoxin, then the PBS, FBA-EM F2 (2 times/day) and FB-Na eye drops (6 times/day) were instilled into rabbit eyes, respectively. 24 h after injection of endotoxin, rabbits were sacrificed. 150 μl of aqueous humor was removed by anterior chamber paracentesis for the leukocyte and prostaglandin E_2 (PGE_2) levels determination. Leukocyte counts were performed by a hemocytometer, and the PGE_2 levels were determined by enzyme-linked immunoassay according to the manufacture's instructions (rabbit PGE_2 ELISA kit, Shanghai DoBio Biotech Co. Ltd., Shanghai, China).

After the aqueous humor was taken, the animals were sacrificed immediately. The eyeballs were enucleated, washed with PBS, and fixed with a formalin solution 10% (w/v) immediately. The specimens were dehydrated with an alcohol gradient, put in melted paraffin and solidified in block form. Sagittal section (<5 μm) were cut, stained with hematoxyline and eosine (H and E) and microscopically observed for pathological examination (He et al., 2006).

Table 2
Particle size, polydispersion index (P.I.) and encapsulation efficiency (E.E.) of the formulations.

Formulation	Mean size (nm)	P.I.	E.E. (%)
F1	152.1 ± 2.25	0.196 ± 0.08	98.6 ± 0.22
F2	143.6 ± 1.89	0.230 ± 0.12	98.1 ± 0.18
F3	159.7 ± 2.20	0.256 ± 0.13	99.2 ± 0.07
F4	238.7 ± 2.24	0.216 ± 0.09	98.5 ± 0.12

2.8. Statistical analysis

Experiments were performed in replicates for validity of statistical analysis. The results were expressed as mean ± S.D. Student's *t*-test was performed on the data sets using Origin for Windows®. Differences were considered statistically significant for p values < 0.05.

3. Results and discussion

3.1. Particle size, polydispersion index and encapsulation efficiency analysis

As shown in Table 2 and Fig. 2, the mean particle sizes of FBA-EM F1–F3 were about 150 nm. With the elevation of oil content, the mean size of FBA-EM F4 enlarged to about 238.7 nm. It was clearly listed in Table 2 that various FBA-EM formulations had similarly high drug encapsulation efficiency (>98%), which could be due to the high solubility of lipophilic FBA in the caster oil.

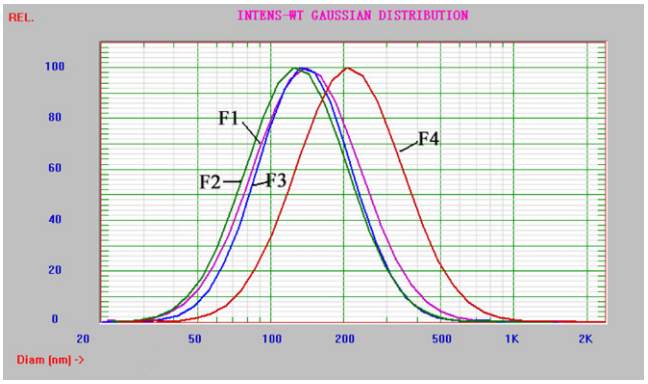

Fig. 2. Particle size distribution of FBA-EM F1–F4.

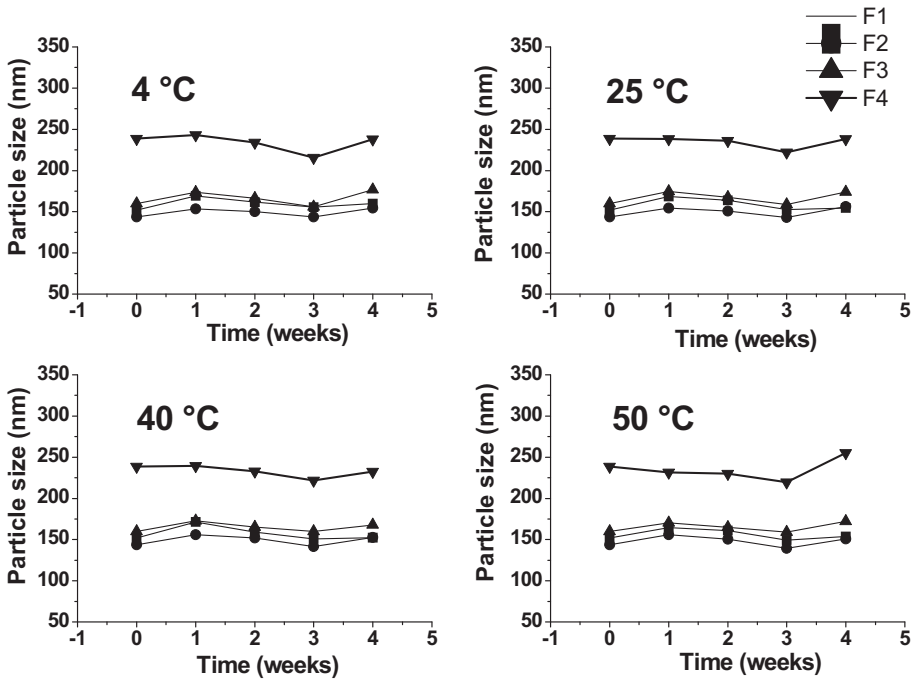

Fig. 3. The changes of particle size of FBA-EM F1–F4 against storage time at 4, 25, 40 and 50 °C, respectively.

3.2. Physical and chemical stability

Physical stability of FBA-EM was indicated by the results of particle size and pH after storage for 4 weeks under 4, 25, 40 and 50 °C. For the FBA-EM formulations F1–F3 (Fig. 3), no significant changes was found in particle size after storage. Only for FBA-EM F4 stored for 4 weeks under 50 °C, particle size slightly increased to above 250 nm, which indicated merging of some oil droplets under the temperature above 40 °C. As shown in Fig. 4, the pH of FBA-EM F1–F4 kept stable at about 5.5 under various storage condition.

Changes in hydrolytic extent (HE%) of FBA was investigated to evaluate the chemical stability of the FBA-EM. Under various temperatures, great difference in the changing tendency of HE% could be seen in Fig. 5. At 4 °C, HE% of all FBA-EM formulations kept at about 0.75% for 4 weeks. After storage at 25 °C, the HE% elevated slightly from about 0.75 to 1.0%. After 4 weeks storage at higher

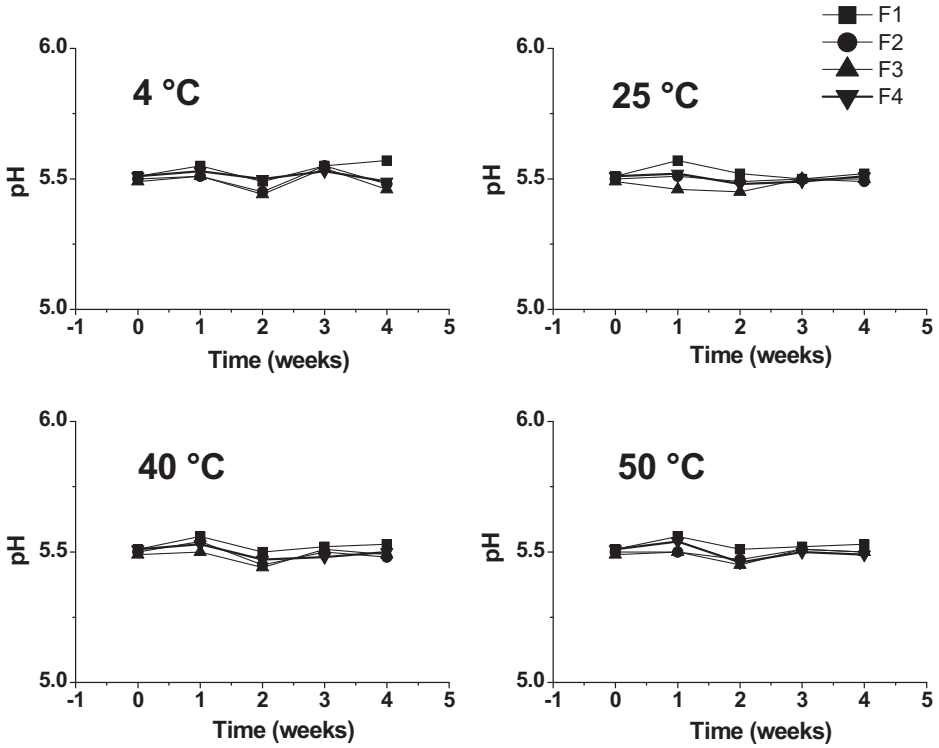

Fig. 4. The changes of pH of FBA-EM F1–F4 against storage time at 4, 25, 40 and 50 °C, respectively.

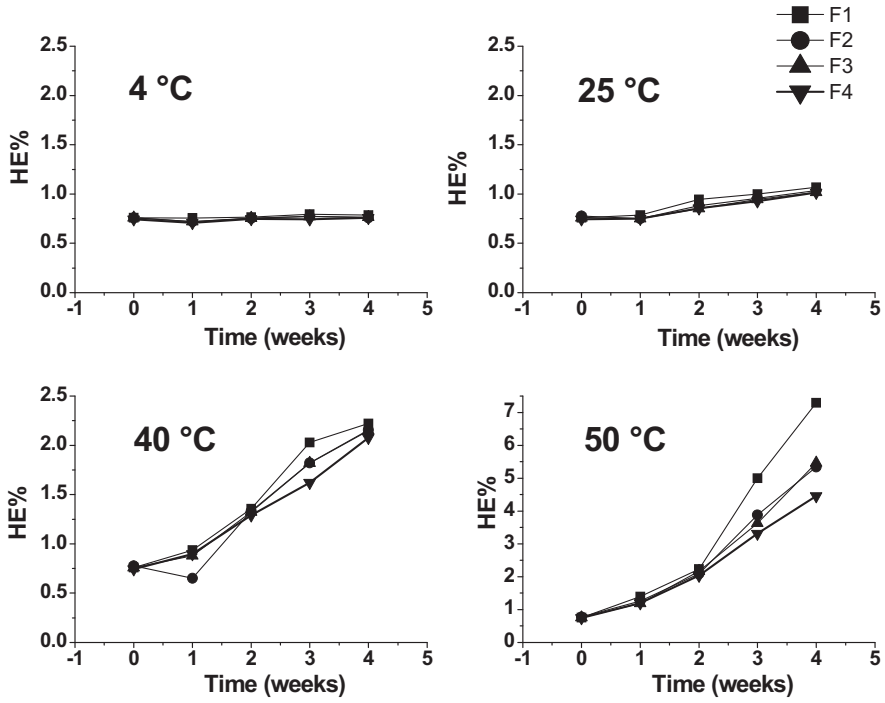

Fig. 5. The changes in hydrolytic extent (HE%) of FBA-EM F1–F4 against storage time at 4, 25, 40 and 50 °C, respectively.

temperature of 40 °C and 50 °C, HE% increased greatly to about 2.0% and above 4.0%, respectively.

HE% indicated the hydrolysis of FBA dissolved in water phase of the lipid emulsion, although FBA solubility in the water phase was low. FBA was supplied continuously from oil phase to water phase, when FBA in water phase was hydrolyzed to FB. With the elevation of storage temperature, the hydrolysis of FBA was accelerated.

Meanwhile, significant differences in HE% of F1 and F4 could be observed. After storage at 50 °C for 4 weeks, HE% of F1 was about 7.0%, which of F4 was about 4.0%. This result suggested the protect effect of oil droplets on the stability of FBA, as most of the drugs participated in the oil droplets. With the raise of oil content from 0.1 (F1) to 2.5% (F4) in the lipid emulsion formulation, the HE% of FBA was significantly reduced. Hence we can see that in case of ester drugs sensitive to the aqueous environment, formulation of lipid emulsion with relatively higher oil content might effectively protect the drugs from degradation.

During the investigation period, no emulsions showed phase separation indicating the good storage stability of FBA-EM. As the chemical stability of FBA-EM F1 was relatively poor, it was therefore not investigated in the pharmacokinetic studies.

3.3. Aqueous humor pharmacokinetic studies

The pharmacokinetic profiles of FB in aqueous humor after instillation of FBA-EM (F2–F4), FBA-oil solution and 0.03% FB-Na eye drops were investigated. Due to the strong ocular irritancy, 0.1% FB-Na eye drops was not evaluated. Since FBA was quickly hydrolyzed to an active metabolite of FB (Ohmukai, 1996), it was not detected in the aqueous humor by HPLC. FB was a major metabolite in the aqueous humor. Hydrolysis of FBA to FB was probably due to the action by esterase in the eye. The limit of determination (LOD) and limit of quantification (LOQ) of FB was 7.15 ng/ml and 23.76 ng/ml, respectively, which of FBA was 0.49 μg/ml and 1.67 μg/ml, respectively.

Concentration–time profiles of FB in aqueous humor after topical instillation of the formulations were shown in Fig. 6, with parameters been summarized in Table 4. The $AUC_{0\rightarrow 10\,h}$ of FB administered in FBA-EM F2–F4 and FBA-oil solution were 151.20 ± 68.9, 100.91 ± 51.95, 103.85 ± 4.28 and 22.43 ± 2.74 μg min/ml, respectively. Compared with FBA-oil solution, the $AUC_{0\rightarrow 10\,h}$ of FB administered in FBA-EM exhibited 6.7-fold (F2), 4.5-fold (F3) and 4.6-fold (F4) increase, *Cmax* of which also showed significant increment ($p < 0.05$).

The effect of lipid emulsion to improve ocular bioavailability has been already reported by many researchers (Baranano et al., 2009; Lallemand et al., 2003; Lv et al., 2006). From a medical point of view, lipid emulsions for ophthalmic use aim to enhance drug bioavailability either by providing prolonged delivery to the eye or by facilitating transcorneal/transconjunctival penetration (Luma et al., 2004). It has been proved that the spreading coefficient values of one droplet could be reduced by emulsions, therefore they have better wettability properties on the cornea compared to saline. The lipid emulsion may then prolong the residence time of the droplet

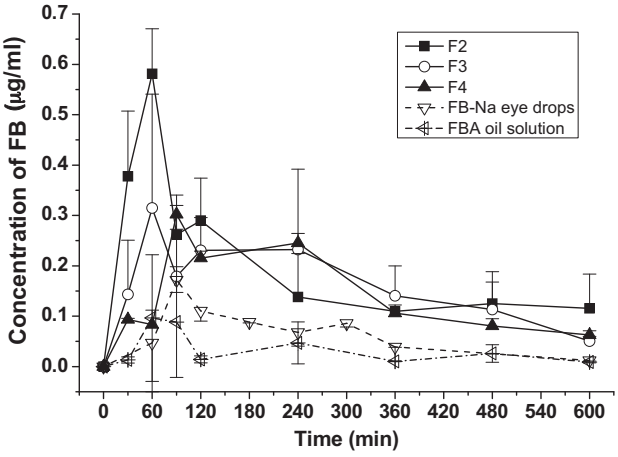

Fig. 6. Concentration–time profiles of FB in the aqueous humor after instillation of FBA-EM F2–F4, FB-Na eye drops and FBA-oil solution in rabbits.

Table 3
Aqueous humor pharmacokinetic parameters of different formulations after ophthalmic instillation ($n = 3$).

Formulations	*Cmax* (μg/ml)	*MRT* (h)	$AUC_{0\rightarrow 10\,h}$ (μg min/ml)	*F* (%)
F2	0.58 ± 0.02[a]	5.14 ± 2.23	151.20 ± 68.9[a]	105
F3	0.35 ± 0.18[a]	5.73 ± 3.35	100.91 ± 51.95[a]	70
F4	0.30 ± 0.02[a]	8.71 ± 0.94[b]	103.85 ± 4.28[a]	72
FBA-oil solution	0.10 ± 0.13	4.73 ± 0.34	22.43 ± 2.74	–
FB-Na eye drops	0.18 ± 0.01	4.60 ± 0.30	43.25 ± 6.90	–

[a] Statistically difference to FBA-oil solution, $p < 0.05$.
[b] Statistically difference to F2, $p < 0.05$.

on the epithelial layer of the cornea, thereby enabling better drug penetration through the cornea to the internal tissues of the eye (Klang et al., 2000). While, for the oil solution formulation, the poor wettability property and fast clearance from preocular surface led to the limited ocular drug bioavailability.

Another phenomenon needs to be paid attention was the *MRT* of FB administered in FBA-EM formulations, which of FBA-EM F2–F4 were 5.14 ± 2.23, 5.73 ± 3.35 and 8.71 ± 0.94 h, respectively. With the increment of oil content, the *MRT* also prolonged. Significant difference could be seen between the *MRT* of FB administered in FBA-EM F2 and F4 (Table 3). As the log *P* of FBA was about 4.8, it is relatively lipophilic. The partition rate of FBA from the oil droplet to the epithelia membrane might be reduced with the increase of oil content. Therefore, this result might provide a hint for the future ophthalmic lipid emulsion formulation design as the sustained release behavior would be obtained by properly elevating the oil content. What is more, the sustained release behavior should be a patient-friendly property with reduced dosage frequency.

The relative bioavailability (*Fr*) of FBA-EM (F2–F4) versus marketed product 0.03% FB-Na eye drops was calculated according to the formula (3):

$$Fr = \frac{AUC_{FBA-EM}/dose_{FBA-EM}}{AUC_{FB-Na\ \ eye\ \ drops}/dose_{FB-Na\ \ eye\ \ drops}} \times 100\% \quad (3)$$

The *Fr* of FBA-EM F2–F4 were 105%, 70% and 72%, respectively. It was shown that no significant difference was found between the bioavailability of FBA-EM F2 and 0.03% FB-Na eye drops. Ocular bioavailability of the FBA-EM F3 and F4 were relatively reduced, compared with 0.03% FB-Na eye drops. Higher content of oil would perform a good sustained release effect, but might simultaneously restrict the transcorneal delivery of drugs. As could be seen, the bioavailability of the emulsion formulations was equal to or less than the eye drops at the same dose. This might be due to the difference of log *P* between FBA and FB. It has been shown that the optimal lipophilicity for corneal permeation corresponds to log *P* values of 2–3 (Mannermaa et al., 2006). The esterification of FB to FBA would also lead to the increase of lipophilicity. The log *P* of FBA

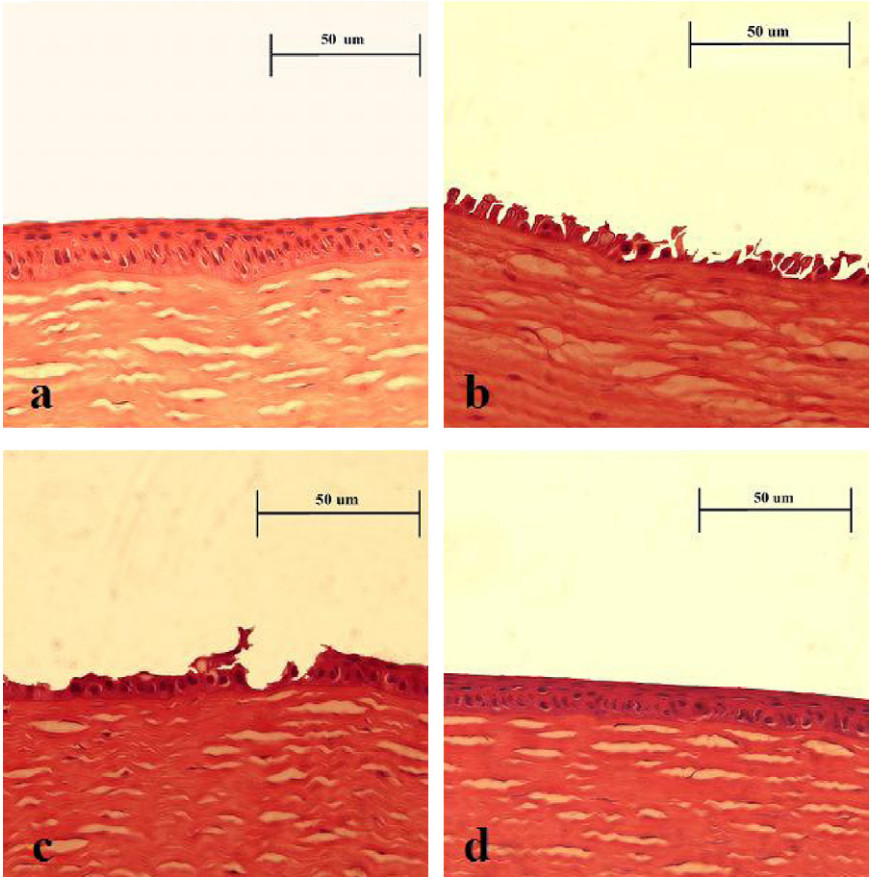

Fig. 7. Histologic cross sections of excised rabbit cornea showing epithelium (EP) and stroma (ST), stained with hematoxylin-eosin (scale bar 50 μm) after incubation at 37 °C. (a) PBS, pH 7.4; (b) sodium dodecylsulfate (SDS) solution 0.1% (w/w); (c) 0.03% FB-Na eye drops, pH 7.0; and (d) FBA-EM F2, pH 5.5.

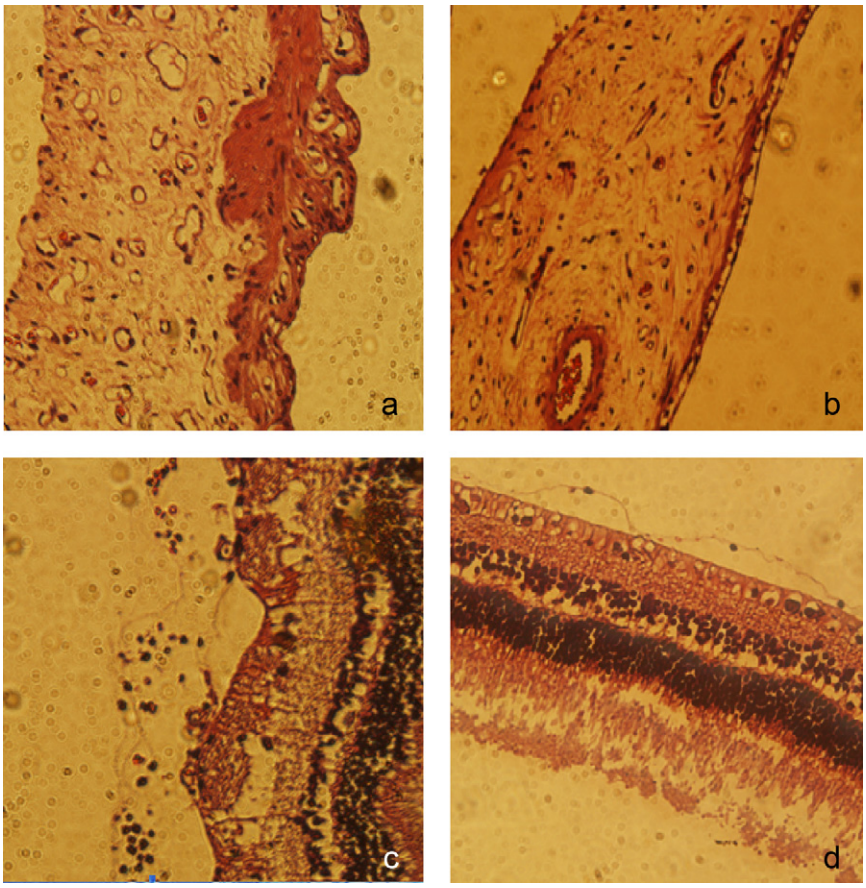

Fig. 8. Results of histological examination of iris and retina of rabbit eyes with experimental uveitis after treatment with FBA-EM F2. (a) Iris of untreated group; (b) iris of FBA-EM F2 treated group; (c) retina of untreated group; (d) retina of FBA-EM F2 treated group.

was 4.8, which of FB was just 3.6. It was too lipophilic for FBA to penetrate through the cornea than FB, therefore leaving the equal or less bioavailability than the FB eye drops.

3.4. Safety evaluation based on histological examination

Fig. 7 presented corneal cross sections after incubation of freshly excised rabbit corneas with various formulations to investigate their influence on the keratocyte structure and tissue integrity. After incubation in PBS pH 7.4 (Fig. 7a), epithelium (EP) and stroma (ST) structure was maintained. Typical stratified epithelial layer can be recognized by the basal columnar cells and the squamous surface cells appearing with a bulge at the nuclei (NU). When corneal epithelium was exposed to an irritant, as 0.1% SDS (Fig. 7b), previously narrow intercellular spaces are clearly widened, cells and nuclei are deformed and superficial epithelial cells are detached from tissue assembly. There were also some superficial epithelial cells detached in case of incubating with 0.03% FB-Na eye drops (Fig. 7c). While the Fig. 7d showed a cornea cross section after incubation in FBA-EM F2, which leaves corneal structure and integrity visibly unaffected.

As could be seen from the results, FBA-EM F2 had better ocular biocompatibility than 0.03% FB-Na eye drops, even though the FBA concentration was up to 0.1%. The better biocompatibility may thank to the carboxy-group shielding effect by the emulsion carrier. Encapsulation efficiencies of FBA-EM formulations were above 98% (Table 2), which meant most of the drug was encapsulated in the oil droplets. Thus, few drugs partitioned in the aqueous phase, leaving few carboxy groups to exert ocular irritation effect (Han et al., 2010).

3.5. Results of intraocular anti-inflammation effect

Injection of endotoxin into the rabbit vitreous induced a robust inflammatory response at twenty-four hours, reflected in a mean aqueous total leukocyte count of 4560.0 ± 176.3 cells/μl (Table 4), conformed to the date reported by David (Lallemand et al., 2003). Administration of FBA-EM F2 significantly reduced the inflammatory response to 890.0 ± 353.6 cells/μl ($p < 0.05$), which of FB-Na eye drops treated group was 3567.5 ± 802.6 cells/μl. The reduction of $80.9 \pm 7.6\%$ by FBA-EM F2 was significantly ($p < 0.05$) higher than that ($23.4 \pm 17.2\%$) of FB-Na eye drops.

At 24 h after induction by endotoxin, aqueous PGE_2 levels raised to 1023.4 ± 133.6 ng/l. The level of PGE_2 was significantly reduced to 562.1 ± 50.1 ng/l in eyes treated with FBA-EM F2 ($p < 0.05$), which was lower than that of FB-Na eye drops treated group (722.5 ± 202.4 ng/l). It was suggested that FBA-EM F2 had a quite good anti-inflammation effect.

Histopathologic examination was performed to illustrate the anti-inflammation effect of FBA-EM F2 on experimental uveitis. Typical histological photographs showed marked inflammatory cell infiltration in the iris and retina. In particular, the iris in untreated

Table 4
Pharmacodynamic assessment of leukocyte counts and PGE_2 levels ($n = 3$).

Group	leukocyte counts (cells/μl)	PGE_2 levels (ng/l)
Normal	415.0 ± 33.2	490.7 ± 22.4
Control (*EIU* model)	4560.0 ± 176.3	1023.4 ± 133.6
FBA-EM F2	890.0 ± 353.6[a]	562.1 ± 50.1[a]
FB-Na eye drops	3567.5 ± 802.6	722.5 ± 202.4

[a] Statistically difference to control group, $p < 0.05$.

eyes was markedly swollen with proteinaceous exudates (Fig. 8a), and retinal architectures were severely disorganized with photoreceptor outer segments loss, subretinal exudation, and serous retinal detachment (Fig. 8c). However, in the FBA-EM F2 treated group (Fig. 8b and d), iris and retina were less disorganized with minimal inflammatory cell infiltration and showed well-preserved architectures (Shin et al., 2009).

4. Conclusions

In this study, a novel low-irritant NSAIDs ophthalmic formulation of FBA emulsion was designed and prepared. With the elevation of oil content from 0.1 to 2.5%, the physical and chemical stability of FBA-EM was improved. Aqueous humor pharmacokinetic studies revealed that no significant difference was found between the ocular bioavailability of FBA-EM F2 (with oil content of 0.5%) and 0.03% FB-Na eye drops. As could be seen from ocular irritation elevation based on histological examination, FBA-EM F2 had better ocular biocompatibility than 0.03% FB-Na eye drops, even though the FBA concentration was up to 0.1%. In vivo experiments based on rabbit model of endotoxin-induced uveitis proved that FBA-EM F2 had quite good anti-inflammation effect. In conclusion, FBA-EM F2 with elevated FBA concentration to be 0.1% might represent a promising NSAIDs ophthalmic emulsion with low irritancy and improved anti-inflammation effect.

Acknowledgements

We thank the National Basic Research Program of China (No. 2009CB930300) and National Science and Technology Major Project “Key New Drug Creation and Manufacturing Program” (No. 2009ZX09301-001) for their financial support.

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