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European Journal of Pharmaceutics and Biopharmaceutics 56 (2003) 189–196
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Solid lipid nanoparticle and microemulsion for topical delivery of triptolide

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Received 4 September 2002; accepted in revised form 4 April 2003

Abstract

Triptolide (TP) has been shown to have anti-inflammatory, immunosuppressive, anti-fertility and anti-neoplastic activities. However, its clinical use is restricted to some content due to its poor water solubility and some toxic effects. In order to find innovative ways for administering TP and alleviating its disadvantages, the controlled release delivery systems such as solid lipid nanoparticle (SLN) and microemulsion have been developed. In the present paper we describe the preparation and some characterization of specialized delivery systems for TP. The transdermal delivery capacity and anti-inflammatory activity were also evaluated. The results indicated that these SLN dispersions and microemulsions could serve as efficient promoters for the TP penetrating into skin. Furthermore, different formulations were optimized in this study. The best formulation of SLN dispersion consisted of 5% tristearin glyceride, 1.20% soybean lecithin and 3.60% polyethylene glycol (400) monosterate, while the best formulation of microemulsion consisted of 40% isopropyl myristate, 50% Tween-80: 1,2-propylene glycol (5:1, v/v) and water. The steady-state flux (J_s) and permeability coefficient (K_p) of triptolide for the SLN dispersion of the first 6 h were 3.1 ± 0.4 μg/cm^2 per h and 0.0124 ± 0.001 cm/h or 6.4 ± 0.7 μg/cm^2 per h and 0.0256 ± 0.002 cm/h for the microemulsion, which was 3.45 and 7.02 times higher than those of triptolide solution, respectively. The anti-inflammatory activity of SLN dispersion was stronger than that of microemulsion in carrageenan induced rat paw edema. However, the results were the reverse in complete Frenud's adjuvant induced paw edema. Further investigations should be carried out on the toxicity of different formulations of triptolide to tissues.

Keywords: Triptolide; Solid lipid nanoparticle; Microemulsion; Anti-inflammatory; Transdermal absorption

1. Introduction

Triptolide (diterpenoid triepoxide, TP), a purified component of a traditional Chinese medicine, was isolated from a shrub-like vine named *Tripterygium wilfondil* Hook F (TWHF). Two extracts of TWHF, methanol/chloroform (T2) and ethyl acetate (EA) have been reported to be effective in the treatment of patients with a variety of inflammatory and autoimmune diseases, especially rheumatoid arthritis (RA). The extracts were found to contain TP that accounts for the immunosuppressive activity in its extracts [1–2]. Besides, TP has been shown to have other functions, such as anti-fertility and anti-neoplastic activity. However, the clinical use of TP has some practical disadvantages mainly due to scarce water solubility and toxic effects. The incidence of adverse drug reaction (ADRs) was significantly higher than other drugs. The organic system affected by ADRs of TP including gastrointestinal, urogenital, cardiovascular, blood circulatory system, bone marrow as well as hypersusceptibility of skin. In China, there are thousands of adverse events about TWHF or TP reported, especially in the gastrointestinal tract, such as nausea, vomiting, bellyache, diarrhea, duodenal ulcer, and gastrointestinal bleeding. Therefore, the development of novel types of delivery systems could lead to significant advantages in the clinical use of the drug.

With the aim of using innovative ways to administer TP, possibly overcoming or alleviating the solubility and toxicity problems associated with its usage, we compare the use of solid lipid nanoparticles (SLN™) [3–4] and microemulsion [5–7] for topical drug delivery systems. The

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doi:10.1016/S0939-6411(03)00067-5

development of topical drug delivery systems for systematic effects has gained more and more interest in the past few years due to its obvious advantages that transdermal delivery systems offer a better way than conventional routes for drug administration, such as the possibility of systemic drug therapy, the avoidance of first pass metabolism and the minimization of side effects. However, the relative impermeability of the stratum corneum provides the principal resistance to percutaneous absorption. SLN and microemulsion appears as a promising drug carrier system for topical application. The small particle size of SLN ensure the nanoparticles are in close contact to the stratum corneum, thus can increase the amount of encapsulated agents penetrating into the viable skin. The microemulsion can reduce the diffusional barrier of the stratum corneum by acting as permeation enhancer.

The present study focused on the preparation and some characterization of SLN and microemulsion associated with TP, in vitro permeation studies and anti-inflammatory activity of the specialized delivery systems. The long-term goal of this work is to develop topical TP formulations for clinical use to increase therapeutic index.

2. Materials

Tristearin glyceride (TSG) and stearic acid (SA) were purchased from the first chemical plant of Shanghai, polyethylene glycol (400) monosterate (PEG400MS, Baoan chemical factory). Medicinal grade soybean lecithin was provided by Shanghai No. 1 Oils and Fats Factory. Poloxamer 188 was obtained from China Pharmaceutical University. TP was obtained from the Fujian Institute of Medicinal Sciences. Carrageenan and complete Frenud's adjuvant (CFA) were purchased from Sigma. All other chemicals were obtained from Shanghai Chemical Reagent Corporation.

3. Methods

3.1. Preparation of SLN [8]

Lipid (tristearin glyceride, stearic acid) was heated to 80 ° and TP was dissolved in the melt lipid, lecithin or poloxamer 188 was dispersed in the melting lipids until the dispersion appeared optically clear. PEG400MS was dissolved in double-distilled water containing 0.01% thiomersal as a preservative. The aqueous phase was heated to 80 °. Then the heated aqueous phase was added to the melt lipid and emulsified by probe sonication for up to 20 min (Table 1) [9]. In addition, the TP solution was prepared by dissolving TP into a mixture solution of 1,2-propylene glycol and water (1:4, v/v).

3.2. Preparation of the microemulsions [10]

All microemulsions were formulated with double-distilled water to avoid surface-active impurities. The appropriate amount (Table 2) of water (water phase, W), isopropyl myristate and TP (oil phase, IPM) and Tween-80: 1,2-propylene glycol [5:1, v/v, surfactant + cosurfactant phase (S + C)] was weighed into 10-ml Teflon-sealed screw-cap glass vials. Transparent systems were obtained by vortexing the mixtures vigorously.

3.3. Particle size analysis and zeta potential measurements

The particle size and zeta potential of SLN dispersion was performed by Zetapals (Zetapals, Brookhaven, USA). The samples for zeta potential measuring were diluted with double-distilled water adjusted to a conductivity of 50 μS/cm with sodium chloride. The measured electrophoretic mobility was converted to zeta potential using the Helmholtz–Smoluchowski equation. This processing was done by the software included within the system. In order to evaluate the stability of the systems and to exclude drug crystallization on the boundary, each formulation was measured five times in triplicate over a period of 15 days.

3.4. In vitro cutaneous permeation studies

In vitro permeation studies were performed with a Franz diffusion cell. The diffusion cells were thermoregulated with a water jacket at 37 °C. Full-thickness abdominal skin removing subcutaneous fat tissue was excised from rats whose hair had been removed beforehand by an electric clipper. The excised skin was mounted on a Franz diffusion

Table 1
The compositions of the tested SLN dispersion formulations

Formulation	TSG	SA	P188	SL	PEG400MS	Particle size	PI	ζ (mV)	J_s (μg/cm^2 per h)	K_p (cm/h)
A	5.00	–	1.20	–	3.60	147 ± 1.5	0.27	−42	2.8 ± 0.3	0.0112 ± 0.001
B	5.00	–	–	1.20	3.60	123 ± 0.9	0.19	−45	3.1 ± 0.4	0.0124 ± 0.002
C	–	5.00	–	1.20	3.60	157 ± 1.2	0.29	−40	2.3 ± 0.8	0.0092 ± 0.004
D	–	5.00	1.20	–	3.60	173 ± 2.3	0.24	−39	1.9 ± 0.4	0.0076 ± 0.002

TSG, tristearin glyceride; SA, stearic acid; SL, soybean lecithin; P188, poloxamer 188; PI, polydispersity index; ζ zeta potential; J_s, flux for the first 6 h; K_p, permeability coefficient for the first 6 h.

Table 2
The compositions of the tested microemulsion formulations and skin permeation parameters

Formulation		TP	IPM	S + C	Water	J_s (μg/cm^2 per h)	K_p (cm/h)
E	W/O	0.025%	40%	50%	9.975%	6.4 ± 0.7	0.0256 ± 0.002
F	O/W	0.025%	20%	40%	39.975%	6.1 ± 0.9	0.0243 ± 0.005
G	O/W	0.025%	10%	35%	54.975%	5.2 ± 1.2	0.0209 ± 0.007
H	Solution	0.025%				0.9 ± 0.1	0.0034 ± 0.0009

IPM, isopropyl myristate, oil phase; S + C, Tween-80/1,2-propylene glycol (5:1 w/w, surfactant + cosurfactant phase); J_s, steady-state flux; K_p, permeability coefficient.

cell where the stratum corneum side was facing upwards into the donor compartment and the dermal side was facing downwards into the receptor. A mixture of saline and ethanol (9:1, v/v) was used as the receptor fluid. Then, 1 ml of SLN dispersion or microemulsion was applied to the donor compartment. At 2, 4, 6, 8, 10 and 12 h after this. 0.5-ml aliquots were drawn from the receiver compartment. Thereafter, an equivalent volume of receptor fluid was supplied to the receiver compartment. The amounts of TP in receptor fluids were analyzed by HPLC.

3.5. HPLC analysis of TP

The amount of TP penetrated into the receptor compartment was determined with a slight modification of the reversed-phase HPLC method described previously [11]. HPLC (Waters, USA) was equipped with a Hypersil C_{18} column and the mobile phase consisted of methanol–water (40:60, v/v) at a flow-rate of 1.0 ml/min. The retention time was 14.1 min. Detection was accomplished using UV absorbance at 218 nm. The assay was linear ($r^2 > 0.996$) in the concentration range 50–1000 ng/ml with a lowest detection limit at 20 ng/ml of TP. The percentage recoveries ranged from 98.7 to 101.8. The stability studies were performed for TP solution placed on the laboratory bench and in refrigerator for 50 days. The samples were found to be stable for the study periods.

3.6. Data treatment

According to Fick's second law of diffusion, the total amount of drug (Q_t) appearing in the receptor solution in time t is expressed as [12]:

$$Q_t = \mathrm{AKLC}_0\left[\left(\frac{D_t}{L^2}\right) - \left(\frac{1}{6}\right) - \left(\frac{2}{\pi^2}\right) - \sum\left(\frac{(-1)^n}{n^2}\right)\exp\left(\frac{D^n 2\pi^2 t}{L^2}\right)\right] \quad (1)$$

where A is the effective diffusion area, C_0 represents the drug concentration which remains constant in the vehicle, D is the diffusion coefficient, L denotes the thickness of the membrane and K is the partition coefficient of the drug between membrane and vehicle. At steady state, Eq. (1) is expressed as follows:

$$\frac{Q_t}{A} = KLC_0\left[\left(\frac{D_t}{L^2}\right) - \left(\frac{1}{6}\right)\right] \quad (2)$$

The flux, J, is determined from the slope of the steady-state portion of the amount of the drug permeated divided by A versus time. From Eq. (2), the flux is expressed as:

$$J = C_0\frac{KD}{L} = C_0K_p \quad (3)$$

where K_p is the permeability coefficient.

3.7. Suppression of carrageenan-induced inflammation

Male Wistar rats, body weight between 150 and 180 g (provided by the Central Animal Laboratory of Tongji Medicinal University) were randomly divided into 10 groups of six rats for receiving topical treatment. The rats of the standard group were treated with diclofenac emulgel (Beijing Novartis Pharma Ltd). The eight experimental groups received different formulations of TP loaded SLN dispersion (formulations A–D), microemulsion (formulations E–G) and solution (formulation H), respectively, while the control group was treated with saline only. Diclofenac emulgel (10.0 mg/kg), different formulations of TP (0.6 mg/kg) and the control were applied to the shaved abdominal skin of rats. After 30 min, 0.05 ml of 1% carrageenan was injected into the right hind foot of each rat under the planter aponeurosis. Measurements of foot volume were performed by the displacement technique using a calibrated glass tube immediately before and 1 h, 2 h, 3 h, 4 h, 5 h and 6 h after the injection of carrageenan [13]. The edema rate and inhibition rate of each group were calculated as follows [14]:

$$\text{Edema rate } (E)\% = \frac{V_t - V_o}{V_o} \quad (4)$$

$$\text{Inhibition rate } (I)\% = \frac{E_c - E_t}{E_c} \quad (5)$$

where V_o is the mean paw volume before carrageenan injection (ml), V_t is the mean paw volume after carrageenan injection (ml), E_c is the edema rate of control group, and E_t is the edema rate of the treated group.

3.8. Adjuvant-induced arthritis in rats

Experimental arthritis was induced in rats according to the method proposed by Newbould [15] with some modifications. The left footpad of each rat was injected (s.c.) with 0.05 ml of complete Freund's adjuvant (CFA). Rats in the drug test groups were treated with different formulations of TP (0.6 mg/kg) or diclofenac emulgel (10.0 mg/kg) and the control group was treated with saline as mentioned above. Drugs and control were applied to the shaved abdominal skin of rats 24 h before the injection of CFA and then with daily treatment until 14 days after CFA challenge. The edema and inhibition rate were also measured with the same method as described above.

3.9. Statistical analysis

Data were expressed as mean ± S.D. and statistically assessed by one-way analysis of variance (ANOVA). Differences between drug treated groups and the control group were evaluated by Dunnett's *t*-test. $P < 0.05$ was considered significant. Further analysis between the drug treated groups was evaluated by Newman–Keuls test.

4. Results

4.1. The characterization of SLN and drug release

From Table 1, it can be seen that the particle size of TSG-SLN dispersion was smaller than that of SA-SLN dispersion, the particle size of SLN that used poloxamer 188 as emulsifier was larger than that of soybean lecithin. For example, the particle size of formulation B consisting of TSG and soybean lecithin was 123 ± 0.9 nm, while formulation D, which consisted of SA and poloxamer 188, was 173 ± 2.3 nm. An identical dependency of the release on the size was obtained for different size TP loaded nanoparticles. The cumulative amounts of TP at different times in the receptor fluids are shown in Fig. 1, and the steady-state flux (J_s), permeability coefficient (K_p) for the first 6 h according to Eqs. (1)–(3) are shown in Table 1. The J_s of the best SLN dispersion (formulation B) was 3.1 ± 0.4 μg/cm^2/h, K_p was 0.0124 ± 0.001 cm/h. The release of encapsulated TP differed to TP in microemulsion (Fig. 2) because of the solid matrix of the nanoparticles and the subsequent drug immobilization. The highest cumulative amounts of drug were obtained from the smaller particle size of TSG-SLN dispersion (formulation B), the lowest cumulative amounts of drug were obtained from the larger particle size of SA-SLN dispersion (formulation D). However, the cumulative transdermal absorption amounts of formulation D were much higher than that of free TP (formulation H). In addition, the total cumulative amount during the first 6 h of formulation B (19.3 μg/cm^2) was higher than that of the microemulsion formulations at the same periods.

4.2. Drug release from microemulsion

Table 2 shows the three formulations composed of IPM, Tween-80/1,2-propylene glycol and water at different concentrations. The effects of the content of oil and surfactant mixture on the skin permeation of TP were evaluated. The skin permeation profiles are presented in Fig. 2; the J_s and K_p values are shown in Table 2. Among the formulations tested, formulation E, which was composed of 0.025% TP, 40% IPM and 50% Tween-80/1,2-propylene glycol (5:1, v/v) mixture, showed the highest permeation profile, followed by formulations F and G. The J_s of TP from formulation E was 6.4 ± 0.7 μg/cm^2 per h and K_p was 0.0256 ± 0.002 cm/h, 7.02 times higher than those of the TP solution (formulation H), which were 0.9 ± 0.1 μg/cm^2 per h and 0.0034 ± 0.0009 cm/h, respectively.

4.3. Anti-inflammatory effects on carrageenan induced rat paw edema

The rat's footpad became edematous soon after injection of carrageenan. Edema rate of the left footpad reached its peak at 4 h (63.2%). The results obtained with different formulations of TP and the reference drug (Diclofenac

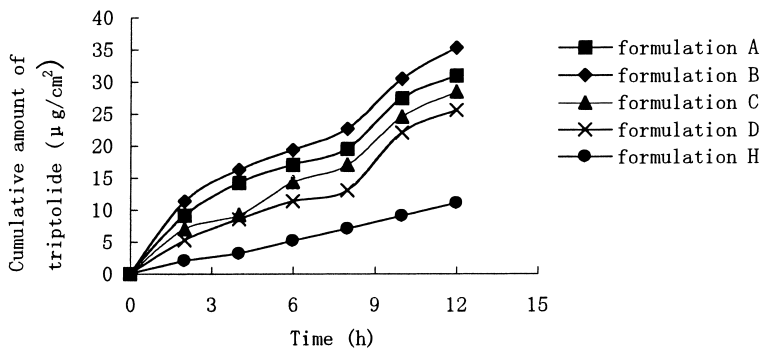

Fig. 1. Permeation profiles of triptolide through excised rat skins from solid lipid nanoparticles (SLN), the compositions of the tested SLN dispersion formulations are shown in Table 1, while formulation H was a triptolide solution.

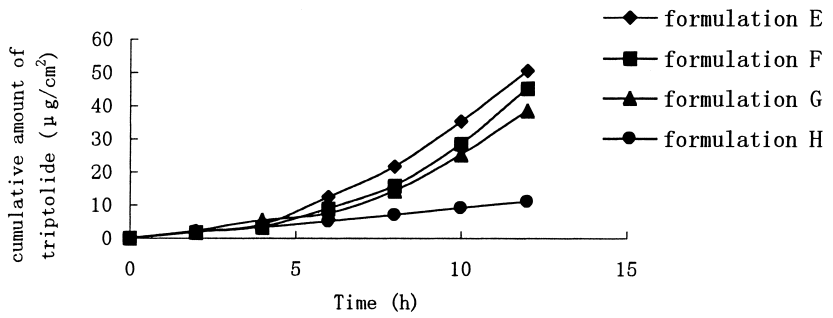

Fig. 2. Permeation profiles of triptolide through excised rat skins from microemulsions, the compositions of the tested microemulsion formulations are shown in Table 2, while formulation H was a triptolide solution.

emulgel) in the carrageenan-induced edema test at specific time intervals are shown in Table 3. Results show the anti-acute inflammatory activity of TP loaded SLN and microemulsion delivery systems as well as TP solution. It was of interest to see that the SLN dispersion with the smallest particle size (123 ± 0.9 nm, formulation B) possessed the strongest anti-acute inflammatory activity, nearly twofold higher than that of TP solution (formulation H), formulations A, C, D, E, F were also stronger than formulation H. There was no significant difference between formulations G and H. These data were evaluated by the Newman–Keuls test.

4.4. Anti-inflammatory effects on adjuvant induced rat paw edema

Table 4 shows the time course of edema rate and inhibition rate after administration of CFA and different formulations of TP. The footpad injected with CFA gradually became swollen for more than 25 days. Administration of TP significantly inhibited the development of joint swelling induced by CFA. The anti-inflammatory activity of TP was obvious as early as 1 day after CFA injection and was maintained until the experiment was terminated after 25 days. It was shown that the formulation E (W/O microemulsion) has the strongest chronic anti-inflammatory activity, nearly threefold higher than that of formulation H (TP solution), while formulations A–D and F and G were stronger than formulation H.

5. Discussion

Burst release as well as sustained release has been reported for SLN dispersions. For transdermal application, both features are of interest. Burst release can be useful to

Table 3
The anti-inflammatory effect of carrageenan-induced paw edema

Formulation	Edema rate (%)					
	1 h	2 h	3 h	4 h	5 h	6 h
Control	37.1 ± 5.6	57.8 ± 10.1	62.0 ± 9.2	63.2 ± 12.4	50.5 ± 9.3	67.0 ± 7.3
A	18.1 ± 3.6*	28.5 ± 7.2*	37.6 ± 5.6*	30.6 ± 5.3*	26.1 ± 4.9*	38.2 ± 4.9*
	(51.2)	(50.1)	(64.9)	(51.6)	(48.3)	(42.9)
B	11.2 ± 2.3*	20.0 ± 5.6*	22.5 ± 3.6*	27.5 ± 8.9	24.6 ± 1.5*	36.2 ± 8.6*
	(68.8)	(65.4)	(63.7)	(56.5)	(51.3)	(45.9)
C	22.5 ± 4.3*	23.8 ± 8.2*	30.0 ± 3.5*	37.5 ± 5.9*	33.8 ± 2.6*	46.3 ± 2.6*
	(39.4)	(58.8)	(51.6)	(40.1)	(33.1)	(30.9)
D	24.9 ± 3.2*	33.4 ± 7.1*	41.9 ± 7.3*	50.7 ± 7.4**	45.6 ± 8.6	57.8 ± 6.1
	(32.8)	(42.2)	(32.4)	(19.8)	(9.7)	(13.7)
E	25.5 ± 2.7*	38.5 ± 9.2*	44.6 ± 12.0*	45.1 ± 4.9*	35.3 ± 4.2*	50.7 ± 3.3
	(31.2)	(33.4)	(28.1)	(28.6)	(30.1)	(24.3)
F	28.0 ± 4.9**	39.1 ± 6.1*	40.8 ± 6.3**	49.5 ± 8.3**	48.7 ± 3.9	54.9 ± 2.2*
	(24.5)	(32.3)	(22.6)	(21.7)	(3.6)	(18.1)
G	30.4 ± 5.9**	38.2 ± 5.2*	49.3 ± 5.5**	58.4 ± 7.1	50.1 ± 2.3	60.9 ± 1.6
	(18.1)	(33.9)	(20.5)	(7.6)	(0.8)	(9.1)
H	34.3 ± 5.4	49.2 ± 7.6	50.1 ± 3.9**	55.6 ± 9.3	45.3 ± 1.9	58.2 ± 3.3
	(7.5)	(14.9)	(19.2)	(12.0)	(10.3)	(13.1)
Diclofenac	24.2 ± 3.5*	33.7 ± 3.6*	40.2 ± 5.3*	45.6 ± 6.4*	39.6 ± 3.9*	47.8 ± 2.6*
emulgel	(34.8)	(41.7)	(35.2)	(27.8)	(21.6)	(28.6)

Values represent the mean ± S.D. of six animals for each groups. Each value in parenthesis indicates the percentage inhibition rate. Statistically significant from control: *$P < 0.01$ and **$P < 0.05$ (Dunnett's t-test).

Table 4
Effect of different formulations on CFA induced paw edema in rats

Group	Edema rate (%)							
	1 day	3 days	6 days	9 days	12 days	16 days	21 days	25 days
Control	37.2 ± 4.7	44.2 ± 4.2	39.7 ± 5.8	56.0 ± 4.8	54.7 ± 4.0	65.5 ± 4.9	85.3 ± 10.3	75.8 ± 8.9
A	24.5 ± 3.3* (34.1)	23.9 ± 4.3* (45.9)	29.1 ± 4.1* (26.7)	39.2 ± 7.4* (30.0)	39.2 ± 4.2* (28.3)	44.4 ± 9.0* (32.2)	61.3 ± 8.7* (28.1)	66.0 ± 7.2 (12.9)
B	23.9 ± 2.0* (35.8)	24.0 ± 2.5* (45.7)	33.7 ± 2.3 (13.6)	36.8 ± 2.3* (34.2)	36.1 ± 3.2* (34.0)	46.8 ± 5.3* (28.5)	62.9 ± 2.2* (26.2)	57.0 ± 3.4* (24.8)
C	25.6 ± 4.6* (31.1)	27.2 ± 3.8* (38.5)	24.6 ± 4.6* (38.0)	38.7 ± 8.0* (30.1)	32.1 ± 5.8* (41.3)	46.8 ± 13.2* (28.5)	64.7 ± 5.8* (24.1)	53.0 ± 7.8* (30.1)
D	28.5 ± 4.3* (23.4)	38.2 ± 4.9 (13.6)	34.1 ± 6.4 (14.1)	43.4 ± 9.5* (22.5)	44.9 ± 11.7 (17.9)	57.3 ± 7.9 (12.5)	71.8 ± 3.7*** (15.8)	60.3 ± 11.2** (20.5)
E	16.0 ± 2.4* (56.9)	19.7 ± 3.8* (55.4)	21.7 ± 1.9* (43.5)	32.4 ± 4.1* (42.1)	32.6 ± 3.7* (40.4)	43.5 ± 6.2* (33.8)	54.8 ± 9.5* (35.7)	45.8 ± 5.3* (39.6)
F	16.2 ± 1.6* (56.6)	27.3 ± 3.2* (38.2)	22.7 ± 4.6* (42.8)	38.7 ± 8.0* (30.9)	39.1 ± 5.8* (28.5)	44.8 ± 9.9* (31.6)	64.7 ± 9.5* (24.1)	53.0 ± 9.2* (30.1)
G	20.0 ± 4.4* (46.3)	30.1 ± 4.8* (31.9)	30.2 ± 3.8* (23.9)	41.3 ± 4.9* (26.2)	40.2 ± 3.6* (26.5)	50.4 ± 4.3* (23.0)	60.1 ± 6.5* (29.5)	59.4 ± 9.8* (21.6)
H	30.2 ± 3.2** (18.8)	35.2 ± 4.7* (20.4)	32.2 ± 3.0** (18.9)	47.3 ± 1.1*** (15.5)	42.3 ± 3.6* (22.6)	53.2 ± 1.9** (18.7)	70.5 ± 3.2** (17.3)	60.2 ± 4.3* (20.6)
Diclofenac emulgel	23.7 ± 2.2* (36.3)	32.4 ± 1.3* (26.7)	28.5 ± 1.5* (28.2)	43.1 ± 2.5* (23.0)	40.9 ± 7.0* (25.2)	45.0 ± 4.6* (31.3)	62.2 ± 9.5* (27.1)	54.0 ± 10.5* (28.8)

Values represent the mean ± S.D. of six animals for each group. Each value in parenthesis indicates the percentage inhibition rate. Statistically significant from control: *$P < 0.005$, **$P < 0.01$ and ***$P < 0.05$ (Dunnett's t-test).

improve the penetration of drugs, while sustained release becomes important for active ingredients with irritating effects at high concentrations or to supply the skin over a prolonged period of time with a drug. Fick's law of diffusion does not seem to be applicable in this case but may be applied for different parts of the release. In the first fraction, before 6 h most of the amount released from SLN dispersion was found in the receiver compartment, which was higher than that of microemulsions. Furthermore, the flux was not constant but increased over the next 6 h. A possible explanation for the release profile from SLN was that within the first 6 h the carrier remained essentially unchanged and burst drug release because of the solid matrix of the particles, due to the experimental settings, water evaporated from the SLN dispersions during the experiment. Within 12 h the fluid SLN dispersion slowly turned into a semisolid gel. Gel formation of SLN could be correlated with polymorphic transitions of the lipid matrix [16–17]. Since different polymorphic forms differ in their ability to include host molecules [18] in their lattice, drug expulsion as a consequence of this transition was likely. The expelled TP was poorly soluble in water and hence increased thermodynamic activity of TP. The increase in thermodynamic activity could explain the higher diffusion velocity of TP as compared to the first 6 h. The different cumulative transdermal amounts for different formulations of TP loaded SLN dispersion might be due to the particle size. The smaller the size of the nanoparticles, the easier contact to the stratum corneum. Hence the amount of encapsulated agents penetrating into the viable skin.

Microemulsion is a transparent O/W or W/O emulsion with a droplet size <100 nm and does not have a tendency to coalesce. It consists of an oil phase, surfactant, cosurfactant and aqueous phase. The construction of a phase diagram makes it easy to find out the concentration range of components to form microemulsion (data not given). Several mechanisms have been proposed to explain the advantages of microemulsion for the transdermal delivery of drugs. First, a large amount of drugs can be incorporated in the formulation due to the high solubilizing capacity. Second, the steady-state flux of the drug from microemulsion may be increased, since the affinity of a drug to the internal phase in microemulsion can be easily modified to favor partitioning into stratum corneum, using a different internal phase, changing its portion in microemulsion or adjusting its property. Furthermore, the surfactant and cosurfactant in the microemulsions may reduce the diffusional barrier of the stratum corneum by acting as permeation enhancers [5]. Therefore, TP incorporated microemulsion was also evaluated in this article. Using flow-through Franz diffusion cells and an infinite dose technique, the TP flux through the membrane was almost constant in the case of the microemulsions. This result was expected because of a surplus of TP at the donor side. Therefore, the concentration gradient between receptor and donor phase over 12 h remained constant, resulting in a constant flux. The application of Fick's law of diffusions was appropriate in describing the release properties of microemulsion.

In this study, it was found that the different formulations

of TP significantly inhibited rat paw swelling induced by carrageenan. It has been reported that the carrageenan-induced edema can be divided into two phases. The first phase occurs during 1 h after carrageenan injection. It derives from the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin in their inflammatory area. The second phase occurs 3–5 h after carrageenan injection. In this phase, the macrophages in carrageenan-insulted dermal tissue release Interleukin-1 (IL-1) to induce accumulation of polymorphic nuclear cells (PMNs) into the inflammatory area. This then releases the lysomal enzymes and active oxygen to destroy connective tissues and induce paw swelling [19]. The carrageenan induced rat paw edema could be significantly suppressed by TP. The results exhibit the anti-inflammatory effects of TP loaded delivery systems as well as TP itself. Of special interest is the fact that some of the formulations are more active than diclofenac emulgel.

Furthermore, the curves of adjuvant induced rat paw edema rate versus time could be divided into two phases. In the first phase, edema rate of the injected footpad increased and reached a peak during the first 3 days. Thereafter, the swelling slowly subsided until the 9th day when the paw began to swell again and peaked in the 3rd week (second phase). Adjuvant-induced arthritis is the most frequently used chronic inflammatory model. It seems that bacterial peptidoglycan and muramyl dipeptide are responsible for its induction [20]. Since the composition of bacterial adjuvant is complex and the immune response is a multi-stage process of intercellular cooperation, the mechanism is unclear. However, either superoxide released by PMNs, or IL-1 released by activating macrophages plays an important role in the development of chronic arthritis [21]. According to Lin [22], the therapeutic effects of TP in rheumatic disease were due in part to the novel chondroprotective effects of TP via the direct suppression of the production of pro-matrix metalloproteinase 1 and 3 (proMMPs 1 and proMMPs 3) and the simultaneous up-regulation of metalloproteinases (TIMPs) in IL-1-treated synovial fibroblasts. TP's interference with gene expression of proinflammatory cytokines and its known inhibitory effects on PGE2 production were also probably very effective. TP also inhibited expression of the phorbol 12-myristate 13-acetate (PMA)-induced gene tumor necrosis factor-alpha, IL-8, macrophage inflammatory protein-2 alpha, intercellular adhesion molecule-1, integrin beta (6), vascular endothelial growth factor, granulocyte-macrophage colony-stimulating factor, GATA-3, fra-1, and NF45. Furthermore, TP could inhibit constitutively expressed cell cycle regulators and survival gene cyclins D1, B1, and A1, cdc-25, bcl-x, and c-jun. So it could be concluded that TP exerted an anti-inflammatory role by immunosuppression. The results of this study indicated that some formulations of TP loaded SLN and microemulsion could enhance TP's anti-inflammatory activity. This is a hallmark of a successfully encapsulated drug.

As transdermal delivery system, SLN and microemulsion possess many obvious advantages that have been mentioned above. It is assumed that SLN dispersion, when administered directly onto skin in a small but sufficient quantity, would cause less side effects, if any, than the currently available formulations. SLN seem to be well suited for use on damaged or inflamed skin because they are based on non-irritative and non-toxic lipids. With respect to their use as carriers for topical applications, the occlusive effect due to film formation on the skin surface that reduces transepidermal water loss (TEWL). Increasing the water content in the skin can reduce the symptoms of atopic eczema and improve the appearance of healthy human skin. Occlusion can also enhance the penetration of drugs through the stratum corneum by increased hydration. Apart from a non-specific occlusion effect on penetration, penetration might also be affected by the SLN carrier itself, the high specific surface area of nanometer sized SLN facilitates contact of encapsulated drugs with the stratum corneum [3]. Furthermore, stabilization of chemically unstable drugs by incorporating them into a lipid matrix might be possible. On the other hand, soybean lethicin is also known to improve the safety of its co-applied agents, when the latter is surfactant-like [23]. Moreover, the co-applied lipids are likely to minimize the danger of allergic contact dermatitis that may be induced by the drug [24]. As for microemulsion, the absorption enhancing mechanism of microemulsion was closely related to the very high surfactant concentration that modified the lipid membranes or membrane proteins. In addition, the modified lipid membranes or membrane proteins can be recovered by the turnover of lipid membranes. It has been pointed out that whether the membrane can recover immediately or not is more important. From this viewpoint, sodium dodecyl sulfate (SDS) and polyoxyethylene (9) monolauric ether (BL-9EX) are classified as toxic surfactants. It has been reported that BL-9EX microemulsion caused the most serious damage, followed by polyoxyethylene (40) hydrogenated castor oil (HCO60) microemulsion, which contains SDS [25]. According to our macroscopic observations, no noticeable erythema of the shaved abdominal skin was visually observed when using Tween 80 as surfactant. However, it should be considered that whether the efficiency we expected was really deserved to taking such a risk when microemulsion formulations were employed. The toxicity of different TP formulations on tissues is under investigation.

6. Conclusion

In this study, the utility of SLN dispersions and microemulsions as carriers for topical delivery of TP were exploited. The results suggest that these SLN dispersions and microemulsions can serve as efficient promoters for TP to penetrate into skin. Furthermore, different formulations were optimized in this study. The best formulation of SLN

dispersion consisted of 5% TSG, 1.20% soybean lecithin and 3.60% PEG400MS, while the best formulation of microemulsion consisted of 40% isopropyl myristate (IPM), 50% Tween-80/1,2-propylene glycol (5:1, v/v) and water. Comparatively, the anti-inflammatory activity of SLN dispersion was stronger than that of microemulsion in carrageenan induced rat paw edema. However, the results were reverse in complete Frenud's adjuvant (CFA) induced paw edema. Unlike the SLN, the absorption enhancing mechanism of microemulsion was closely related to the very high concentration surfactant that modified the barrier of the skin. Therefore, applying SLN with higher surfactant concentrations might even create a better penetration effect than the microemulsion. Nevertheless, significant work still needs to be carried out to confirm these interesting conclusions. The toxicity of different formulations of TP on tissues is under further investigation.

Acknowledgements

The authors would like to thank States Development Plan of High Technology ('863' Plan) for its financial support and Prof. K. Mäder, Free University of Berlin, Germany, and Dr H. Bunjes, Friedrich Schiller University, Germany for their helpful discussions.

References

[1] X. Tao, P.E. Lipsky, The Chinese anti-inflammatory and immunosuppressive herbal remedy, *Tripterygium Wilfordii* Hook F, Rheum Dis. Clin. North Am. 26 (2000) 29–50.

[2] X. Tao, L. Ma, Y. Mao, P.E. Lipsky, Suppression of carrageenan induced the inflammation in vivo by an extract of the Chinese herbal remedy *Tripterygium Wilfordii* Hook F, Inflamm. Res. 48 (1999) 139–148.

[3] V. Jenning, A. Gysler, K.M. Schäfer, S.V. Gohla, Vitamin A loaded solid lipid nanoparticles for topical use: Occlusive properties and drug targeting to the upper skin, Eur. J. Pharm. Biopharm. 49 (2000) 211–218.

[4] V. Jenning, K.M. Schäfer, S.V. Gohla, Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties, J. Controlled Release 66 (2000) 115–126.

[5] B. Bianca, M.A.L. Quintela, M.A.D. Charro, M.B. Fadda, A.M. Fadda, J.B. Mendez, Microemulsions for topical delivery of 8-methoxsalen, J. Controlled Release 69 (2000) 209–218.

[6] M.R. Gasco, Microemulsions in the pharmaceutical field: perspectives and applications, Industrial Applications of Microemulsions, Marcel Dekker Inc, New York, 1997, pp. 97–122.

[7] M.B.D. Charro, G.I. Vilas, J.B. Mendez, M.A.L. Quintela, J.P. Marty, R.H. Guy, Delivery of a hydrophilic solute through the skin from novel microemulsion systems, Eur. J. Pharm. Biopharm. 43 (1997) 37–42.

[8] W. Mehnert, K. Mäder, Solid lipid nanoparticles: Production, characterization and applications, Adv. Drug. Deliv. Rev. 47 (2001) 165–196.

[9] R.H. Müller, J.S. Lucks, Arzeistoffträger aus festen lipid teilchen, Feste lipid nanosphären (SLN), European application PCT 92,02, 132(1992)

[10] Y.S. Rhee, J.G. Choi, E.S. Park, S.C. Chi, Transdermal delivery of ketoprofen using microemulsions, Int. J. Pharm. 228 (2001) 161–170.

[11] K. Li, Y. Yuan, X. Dai, X. Qao, Determination of triptolide in extract from leigongteng (*Tripterygium Wilfordii* Hook.F) by RP-HPLC, Se Pu. 16 (1998) 356–357.

[12] J. Shokri, A. Nokhodchi, A. Dashbolaghi, D. Hassan-Zadeh, T. Ghafourian, M.J. Barzegar,, The effect of surfactants on the skin penetration of diazepam, Int. J. Pharm. 228 (2001) 99–107.

[13] C.T. Chin, C.L. Chun, Anti-inflammatory effects of Taiwan folk medicine 'Teng-Khia-U' on carrageenan-and adjuvant-induced paw edema in rats, J. Ethnopharm. 64 (1999) 85–89.

[14] M.S.A. Ghamdi, The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*, J. Ethnopharm. 76 (2001) 45–48.

[15] B.B. Newbould, Chemotherapy of arthritis induced in rats by mycobacterial adjuvant, Br. J. Pharm. 21 (1963) 127–136.

[16] Z.A. Mühlen, C. Schwarz, W. Mehnert, Solid lipid nanoparticles (SLN) for controlled drug delivery-Drug release and release mechanism, Eur. J. Pharm. Biopharm. 45 (1998) 149–155.

[17] C.S. Maia, W. Mehnert, M.S. Korting, Solid lipid nanoparticles as drug carriers for topical glucocorticoids, Int. J. Pharm. 196 (2000) 165–167.

[18] H. Bunjes, K. Westesen, M.H.J. Koch, Crystallization tendency and polymorphic transitions in triglyceride nanoparticles, Int. J. Pharm. 129 (1996) 159–173.

[19] G. Zhao, L.T. Vaszar, D. Qiu, Anti-inflammatory effects of triptolide in human bronchial epithelial cells, Am. J. Physiol. Lung. Cell. Mol. Physiol. 279 (2000) L958–L966.

[20] L.J. Crofford, R.L. Wilder, Arthritis and autoimmunity in animals, in: D.J. Mcarty, W.J. Koopman (Eds.), Arthritis and Allied Conditions, Lea and Febiger, London, 1993, pp. 525–539.

[21] T. Yoshikawa, H. Tanaka, M. Kondo, The increase of lipid peroxidation in rat adjuvant arthritis and its inhibition by superoxide dismutase, Biochem. Med. 33 (1985) 320–326.

[22] N. Lin, T. Sato, A. Ito, Triptolide, a novel diterpenoid triepoxide from *Tripterygium wilfordii* Hook. f., suppresses the production and gene expression of pro-matrix metalloproteinases 1 and 3 and augments those of tissue inhibitors of metalloproteinases 1 and 2 in human synovial fibroblasts, Arthritis Rheum. 44 (2001) 2000–2193.

[23] G. Cevc, G. Blume, New, highly efficient formulation of diclofenac for the topical transdermal administration in ultradeformable drug carriers, Transfersomes, BBA 1514 (2001) 191–205.

[24] J. Shokri, A. Nokhodchi, A. Dashbolaaghi, D.H. Zadeh, T. Ghafourian, J.M. Barzegar, The effect of surfactants on the skin penetration of diazepam, Int. J. Pharm. 228 (2001) 99–107.

[25] K. Kawakami, T. Yoshikawa, T. Hayashi, Y. Nishihara, K. Masuda, Microemulsion formulation for enhanced absorption of poorly soluble drugs II. In vivo study, J. Controlled Release 81 (2002) 75–82.