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A controlled-release ocular delivery system for ibuprofen based on nanostructured lipid carriers

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ABSTRACT

The objective of this study was to develop an ocular drug delivery system based on nanostructured lipid carrier and investigate its in vitro and in vivo characteristics. Ibuprofen was chosen as the model drug. Four different formulations of Ibuprofen nanostructured lipid carriers were prepared by melted-ultrasonic methods; Gelucire 44/14 was screened as one of the solid lipid matrix materials due to the good particle size dispersion and excellent contribution to the corneal permeability of the model drug. The modified Franz-type diffusion cells and isolated corneas were used in the test of drug corneal permeability and the in vivo releasing tests were carried out using microdialysis method. Gelucire 44/14 and Transcutol P could enhance the corneal permeability by different mechanisms. The corresponding apparent permeability coefficients (P_{app}) were 1.28 and 1.36 times more than that of the control preparation. Stearylamine could prolong the pre-cornea retention time of the model drug to some extent. Ibuprofen nanostructured lipid carriers displayed controlled-release property. The AUC of the optimized formulation of Ibuprofen nanostuctured lipid carriers was 3.99 times more than that of Ibuprofen eye drops).

1. Introduction

The bioavailability of traditional ocular drug delivery systems such as eye drops is very poor because eye is protected by a series of complex defense mechanisms that make it difficult to achieve an effective drug concentration within the target area of the eye. Many approaches have been developed to solve the problem in recent decades, of which colloidal drug delivery system has been paid much attention. For example, liposome and emulsion are reported as effective ocular drug carries (Assil et al., 1991; Meisner and Mezei, 1995; Nagarsenker et al., 1999; Law et al., 2000); But they still have many disadvantages like expensive excipients and poor stability. Nanostructured lipid carrier (NLC), a new generation of solid lipid nanoparticles (SLN), was developed in early 1990s; now it is a good alternative carrier of traditional colloidal controlled-release drug delivery system (Müller et al., 2000). NLC is composed of a solid lipid matrix with certain content of liquid lipid; it has many advantages such as good biocompatibility due to the use of physiological and biodegradable lipids of low systemic toxicity, possibility of production on large industrial scale and reduction of drug leakage during storage. Some authors reported that SLN drug delivery system can improve ocular bioavailability (Cavalli et al., 2002; Hu et al., 2005) but there have not been reports on NLC as an ocular drug delivery system until now.

Another approach is to increase the transcorneal passage of drugs by incorporating permeation enhancers into formulations (Diane et al., 1994). Gelucire 44/14 was used as one of the solid lipids in this study and it was found that it could enhance drug corneal permeability; Transcutol P is(has been) exploited as a corneal permeability enhancer recently and was used in this study (Liu et al., 2006). Ibuprofen was selected as a model drug of a highly lipophilic nature.

This work was focused on the preparation of a controlled-release Ibuprofen NLC ocular drug delivery system.

2. Materials and methods

2.1. Materials

Compritol ATO 888; Gelucire 44/14 and Trancutol P were kindly gifted by Gattefosse; Miglyol 812 (Guangdong, China), stearylamine (Ningbo, China), LM-10 microdialysis probe (Bioanalytical Systerm, USA).

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2.2. Preparation of free and drug-loaded NLCs

The Ibuprofen NLCs were prepared by melted-ultrasonic method. Table 1 showed the four formulations investigated. For all formulations water with 2% Crempher EL and appropriate permeability enhancer were added to the hot lipid phase slowly at 75 °C and a premix was formed by the aid of magnetic stirring at 400 rpm. The crude emulsion was then treated by probe-type ultrasonic for 5 min (active every 3 s for a 3 s duration, 400 W) and subsequently the NLCs were cooled immediately at 0 °C.

2.3. Particle size and zeta potential measurement

The particle size distributions of the prepared NLCs were measured by a LS230 laser particle size analyzer (Beckman-Coulter, USA) and the zeta potentials were measured by a Delsa 440SX zeta potential analyzer (Beckman-Coulter, USA).

2.4. Determination of entrapment efficiency

Entrapment efficiency of Ibuprofen NLCs was measured (Zeisig et al., 1998) by gel permeation chromatography on a Sephadex G-50 column (15 mm × 200 mm). An appropriate amount of NLC sample was loaded onto the gel column and eluted continuously with deionized water at a speed of 1.0 mL/min. The eluants were collected separately in numbered tubes to separate NLC entrapped drug and free drug. The absorbance of the settled eluants was measured at 265 nm with a UV spectrophotometer (UV-9100, shanghai, China). Each determination was run in triplicate. The entrapment efficiency was calculated according to the following equation:

$$E(\%) = \frac{(\mathrm{TD} - \mathrm{UED})}{\mathrm{TD}} \times 100$$

where E (%) was the trapping efficiency, TD the concentration of total amount of drug, and UED the concentration of free drug.

2.5. Stability of NLC

The storage stabilities of four drug-loaded NLCs were determined as follows (Hu et al., 2006). Briefly, a volume of 5 mL of Ibuprofen NLC dispersions were filled into glass vials, and stored at 25 °C and 4 °C for 1 month, and the changes of particle size against storage time were investigated.

2.6. Ocular irritation of NLC

The ocular irritation of the NLC was evaluated by rabbit winking test (Wei et al., 2002). A total of 12 New Zealand white rabbits each weighing 2.5–3.0 kg was used. The 12 rabbits were divided into four groups depending on the type of the Ibuprofen NLC. The NLC samples (25 μL) were instilled into the left eye of the rabbits and the rabbits were forced to wink once to spread the NLC uniformly on the corneas. Then the frequency of the rabbits winking in 5 min after instillation was recorded. As the control, phosphate buffered saline pH 7.4 was instilled into the left eye 24 h after the instillation of NLC.

2.7. Pre-corneal retention of four NLC

The pre-corneal retention of the NLC on rabbits cornea was determined by the following method. After instilling 150 μL NLC samples into the eyes of rabbits, a little piece of filter paper (2 mm × 5 mm) was put into the conjunctival sac for about 10 s, then a 0.5 mL tube was placed immediately, the total weight was determined before and after the tear being adsorbed (the volume of tear could be calculated by the difference of the weight). Then 150 μL of ethanol was added into the tube, ultrasonic for 15 min, and the solution was filtered and the concentration of Ibuprofen in tear was determined by HPLC method. The samples were prepared at 10, 20, 40, 60, 120 and 180 min after instillation.

2.8. Test of permeability of drug through the isolated-cornea

Male New Zealand white rabbits (Animal Experiment Center of Shenyang Pharmaceutical University) weighing 2.5–3.0 kg were used. All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 92–93, revised in 1985), and were approved by the Department of Laboratory Animal Research at Shenyang Pharmaceutical University. The procedures with animals were reviewed and approved by the Animal Ethical Committee at Shenyang Pharmaceutical University. After the rabbits were sacrificed by intravenous air injecting into the marginal ear vein, the fresh corneas were excised immediately, weighed and preserved in glutathione bicarbonate Ringer (GBR) buffer (O'Brien and Edelhauser, 1977). The corneal permeation studies were carried out using Franz-type cells. 2 mL of Ibuprofen NLC (1 mg/mL Ibuprofen) and 7.8 mL of GBR buffer were filled into the donor and receptor chamber, respectively. In reference cell, 2 mL of Ibuprofen eye drops (1 mg/mL Ibuprofen, in 0.9% NaCl) was used as control. For the tests on the effect of solid lipid and penetration enhancers, Ibuprofen NLC with Gelucire 44/14 and Ibuprofen NLC with 0.02% Trancutol P were used when Ibuprofen NLC without them was taken as control. The cells were maintained at 34 °C with magnetic stirring. The corneas should be installed into the Franz-type cells within 0.5 h after excision, and the available area for diffusion was 0.70 cm^2. At time intervals of 40, 80, 120, 160, 200 and 240 min, 1 mL of sample was withdrawn from the receptor chamber, and an equal amount of GBR buffer was added to maintain the original volume. Each experiment was run in triplicate. The concentration of the drug in the samples was determined by HPLC.

The apparent permeability coefficient (P_{app}) was calculated as follow (Schoenwald and Huang, 1983):

$$P_{app} = \frac{\Delta Q}{\Delta t \times C_0 \times A \times 60}$$

$$J_{ss} = C_0 \times P_{app}$$

where the term $\Delta Q/\Delta t$ was the steady-state of the linear portion of the plot of the amount of drug in the receptor chamber *vs* time, A the available cornea area for diffusion (0.70 cm^2), C_0 the initial concentration of drug in the donor cell and 60 the conversion of units from minute to second.

2.9. Determination of corneal hydration levels

The corneal hydration level (HL%) was calculated from (Liu et al., 2005):

$$\mathrm{HL}\% = \left[1 - \left(\frac{W_a}{W_b}\right)\right] \times 100$$

where W_b was the wet cornea weight, and W_a the corresponding dry cornea weight after a desiccation of 6 h at 100 °C. The corneal hydration levels were determined for both newly excised corneas (no later than 30 min after removal from the rabbit eyes) and treated corneas (after the corneal permeation tests).

2.10. Optimized formulation and the microdialysis test

From the result of above tests, an optimized formulation was determined as following: Compritol 888 ATO 400 mg, Gelucire

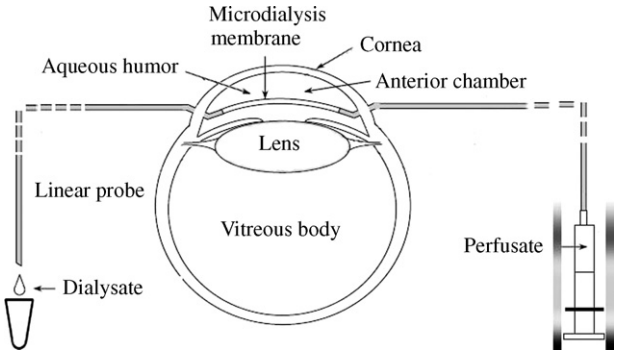

Fig. 1. The schematic of microdialysis probe implantation in the ocular.

44/14 400 mg, Miglyol 812 200 mg, stearylamine 20 mg, Ibuprofen 20 mg and Transcutol P 5 mg in 10 mL distilled water. The optimized formulation of Ibuprofen NLC was prepared by the method described before; the entrapment efficiency, particle size, zeta potential and corneal permeability of it were measured. Then the microdialysis test was carried out using this Ibuprofen NLC as test preparation.

Rabbits ($n = 4$) were anesthetized with the injection of lidocaine hydrochloride. A custom-designed LM-10 microdialysis probe (Bioanalytical System, USA) was implanted into the anterior chamber of each rabbit eye as described (Lonnroth et al., 1987). Probe inlet and outlet lines were tunneled beneath the conjunctiva, under the upper eyelid, as was shown in Fig. 1. The leads were protected with a latex glove pocket affixed to the top of the head. The probe was introduced as described previously (Duchêne and Wouessidjewe, 1996), the anchor was sutured to the sclera with 7-0 Vicryl, and conjunctiva was sutured over the anchor. Exterior wound surface were treated with ofloxacin 0.3% ophthalmic solution. Animals were used for experimentation after >5 days recovery.

Conscious rabbits ($n = 4$) were placed in rabbit restrainers "home made" which permitted free movement of the head. Following a 1 h equilibration period with perfusion of saline through the probe, different concentration standard Ibuprofen saline solutions (2.01, 4.02, 6.03, 8.04, 10.5 μg/mL) were perfused through the probe at a rate of 3 μL/min, and dialysates were collected for 15 min after 30 min of perfusion. A 20 μL aliquot of each fraction was analyzed by HPLC. In vivo recovery was defined as (Lonnroth et al., 1987):

$$R = \frac{(C_{in} - C_{out})}{(C_m - C_{out})}$$

where C_{in} was the concentration of the standard solution, C_{out} the concentration of the dialysate, and C_m the concentration in aqueous humor. A linear equation was plotted by $(C_{in} - C_{out})$ *vs* C_{out}, and the slope of the line gave the recovery (R).

After the disturbance of the standard solution was reduced to be negligible by perfusion of saline through the probe, 40 μL of Ibuprofen NLC as test or Ibuprofen ophthalmic solution as reference was placed in the lower cul-de-sac with a micropipette. In general, the rabbits closed their eyes without blinking after Ibuprofen administration. Immediately 60 μL fractions of effluent were collected every 20 min for 1 h, then 90 μL were collected every 30 min for 6 h. A 20 μL aliquot of each fraction was assessed by HPLC.

Table 2
EE%, particle size and zeta potential of the four studied formulations

Formulations	NLC-1	NLC-2	NLC-3	NLC-4
EE (%)	89.5	95.6	90.3	94.5
Particle size (nm)	160.1	80.6	108.7	120.7
Zeta potential (mv)	−23.2	−33.1	−27.5	25.7

Where NLC-1 is Ibuprofen nanostructured lipid carrier; NLC-2 is Ibuprofen nanostructured lipid carrier with Gelucire 44/14; NLC-3 is Ibuprofen nanostructured lipid carrier with Transcutol p; NLC-4 is Ibuprofen nanostructured lipid carrier with stearylamine.

3. Results and discussions

3.1. NLC preparation and characterization

Ibuprofen is a water-insoluble drug and melted-ultrasonic method is considered to be an appropriate method to prepare its NLC. A prerequisite for good drug accommodation is significant differences between the C chains length of the fatty acid glycerides and general imperfections in the crystal (Müller et al., 2002). So glycerides formed by different fatty acids (e.g. mixture of long chain and short chain, saturated and unsaturated acids) were used as lipid materials. Gelucire 44/14 is a semi-solid lipid with saturated polyglycolized glycerides as active ingredients and has a C chain of 12 carbon atom, so there is difference between the C chain of Gelucire 44/14 (12) and Compritol ATO 888 (22) and Miglyol 812 (8/10) which are often used in the formulation of NLC drug delivery system. Besides, it is also reported that it can enhance the drug permeability across skin. Stearylamine is added into the formulation as a charge-inducing reagent for cationic NLC.

In order to obtain NLC with high entrapment efficiency, stability and corneal permeability, different Ibuprofen NLC formulations were investigated. The formulations were shown in Table 1.

Table 2 displayed the EE%, particle size and zeta potential of the four formulations.

The particle size of the four studied formulations are small, d_{90} of all the four formulations are smaller than 300 nm, and the particle size that human eyes can tolerate is about 10 μm (Andreas Zimmer and Jörg Kreuter, 1995). However, they still have some differences: the Ibuprofen NLC-1 is a little larger than the other three ones. Due to the content of Gelucire 44/14, Transcutol p and stearylamine having emulsifying effect to a certain extent, the emulsion drops of NLC-2, NLC-3, NLC-4 are smaller than NLC-1 when the crude emulsions are formed, and when they are cooled, the particles are correspondingly smaller.

3.2. Stability of NLC

To assess the effect of the storage temperature on the stability, the NLC dispersions were stored at 4 and 25 °C over a period of

Table 1
Composition of the investigated NLCs formulations (10 mL)

	Compritol ATO (mg)	Gelucire 44/14 (mg)	SA (mg)	Miglyol 812 (mg)	Ibuprofen (mg)	Cremphor EL 40 (mg)	Transcutol P (mg)
NLC-1	800			200	20	200	
NLC-2	400	400		200	20	200	
NLC-3	800			200	20	200	10
NLC-4	790		10	200	20	200	

Where SA is Stearylamine, NLC-1 is Ibuprofen nanostructured lipid carrier; NLC-2 is Ibuprofen nanostructured lipid carrier with Gelucire 44/14; NLC-3 is Ibuprofen nanostructured lipid carrier with permeability enhancer Transcutol p; NLC-4 is Ibuprofen nanostructured lipid carrier with charge-inducing reagent stearylamine.

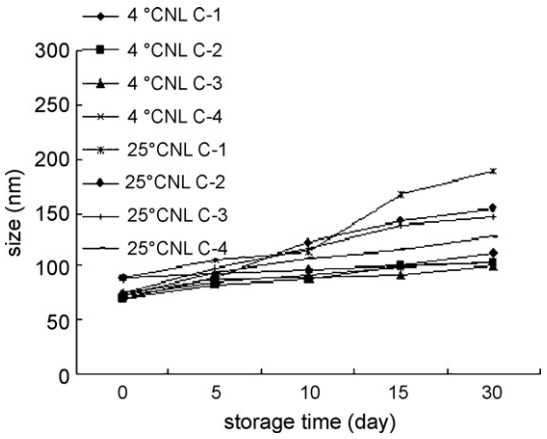

Fig. 2. The changes of the particle size against storage time at 4 and 25 °C.

Table 3
Corneal hydration levels ($n = 3$)

Formulation	Hydration level (%)
Eye drops	76.2 ± 0.74
NLC-1	77.4 ± 0.50
NLC-2	77.7 ± 1.24
NLC-3	82.8 ± 1.82

Data are mean ± S.D., $n = 3$.

30 days. Fig. 2 showed the changes of particle size against storage time. In the case of 25 °C, the particle sizes of NLCs were increased significantly ($P < 0.001$) during the storage period (Fig. 2). The particle growth was slower ($P > 0.05$) when NLCs were stored at 4 °C. In addition, the visible aggregation of NLCs (stored at 25 °C) was found at the 15th and 30th day, respectively. High temperature (25 °C) increased the kinetic energy of system, which could accelerate the collision of particles, and consequently increased the possibility of aggregation for nanoparticles. Moreover, from Fig. 2, it could be seen that the rate of particle growth for NLC-4 (NLC with stearylamine) was slower than that for the other NLCs. This was probably due to positive charges on particle surface of NLC-4 and the repulsion between the particles made the particle growth slower.

3.3. Ocular irritation and corneal hydration levels of Ibuprofen NLCs

Hydration levels of cornea are used to evaluate the corneal damage of substances in vitro. The hydration level of healthy cornea is 76–80% (Schoenwald and Huang, 1983), and when a hydration level higher than 83% is detected, the cornea is considered to suffer a certain degree of injury.

The hydration levels of the excised cornea exposed to the Ibuprofen NLCs (Table 3) presented satisfactory correlation with the result of ocular irritation test (Table 4). Ibuprofen NLC with Transcutol P and Ibuprofen NLC with stearylamine displayed little damage to the corneas, while Ibuprofen NLC with Gelucire 44/14 showed very slight irritation to corneas.

Table 4
Winking counts in 5-min period after instillation of 25 μl NLC samples in rabbit eyes ($n = 3$)

Formulation	Winking counts	
	Control (PBS, pH 7.4)	Preparation
Eye drops	8.00 ± 1.00	7.33 ± 1.52
NLC-1	10.0 ± 2.00	12.0 ± 1.00
NLC-2	10.0 ± 3.00	12.0 ± 1.00
NLC-3	15.7 ± 0.58	17.3 ± 2.51
NLC-4	14.3 ± 2.08	16.3 ± 1.53

Data are mean ± S.D., $n = 3$.

3.4. Pre-corneal retention of NLC

The results of the pre-ocular retention of the four kinds of Ibuprofen NLC showed that NLC-4, the NLC containing stearylamine, displayed a longer pre-ocular retention time. It might suggest that the positive charge on the surface of cationic NLC particles should have a stronger affinity to the negative-charging corneal surface and result in a longer retention time.

3.5. Corneal permeation studies on Ibuprofen NLCs

A total of nine male New Zealand white rabbits were used and divided into three groups. For each rabbit, the cornea of left eye was used for reference preparation, while the cornea of the right eye was for test preparation. The P_{app}, J_{ss} and lag time values were showed in Table 5. In the first group, drug permeation of Ibuprofen NLC in a period of 240 min showed a significant increase compared with that of eye drops. In the case that Gelucire 44/14 was added to the formulation, the preparation showed a higher corneal permeability (about 1.29-fold) than the preparation without it (group 2). In the case of group 3, the addition of Transcutol P made a marked increment of drug permeation comparing to Ibuprofen NLC without it.

Gelucires are saturated polyglycolized glycerides consisting of mono-, di-, and tri-glycerides and mono- and di-fatty acid esters of polyethylene glycol. Some authors reported (Nilüfer et al., 2003) that it could enhance transdermal permeability of many drugs, and the result of this study shows that it can also enhance the corneal permeability of the model drug.

Transcutol P is a surfactant and the results suggest that the mechanism of the action of it on drug corneal transport should involve changes in the structure of the epithelium as a result of Trans producing micelles in the epithelial lipid bilayer. The micelles formed by Trans removed phospholipids from the epithelial cell

Table 5
Permeation parameters of Ibuprofen NLC through the excised corneas ($n = 3$)

Group		Preparation	$P_{app} \times 10^6$/(cm s^{-1})	$J_{ss} \times 10^3$(μg s^{-1} cm)	Lag time/(min)
1	L	Eye drops	5.779 ± 0.057	5.779 ± 0.057	53.53 ± 10.21
	R	NLC-1	18.29 ± 0.781	18.29 ± 0.781	43.02 ± 1.82
2	L	NLC-1	17.51 ± 0.604	17.51 ± 0.604	50.38 ± 2.40
	R	NLC-2	22.58 ± 1.233	22.58 ± 1.233	22.42 ± 5.99
3	L	NLC-1	12.55 ± 0.399	12.55 ± 0.399	34.26 ± 9.82
	R	NLC-3	17.10 ± 1.399	17.10 ± 1.399	51.02 ± 12.81

L, cornea of left eye of each rabbit; R, cornea of right eye of each rabbit; NLC-1 is Ibuprofen nanostructured lipid carrier; NLC-2 is Ibuprofen nanostructured lipid carrier with Gelucire 44/14; NLC-3 is Ibuprofen nanostructured lipid carrier with Transcutol p, date are mean ± S.D., $n = 3$.

Table 6
Aqueous humor pharmacokinetics parameters of Ibuprofen NLC ($n = 4$)

Group	AUC ($\mu g\,mL^{-1}\,h\,\mu g^{-1}$)	C_{max} ($\mu g\,mL^{-1}\,\mu g^{-1}$)	T_{max} (h)	K_e
Reference	0.938 ± 0.413	0.967 ± 0.230	0.209 ± 0.083	0.655 ± 0.061
Test	3.75 ± 0.917	3.15 ± 0.777	0.584 ± 0.17	0.659 ± 0.364

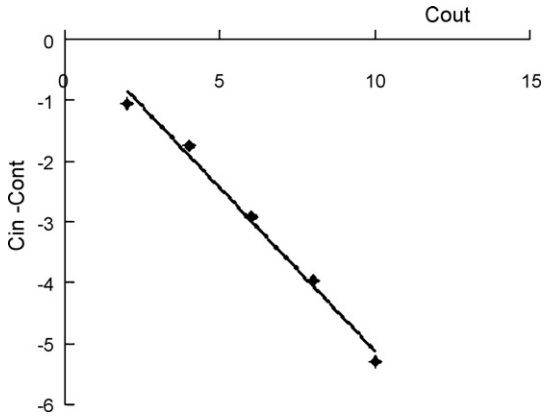

Fig. 3. In vivo recovery of microdialysis test ($n = 3$).

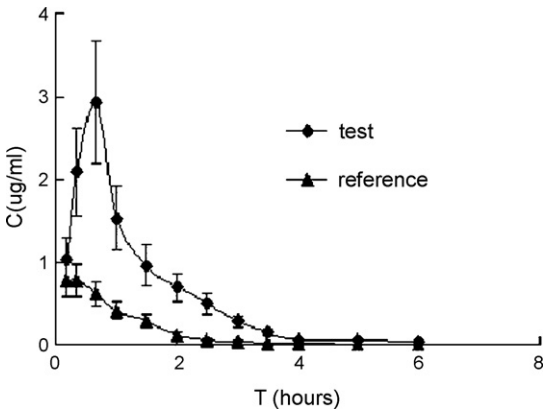

Fig. 4. Ibuprofen concentration profiles in aqueous humor after the administration of the reference solution and of ibuprofen NLC to conscious rabbits ($n = 4$).

membranes, thereby leading to an increase in the transcorneal passage of drug.

3.6. Result of microdialysis test

3.6.1. Optimized formulation

The entrapment efficiency of the optimized formulation was 95.2%; particle size was 69 nm and zeta potential was +28.9 mv. The result of the corneal permeability test showed that the P_{app} and J_{ss} was 4.19-fold and 4.19-fold of Ibuprofen eye drops, respectively. The cornea hydration level was 78.91%, which illustrated that the preparation had no irritation on cornea.

3.6.2. Pharmacokinetics studies

Fig. 3 illustrated the linear regression between perfusate (C_{in}) and dialysate (C_{out}): $C_{in} - C_{out} = -0.5349C_{out} - 0.2142$ ($r = 0.9948$). The in vivo recovery (r) was 53.49%.

There are a number of parameters effecting on in vitro recovery, such as perfusion flow-rate, temperature, perfusate composition, characteristics of the drug, characteristics of the semipermeable membrane, and the surface of the semipermeable membrane. All parameters that influence in vitro recovery will also influence in vivo recovery. However, in vivo, tissue characteristics will play a more important role and may ultimately determine the recovery.

In vivo recovery is determined by diffusion in three regions: probe lumen, dialysis membrane and the periprobe environment (Bungay et al., 1990; Bungay et al., 1991; Benveniste et al., 1991). Diffusion in probe lumen is limiting only with the use of very low flow rates. Diffusion through the dialysis membrane is limited only when transport through the periprobe environment is rapid. Rapid diffusion through the periprobe environment occurs in most flowing system (like blood). In tissues, effective diffusion through the extracellular fluid determines the recovery of the microdialysis probes (Bungay et al., 1990; Bungay et al., 1991). Perfusate also has effect on in vitro recovery which is still under research.

In this study, the in vivo recovery was almost as triple as reported (Sato et al., 1996; Othori et al., 1998; Fukuda et al., 1999), which was only 16–20%. The difference might exist in the lumen of probe and the flow rate of perfusion.

Fig. 4 showed the profiles of Ibuprofen concentration in aqueous humor *vs* time. Concentration at peak (C_{max}), time to peak (T_{max}), and terminal rate constant(K_e) were calculated using noncompartmental techniques. The area under the curve (AUC) was estimated by linear trapezoidal method with extrapoltion to infinite time. All parameters were reported as mean ± S.D.

Aqueous humor pharmacokinetic parameters were presented in Table 6. The AUC of test group was 3.99-fold comparing with the reference group ($P < 0.05$), the C_{max} of test group was 3.25-fold of the control group ($P < 0.1$). The T_{max} of test group was longer than that of reference group, and Ibuprofen could still be detected at 6 h after adminstration in the test group, but it could not be detected at 3.3 h after adminstration in the reference group. So the developed formulation had longer resident time in aqueous humor than conventional ophthalmic solutions.

4. Conclusion

In this study Ibuprofen nanostructured lipid carrier for ocular use was prepared and characterized in vitro and in vivo, and the irritation of the preparations were evaluated. The effect of Gelucire 44/14 (as solid lipid material), Transcutol p (as permeability enhancer) and stearylamine (as charge-inducing reagent) on particle size, zeta potential, ocular irritation and corneal permeability were studied. The result showed that both Gelucire 44/14 and Transcutol P could enhance the drug corneal permeability to some extent; stearylamine could prolong the pre-corneal retention of drug; all the three materials could optimize the formulation of a NLC ocular drug delivery and the preparation showed higher bioavailability comparing with eye drops.

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