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Original Research Paper

Design and evaluation of a novel transdermal patch containing diclofenac and teriflunomide for rheumatoid arthritis therapy

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ABSTRACT

The aim of this study was to design a compound transdermal patch containing diclofenac (DA) and teriflunomide (TEF) for the treatment of rheumatoid arthritis (RA). The various organic amines salts of DA were prepared and their forming was confirmed using DSC and FTIR. The percutaneous permeation of organic amines salt of DA was investigated *in vitro* using a two-chamber diffusion cell with excised rabbit skin as transdermal barrier. The formulation of the patch was optimized in terms of the concentration of percutaneous permeation enhancer and the loading dose of drugs. The pharmacokinetic behavior of the optimal formulation was studied in rabbits and the anti-inflammatory and analgesic effects of the optimal patch were evaluated with the adjuvant arthritis model in rats and the pain model in mice, respectively. The result showed that skin penetration of diclofenac-triethylamine (DA-TEtA) salt was better than other organic amine salts. Based on previous study of our laboratory, teriflunomide-triethylamine (TEF-TEtA) significantly enhanced the skin permeation of TEF. 10% of azone (AZ) was the best enhancer for the two drugs. The optimal patch formulation was composed of 2% of TEF-TEtA, 6% of DA-TEtA and 10% of AZ. The cumulative permeated amount of DA-TEtA *in vitro* was comparable with that of the commercial diclofenac-diethylamine (DA-DEtA) patch. The absolute bioavailability of TEF-TEtA was 42%, which could achieve the therapeutic drug levels. In animal study, the optimized compound patch containing DA-TEtA and TEF-TEtA displayed significant anti-inflammatory and analgesic effect, which indicated the potential of the compound patch.

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1. Introduction

Rheumatoid arthritis (RA) is a long-term and chronic inflammatory disease caused by the immune system attacking joints, which is characterized by symmetrical distribution [1]. Chronic synovitis attacks cartilage articularis, ligament and muscle tendon, leading to joint deformity and functional disorder. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of musculoskeletal disorders such as osteoarthritis and rheumatoid arthritis [2]. However, there is no evidence to show that NSAIDs can delay the progression of RA [3]. On the other hand, disease-modifying antirheumatic drugs (DMARDs) could prevent the pathological process of RA and avoid the joint damage but could not relieve the pain immediately [4]. Considering that monotherapies and fixed-dose can not meet the needs of different patients, NSAIDs and DMARDs are always used in combination for RA treatment clinically since 1950s [5].

Diclofenac(2-[2-(2,6 dichlorophenyl amino) phenyl]acetic acid) is one of the most prospective and commercially successful drug in the family of NSAIDs [6,7] and has an annual turnover over 1 billion US dollars [8]. It is used for the treatment of rheumatoid arthritis, osteoarthritis and relief the pain of varying origin treatment [9]. The main mechanism of action is to inhibit the activity of cyclooxygenase (COX) by interdicting the prostaglandin (PG) synthesis [10]. However the main disadvantage of oral administrated diclofenac dosage form is the serious adverse effects such as gastrointestinal disturbances, nausea, vomiting and stomach pain, etc [11].

Leflunomide (LEF) is one of DMARDs, which has been recommended for RA therapy by the American College of Rheumatology [12]. The LEF is transferred into its active metabolite teriflunomide (TEF) and TEF plays the major role for therapy [13,14]. It was shown TEF could inhibit the production of PGE₂ and the activity of cyclooxygenase-2 (COX-2) [15]. However, up to 50% of patients showed intolerability to this drug after oral administration, especially suffering from some gastrointestinal adverse effects [16]. With regarding to this, another administrating route avoiding the severe adverse effects is desired for delivering of the combination of DA and TEF into the body.

Transdermal drug delivery system (TDDS) is a promising alternative way of drug delivery which can maintain a uniform plasma concentration, reduce dosing frequency associated with improved the patient compliance, and avoid the gastrointestinal action. The compound transdermal patch containing DA and TEF will not only avoid the gastrointestinal irritation, but also provide double response of controlling RA activity. It has been reported that the skin permeation of weak acidic DA and TEF (Table 1) is unsatisfactory, but the addition of organic amines can significantly increase the percutaneous permeability of TEF [12] and DA [17,18]. Therefore, the experiment firstly prepared the different origin amine salts of DA and screened the salt with the optimal permeation. Based on previous study of our laboratory [12], TEF-TetA significantly enhanced the skin permeation of TEF and the effect of different chemical enhancers had been investigated. The best concentration of enhancer for two drugs and the loading dose of drugs were chosen. There is no commercial transdermal

product of TEF, so the pharmacokinetics study of TEF is used to confirm whether the loading dose of TEF-TetA is reasonable or not. To investigate the anti-inflammatory and analgesic effects of the compound patch, the pharmacodynamic study was carried out finally.

2. Materials and methods

2.1. Chemistry and materials

DA, diclofenac-sodium (DA-Na), diclofenac-potassium (DA-K), and diclofenac-diethylamine (DA-DEtA) were purchased from Tiande Pharmaceutical Co. Ltd (Tieling, China). TEF and TEF-TetA were synthesized in our laboratory [12]. Pressure sensitive adhesive (PSA) was supplied by Henkel Corp., (New Jersey, USA). Korean commercial DA-DEtA patch was obtained from Samyang Corp., (Korea). Complete Freund's adjuvant (CFA) was purchased from Sigma-Aldrich Co. LLC., (Missouri, USA). Propylparaben was obtained from Bodi Chemical Holding Co. Ltd., (Tianjin, China). Diethanolamine (DEA), triethanolamine (TEA), triethylamine (TEtA), propylparaben were all obtained from Bodi Chemical Holding Co. Ltd., (Tianjin, China). 1-(2-Hydroxyethyl) pyrrolidine (NL) was purchased from Tianjin Heowns Biochem LLC, N-(2-Hydroxyethyl) piperidine (NP) was supplied by Alfa Aesar (Massachusetts, USA). N-methyl pyrrolidone (NMP), azone (AZ), isopropyl myristate (IPM), Transcutol® P (TP), oleic acid (OA), Span 80, propylene glycol (PG) and L-menthol (MT) were obtained from International Specialty Products Inc., (New Jersey, USA), Tianmen Kejie Pharmacy Co. Ltd., (Hubei China), China National Medicines Co. Ltd., (Shanghai, China), Beijing Chemical Co. Ltd., (Beijing, China), Tianjin Bio Chemical Co. Ltd., (Tianjin, China), Tianjin Bio Chemical Co. Ltd., (Tianjin, China), Nanjing chemical reagent Co. Ltd., (Nanjing, China) and Suzhou Healthytech Bio-Pharmaceutical Co. Ltd., (Jiangsu, China), respectively. All other chemicals and solvents were analytical reagent grade.

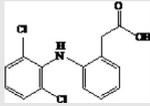
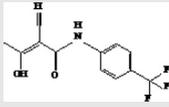
2.2. Animals

Rabbits (male, 1.8–2.2 kg), Wistar rats (male, 180–220 g) and KM mice (male, 18–22 g) used all in the experiments were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The experiments were performed in accordance with the guidelines for animal use published by the Life Science Research Center of Shenyang Pharmaceutical University.

2.3. Synthesis of organic amine salts of DA

DA was completely dissolved in acetone and then equimolar of organic amines was added. After the mixture of DA and organic amine was subjected to ultrasound for 1 h at room temperature, the solvents were removed by using rotary evaporator. The obtained solid product was further dried in a vacuum oven for 24 h at room temperature. The synthesis of organic amine salt was confirmed by DSC (DSC1 STAR® System, Mettler-Toledo International Inc., Schwerzenbach, Switzerland) and FTIR (Spectrum 100, PerkinElmer Inc., Massachusetts, USA) respectively.

Table 1 – Physicochemical properties of DA and TEF.

	Chemical structure	M.W.	log P	pKa	t _{1/2}
Diclofenac (DA)		296.15	4.75	3.6	1.1–1.8 h
Teriflunomide (TEF)		270.21	2.51	5.2	14 day

2.4. Preparation of transdermal patches

Transdermal patches were prepared by the solvent evaporation technique. In brief, specific amount of drug, PSA and enhancer were dissolved in ethanol by mechanically stirred for 1 h. The obtained homogeneous solution was spread onto a silicone-coated release liner (Shanghai Fupeng Adhesive Products Co. Ltd, China) with a thickness of 80 μm . After it was settled at the room temperature for 10 min, the samples were heated in an oven at 50 °C for 10 min to completely remove the solvent. Finally, the samples were covered with a fabric backing film (Hangzhou Xiaoshan Johnson Cloth Factory, Zhejiang, China).

2.5. In vitro permeation experiments

2.5.1. Preparation of excised rabbit skin

After rabbits were anesthetized with urethane (20%, w/v, i.v.), hair on abdominal skin was removed by electric clipper and followed shaved carefully using a razor. Full thickness skin including epidermis with SC and dermis was excised, and the subcutaneous fat was carefully removed using the surgical scissor and scalpel. The excised skin was washed immediately with phosphate-buffered saline and ready for use. If necessary, the skin samples can be frozen at –70 °C for storage for maximum 1 month.

2.5.2. The skin permeation experiments

The rate of DA or its organic amine salts penetrating through the excised rabbit abdominal skin was evaluated by using a two-chamber glass diffusion cell with the effective diffusion area of 0.95 cm^2 . After the device was assembled, the patch was applied on the stratum corneum side of skin that was clamped between the two chambers. The receiver cell was filled with 3 ml of phosphate-buffered saline (PBS, pH 7.4) as receive medium and the media was stirred at the rate of 600 rpm to ensure the uniformity with a magnetic bead. The temperature of the medium was maintained at 32 °C by a circulating-water jacket during the whole experiment. At each predetermined time point, 2.0 ml of received medium was withdrawn from the receptor and an equal volume of fresh PBS buffer was added to keep a constant volume. 200 μl of the withdrawn sample was mixed with 200 μl of internal standard solution (propylparaben, 25 $\mu\text{g}/\text{ml}$) for High Performance Liquid Chromatograph (HPLC) analysis.

2.6. Pharmacokinetic study

2.6.1. The preparation of injection TEF

Weight 10 mg TEF to a 10 ml volumetric flask, dissolve in 3 ml ethanol, dilute with physiologic saline to volume, and mix.

2.6.2. Dosage regimen design

A total of 9 male rabbits (1.8–2.2 kg) were randomly divided into 3 groups for pharmacokinetics study of TEF via i.v. injection, transdermal compound patch and Korean commercial DA-DEtA patch, respectively. Rabbits in group A were i.v. injection 4 mg TEF separately and for group B and C self-prepared and commercial patch with the same area of 20 cm^2 was administrated respectively. Prior to the application of patch the hair on abdominal skin was removed and the skin cleaned. Blank blood samples were withdrawn right before administration and at 5, 10, 15, 30, 45 min, 1, 1.25, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 26, 32, 48, 60, 72 h after the administration of patch blood samples were continuously withdrawn. For group A, the blood samples were withdrawn at 0, 5, 15, 30 min, 1, 2, 3, 4, 6, 8, 10, 12, 14, 24 h. Subsequently, the plasma samples were separated by centrifugation and frozen at –70 °C until analysis.

2.6.3. Plasma sample extraction process

The plasma sample (100 μl) and internal standard (60 $\mu\text{g}/\text{ml}$, 10 μl) were mixed in a 1.5 ml Eppendorf tube by vortex-mixing for 1 min. Protein in the plasma samples was precipitated by the addition of 1 ml acetonitrile [19] followed by extra vortex for 3 min, and centrifugation at 16,000 rpm for 5 min. The supernatant was transferred to another new tube and evaporated to dryness under nitrogen at 40 °C. 100 μl mobile phase was added into the residue for reconstitution and the mixture was vortex-mixed for 3 min and centrifuged at 16,000 rpm for 5 min. Finally, the supernatant was injected into HPLC system for analysis.

2.7. HPLC analysis

The concentration of DA and TEF in the receptor medium and plasma was quantitatively determined by HPLC method. The HPLC system contained a pump L-2130, an ultraviolet detector L-2420, an automatic injector L-2200 and a T2000L workstation. C₁₈ column (200 mm \times 4.6 mm, 5 μm) was used for the separation of samples. Mobile phase was a mixture of

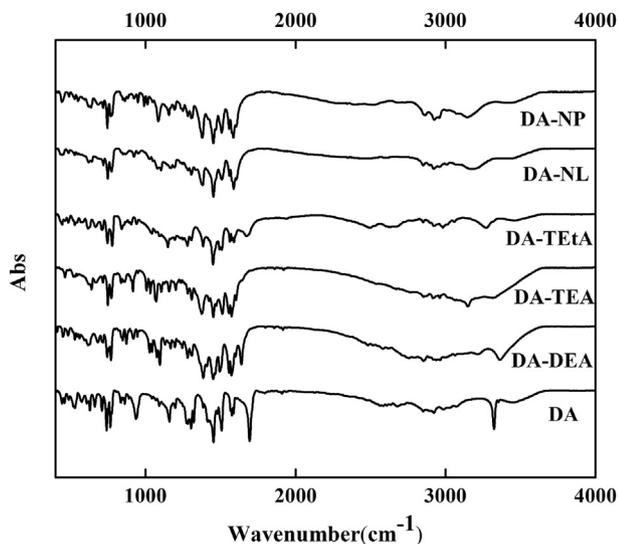


Fig. 1 – FTIR spectra for DA and its organic amine salts.

methanol and distilled water (0.5% acetic) at a ratio of 70:30, adjusted to pH6.8 with TEtA. The flow-rate of mobile phase was 1 ml/min and the analytes were monitored at 280 nm.

2.8. Data analysis

The cumulative amount of drug penetrated per unit area versus time (Q) was plotted. The slope of linear portion of the plot was calculated as the flux (J , $\mu\text{g}/\text{cm}^2/\text{h}$). Enhancement ratio (ER) was the ratio of Q from the vehicle with amine against that without amine or with enhancer against without enhancer. Difference was checked with ANOVA considered to be statistical significance at $P < 0.05$. Pharmacokinetic parameters were determined by non compartmental analysis applying WinNonlin software, version 3.0.

2.9. Pharmacodynamic study

2.9.1. Assessment of anti-inflammatory effect

The adjuvant arthritis model in rats was used to evaluate the therapeutic effect of compound patch for RA [20]. 36 male rats

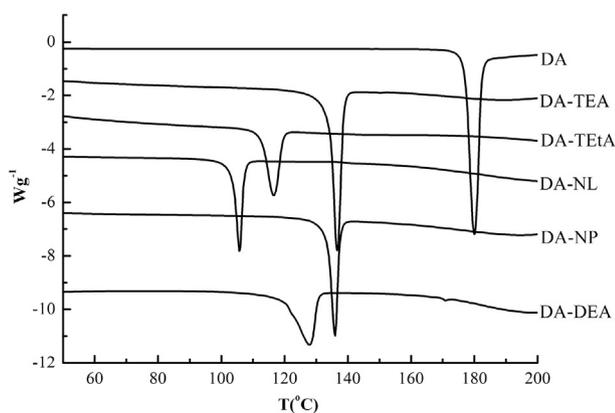


Fig. 2 – DSC curves of the DA and its organic amine salts.

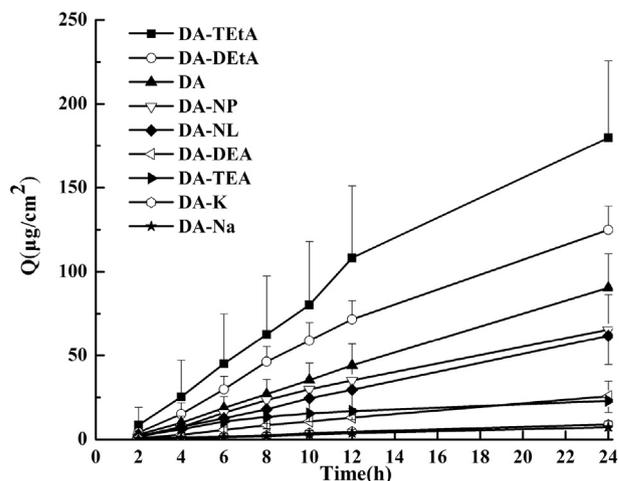


Fig. 3 – The penetration profiles of transdermal patches containing DA or its salts ($n=3$).

weighing 180–220 g were randomly divided into 6 groups. Specific details for each group were as follows:

- Control group: no administration
- Positive group: Korean commercial DA-DEtA patch (10 cm^2 , DA 19.2 mg)
- Negative group: blank patch (10 cm^2 , without any drug)
- DA group: DA-TEtA patch (10 cm^2 , DA 10 mg)
- TEF group: TEF-TEtA patch (10 cm^2 , TEF 3 mg)
- DA/TEF group: the compound patch (10 cm^2 , DA 10 mg, TEF 3 mg)

0.1 ml of the Complete Freund's Adjuvant (CFA) or physiological saline (for the control group) was injected into both side of the hind paw of rats. A water plethysmometer (YLS-7B, Jinan Yiyuan Technology Co. Ltd., Shangdong, China) was used to measure the volume of bilateral hind paw at 0, 0.5, 3, 5, 7, 9, 11 days after injection of CFA. The keen-joint was supplied the transdermal patch at the swelling peak

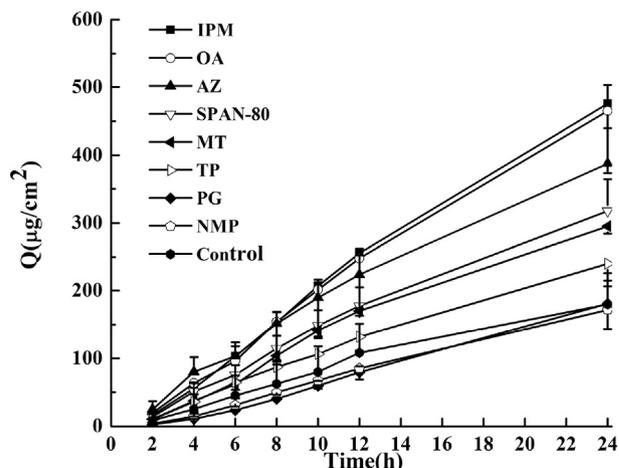


Fig. 4 – The penetration profiles of DA-TEtA transdermal patches containing 10% various chemical enhancers ($n=3$).

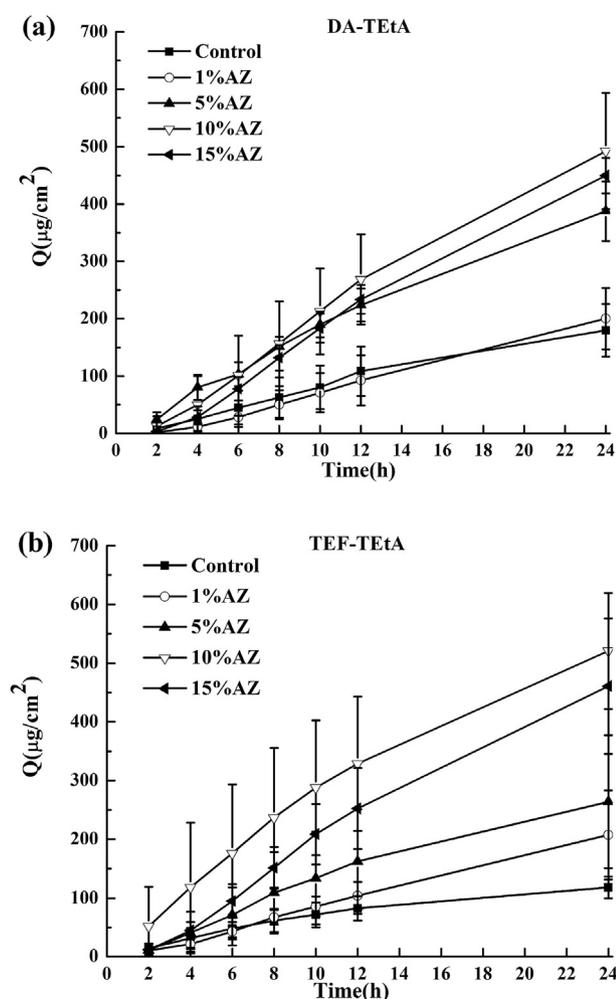


Fig. 5 – (a) The penetration profiles of adding different concentrations of AZ on percutaneous absorption of DA-TEtA. (b) The penetration profiles of adding different concentrations of AZ on percutaneous absorption of TEF-TEtA (n=3).

(the 5th day). The anti-inflammatory effect was assessed by Swelling Degree (S). The Swelling Degree (S) was calculated from the following formula:

$$S(\%) = \frac{V_t - V_n}{V_n} \times 100$$

Where V_n was the paw volume before the injection of CFA or physiological saline; V_t was the paw volume at the t day.

2.9.2. Assessment of analgesic effect

Writhing induced by acetic acid was used to assess the analgesia effect of compound patch for RA. Male mice (18–22 g) were randomly divided into 6 groups and each group was consisted of 10 animals. The grouping scheme was the same as the section 2.9.1 except the area of transdermal patch administrated was 5 cm² instead of 10 cm². The hair on the abdominal skin of the mice was removed 12 h prior to the application of patch. After 2 h's application, the patches were removed and then 0.1 ml/10 g weight of 0.6% (v/v) acetic acid-saline was injected intraperitoneally. Five minutes later, the number of writhing (W) within 20 min was recorded. The pain inhibition ratio (PIR) was calculated according to the following formula:

$$PIR = \frac{W_{\text{blank}} - W_{\text{administration}}}{W_{\text{blank}}} \times 100\%$$

W was the writhing numbers.

3. Results and discussion

3.1. Confirming the formation of organic amine salts of DA

3.1.1. FTIR spectroscopy

The FTIR spectroscopy measurement was carried out to confirm the salts formation between DA and organic amines. The FTIR spectra for DA and its salts were shown in Fig. 1. It was observed that DA showed a strong absorption at 1694 cm⁻¹, which was assigned to stretching vibration of C=O of COOH group [21]. However, when the organic amines were added, this absorption peak shifted to low wavenumber to 1639.4 cm⁻¹ for DA-DEA, 1604.2 cm⁻¹ for DA-TEA, 1674.3 cm⁻¹ for DA-TEtA, 1606.4 cm⁻¹ for DA-NL and 1605.0 cm⁻¹ for DA-NP. It was suggested that addition of organic amines to DA resulted in the change in the chemical shift of the carbonyl. On the other hand, DA had a sharp signal peak at 3323.2 cm⁻¹ which was assigned to the OH stretching vibration. After the addition of organic amines, the corresponding peak became weaker and the peak position was shifted to different wavenumbers. These results indicated that the formation of hydrogen bond between the OH from the parent and the basic N atom from organic amines resulted in the movement of OH stretching vibration absorption peak.

3.1.2. DSC analysis

The DSC curves for DA and its salts were shown in Fig. 2. It could be seen that DA presented a single sharp absorption

Table 2 – The penetration parameters of Korean commercial patch and different compound formulations (n = 3).

Permeants	DA-TEtA		TEF-TEtA	
	Q _{24h} (µg/cm ²)	J (µg/cm ² /h)	Q _{24h} (µg/cm ²)	J (µg/cm ² /h)
F-1	370.96 ± 36.95	8.54 ± 1.41	423.23 ± 42	18.84 ± 2.4
F-2	593.56 ± 40.82	24.36 ± 2.23	436.87 ± 22.45	20.2 ± 3.44
F-3	1007.11 ± 99.96	36.6 ± 1.48	421.35 ± 21.21	13.35 ± 1.21
Commercial patch	1034 ± 46.92	46.25 ± 2.82	–	–

peak at 178.95 °C which indicated that DA had a single melting point. This result was consistent with the reported literature [22]. However, the absorption peaks of DA-DEA, DA-TEA, DA-TetA, DA-NL, DA-NP appeared at 127.52 °C, 136.67 °C, 111.16 °C, 105.22 °C, 135.38 °C, respectively. The melting point of all organic amine salts was lower than DA.

The FTIR spectroscopy and DSC curves confirmed that the organic amine salts of DA has been formed successfully.

3.2. Formulation optimization

Although most acrylic PSAs possessed the advantage of good compatibility with a wide range of drugs, but some of them were resistant to interaction with drugs because of the presence of some polar groups, which could cause the system changed. In order to avoid the destruction, the type of PSA without any polar group was selected as the adhesive matrix in current study.

The cumulative permeation profiles of different salts of DA were presented in Fig. 3. Even though the majority of salts of DA included DA-NL, DA-NP, DA-DEtA did not show enhanced cumulative permeation amount in 24 h (Q_{24}) compared to DA, significant increase of Q_{24} ($ER = 1.99$) was achieved with DA-TetA. Therefore, DA-TetA was used in following studies.

Although the permeation of DA has been significantly enhanced by transforming DA into its salt DA-TetA, the cumulative permeation amount of DA-TetA was not high enough. Therefore, various chemical enhancers such as AZ, OA, MT, IPM, TP, PG, NMP, Span 80 were employed. The effect of enhancers in the vitro penetration of DA-TetA was presented in Fig. 4. Apart from PG and NMP, all the enhancers investigated could increase the penetration of DA-TetA. However, lipophilic enhancers such as AZ, OA, IPM had better transdermal penetrate enhancing effect on DA-TetA. It was well known that the lipophilic stratum corneum was the major barrier for TDDS [23]. While the addition of lipophilic enhancers might disrupt the order of lipid bilayers and change the barrier function of stratum corneum to increase the allocation of DA-TetA to stratum corneum [24]. On the other hand, the enhancers consisting of OH groups moiety may disturb the interaction of DA and organic amine. Considering the safety and effectiveness of enhancer both to DA-TetA and TEF-TetA, AZ was selected as the enhancer for the optimized formulation. The effective of AZ on the permeation of TEF-TetA has been demonstrated previously [12].

Subsequently, we observed the maximum enhancing effect within 1%~15% concentration of range. As shown in Fig. 5, the enhancement effect of AZ was concentration dependent within the range of 1%~15% and there was no significant difference ($P > 0.05$) between 10% and 15% of AZ. Considering the cost and appearance, 10% of AZ was chosen as the enhancer of compound patch.

Since the formulation of the patch we designed to achieve comparable Q_{24} of DA-TetA with that of Korean commercial DA-DEtA patch, the different loading drugs of DA-TetA were screened. Containing 4%, 5% and 6% of DA-DEtA was formulation 1 (F-1), F-2 and F-3, respectively. The results were showed in Table 2. The penetration parameter of F-3 was met the requirement of experimental design. So the loading of DA-TetA was chosen as 6% of the weight of PSA.

3.3. Pharmacokinetic study

Mean plasma concentration-time profiles obtained after intravenous and transdermal patches administration were presented in Fig. 6. The main pharmacokinetic parameters were shown in Table 3.

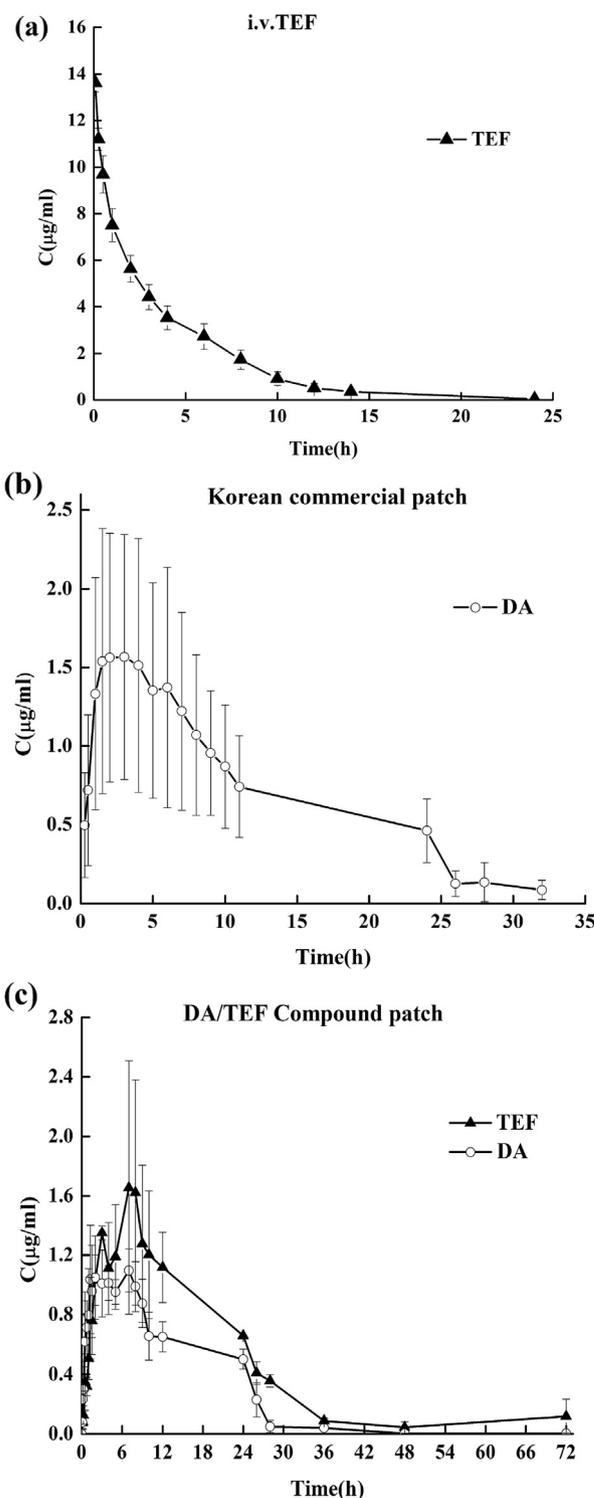


Fig. 6 – Mean plasma concentration-time profiles of different formulations. (a) i.v. TEF; (b) Korean commercial patch; (c) DA/TEF compound patch.

Table 3 – The pharmacokinetic parameters of different formulations (n = 3).

	i.v. TEF	Korean commercial patch	DA/TEF patch	
			DA	TEF
Dose (mg)	4	38.5	24	6
T_{max} (h)	–	2.5 ± 1.32	3.75 ± 2.4	5 ± 2.82
C_{max} ($\mu\text{g/ml}$)	12.91 ± 1.71	1.91 ± 1.04	1.22 ± 0.34	1.90 ± 0.85
AUC ($\text{h} \times \mu\text{g} \times \text{ml}^{-1}$)	39.36 ± 8.62	23.06 ± 8.33	18.1623 ± 2.38	26.14 ± 7.08
AUCM ($\text{h} \times \text{h} \times \mu\text{g} \times \text{ml}^{-1}$)	132.42 ± 52	220.09 ± 18.22	199.11 ± 16.96	324.56 ± 28.88
MRT (h)	3.28 ± 0.57	10.15 ± 2.55	11.15 ± 1.84	12.73 ± 2.34

There was no significant difference between the pharmacokinetic parameters of DA in Korean commercial patch and the prepared DA/TEF patch with T_{max} (time to peak) of 2.5 ± 1.32 h and 3.75 ± 2.4 h, C_{max} of 1.91 ± 1.04 $\mu\text{g/ml}$ and 1.22 ± 0.34 $\mu\text{g/ml}$, AUC (area under the concentration-time curve) of 23.06 ± 8.33 $\text{h} \times \mu\text{g} \times \text{ml}^{-1}$ and 18.1623 ± 2.38 $\text{h} \times \mu\text{g} \times \text{ml}^{-1}$, AUCM of 220.09 ± 18.22 $\text{h} \times \text{h} \times \mu\text{g} \times \text{ml}^{-1}$ and 199.11 ± 16.96 $\text{h} \times \text{h} \times \mu\text{g} \times \text{ml}^{-1}$ and MRT of 10.15 ± 2.55 h and 11.15 ± 1.84 h, respectively. It suggested that the systemic concentration of DA from the compound patch system could be maintained within the therapeutic range and the pharmacokinetic behavior was similar with the commercial patch system. The drug loading of DA from the compound patch was less than the commercial patch, but the relative bioavailability of DA was 126.03%, which implied the compound patch was superior to the commercial patch. The study showed that 20 mg of leflunomide daily was more effective than 10 mg without compromising tolerability [25]. However, the most of RA patients were elder whose liver and kidney functions began to decline, 10 mg was recommended to supply [25]. The absolute bioavailability of TEF in the compound patch was 42% approximately, and the designed daily dosage was 15 mg, which could reach therapeutic drug levels. Therefore, the loading dose of TEF in the optimal formulation was reasonable.

3.4. Pharmacodynamic study

3.4.1. Anti-inflammatory activity

The anti-inflammatory effect of self-made compound transdermal patch was evaluated by continuously measuring the change in paw volume of adjuvant arthritis model rat after the administration of patches. The results were shown in Fig. 7.

Compared with the blank control group, all other groups showed significant swelling at 0.5 d after the injection of CFA of challenging reached a stable volume at the 5th day after injection. The difference in swelling degree between negative group and blank control group remained statistically significant after administration, which indicated the matrix of patch alone had no effect for adjuvant arthritis. Starting from the 7th day, the bilateral paw swellings of TEF group, DA group and DA/TEF group were all reduced significantly compared with the control group, indicating that the all of three types of patches could inhibit the paw swelling of adjuvant arthritis rat. In addition, even though no significant difference was found between TEF group and DA group, the compound DA/TEF group exhibited significantly superiority over the TEF-TEtA and DA-TEtA group ($P < 0.05$). The results

demonstrated both of single TEF and DA patch had anti-inflammatory effect, but their combination had stronger anti-inflammatory effect than these two single drug contained patches. The DA/TEF group was even superior to the positive group in the rate of inhibiting inflammatory. Especially at the 9th day, this difference appeared more significant ($P < 0.05$). DA was a non-selective cyclooxygenase-1/2 inhibitor, which could reduce inflammation [26], and TEF, an isoxazole derivative and inhibitor of de novo pyrimidine synthesis, has been shown to provide comparable suppression of joint inflammation [27]. In conclusion, the compound

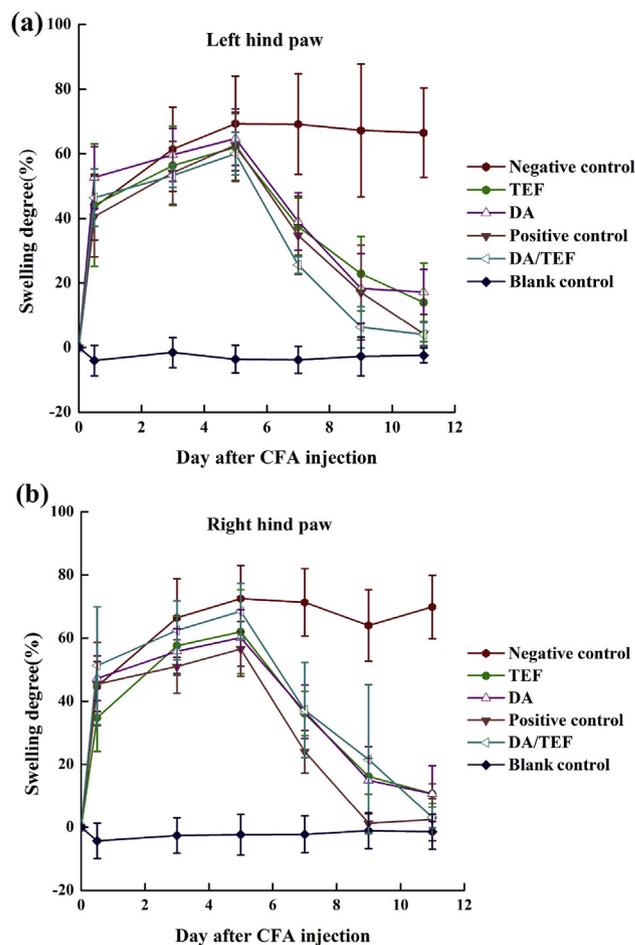


Fig. 7 – The profiles of swelling degree (%) of hind paw of rats after CFA injection and transdermal patches applied on left feet at 5 d: (a) left hind paw; (b) right hind paw (n = 6).

Table 4 – The results of analgesic effect of TEF and DA patch on the pain of mice induced by acetic acid (n = 10).

Group	Dose	WRF	PIR (%)
Control	–	38.33 ± 17.95	–
Negative	–	37.40 ± 21.15	2.43
Positive	10 mg	12.60 ± 11.84***	67.13
TEF	1.5 mg	31.50 ± 12.58	17.81
DA	5 mg	16.10 ± 11.66***	58
DA/TEF	1.5 mg/5 mg	10.00 ± 6.31***	73.91

***P < 0.001 vs. Control.

patch had an excellent anti-inflammatory effect for RA therapy. On the other hand, there was no significant difference for swelling between left hind paw and right hind paw in all groups. From this perspective, we could infer that the transdermal patch's therapeutic effect for RA was via circulatory system [12].

3.4.2. Analgesic activity

Writhing test induced by the acetic acid was a highly sensitive and useful test to evaluate the effect of the analgesic drug. As showed in Table 4, the obvious analgesic effect of the positive group and DA/TEF group was observed, and there was no significant difference ($P > 0.05$) between them. It was suggested that the compound patch had an outstanding analgesic effect. TEF group had little analgesic effect compared with the control group. This is mainly due to different action mechanism of DA and TEF. NSAIDs were preferred over analgesics for pain management [28], but DMARDs prevented the progression of joint destruction and reduced the rate of radiologically detected joint damage and reversed disability [27].

4. Conclusion

The results of this paper showed that DA-TETa, the organic salt of DA confirmed by DSC and FTIR can significantly increase the percutaneous permeability of DA. DA-TETa and TEF-TETa contained patch was prepared and the formulation was optimized. The optimal formulation is 2% of TEF-TETa, 6% of DA-TETa and 10% of AZ. The optimal compound patch presented better anti-inflammatory and analgesic effects in the study of adjuvant arthritis in rats and acetic acid-induced writhing syndrome in mice, respectively. In conclusion, the combined administration of DA-TETa and TEF-TETa for RA treatment is feasible and promising.

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FURTHER READING

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