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Evaluation of ketoprofen formulations via penetration rate and irritation in vivo study

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Abstract

The purpose of the present study was to design an optimal ketoprofen gel with appropriate penetration rate, shortened lag time and acceptable skin irritation. The combination of different mechanism enhancers including nonivamide, menthol and ethanol were used as multi-enhancers for producing a synergistic enhancement effect and reducing the skin irritation via diminishing the used amount of enhancers. The central composite design was applied to prepare a systemic formulation. The penetration rate (PR), lag time (LT) and skin irritation score (TIS) of a commercial product (Formax plus® gel containing 3% ketoprofen) were determined by in vivo study and used as a criterion for designed formulations. The PR, LT and TIS of commercial product were 462.2 ± 162.5 μg/h, 0.6 ± 0.1 h and 12.7 ± 0.6, respectively. Among these designed experimental formulations, four formulations including F07 (code: −1/+1/−1), F11 (code: +1/+1/−1), F13 (code: 0/0/−1.732) and F14 (code: 0/+1.732/0), their PR was not smaller and LT and TIS were not greater than that of commercial product, indicating that these experimental ketoprofen gels could be used in the clinical situation.

Keywords: Ketoprofen; Menthol; Nonivamide; Penetration rate; Total irritation score

1. Introduction

Ketoprofen is a non-steroidal anti-inflammatory, antipyretic and analgesic agent frequently used in the treatment of arthritis and mild to moderate pain (Lacy et al., 1998). Oral therapy of ketoprofen is very effective, but the clinical use is often limited because of adverse effects such as irritation and ulceration of the gastrointestinal tract. Ketoprofen possesses lower molecular mass (254.29) and a relatively short half-life (1–3 h) in plasma and has the potential to be delivered topically (Jamali and Brocks, 1990). Furthermore, topical administration via the dermal route can bypass disadvantages of the oral route. Therefore, transdermal drug delivery has been considered to be an ideal route for ketoprofen administration. However, the most difficult aspect of transdermal delivery system is to overcome the barrier of stratum corneum against foreign substances. The use of penetration enhancer is valuable and important for achieving therapeutic plasma levels for many drugs (Kabayashi et al., 1994; Degim et al., 1999; Wu et al., 2001a; Peltola et al., 2003; Chang et al., 2006), but penetration enhancer causes extensive damage to skin along with the large increase in transdermal penetration rate. Hence, appropriate penetration rate and an acceptable level of irritation must both be jointly considered in the design of an optimum transdermal formulation.

In our preliminary study, nonivamide and menthol had potential enhancement on percutaneous absorption of ketoprofen through rat skin (Wu et al., 2001a). However, nonivamide and menthol are hydrophobic compounds and need a cosolvent to

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help them dissolve in formulation. Additionally, some reports (Obata et al., 1991; Williams and Barry, 1991; Katayama et al., 1992) have stated that specific combinations of menthol and ethanol possess a synergistic enhancement penetration effect. Therefore, in this study, the combination of ethanol, menthol and nonivamide were used as multi-enhancers to produce a synergistic enhancement effect on penetration rate of ketoprofen and to decrease the skin irritation via diminishing the used amount of enhancers. The penetration rate through rat skin in vivo study and the skin irritation at the end of the experiment (10 h) determined by pathologic biopsy were used to assess the possibility of experimental formulations in clinical use by reference to a commercial product (Formax plus® gel containing 3% ketoprofen).

2. Materials and methods

2.1. Materials

The following reagents were used: ketoprofen (Sigma Chemical Company, USA), carbamic acid ethyl ester, hydroxypropyl cellulose (HPC), menthol and nonivamide (TCI, Japan), Formax plus® gel containing 3% ketoprofen (Shiteh, Taiwan). All other chemicals and solvents were analytical reagent grade.

2.2. Preparation of ketoprofen gels

For systemic evaluation of the influence of each enhancer on the desired goals including penetration rate, lag time and skin irritation, the central composite design (Hamed and Sakr, 2001) was applied to various systematic model formulations which were composed of three formulation factors: the content of nonivamide (X_1), menthol (X_2) and ethanol (X_3). The range of each process variable was set according to our preliminary experiments (Wu et al., 2001a). Sixteen model formulations were suggested and randomly arranged by Design-Expert® software. The compositions of all model formulations are summarized in Table 1.

Hydroxypropyl cellulose of 3% was dissolved in a mixed solution of water and propylene glycol. Ketoprofen was dissolved in ethanol containing transdermal enhancers, separately. Then, both components were mixed well. The ketoprofen hydrogels were stored in airtight containers at room temperature prior to use.

2.3. In vivo pharmacokinetics evaluation

Male Wistar rats weighting 180–200 g were anesthetized throughout the whole investigation with a 25% carbamic acid ethyl ester solution (about 3 ml/kg, intraperitoneally) secured on their backs. The experimental ketoprofen gel (45 mg/2.3 cm^2) was applied to the shaven abdomen by the occlusive dressing technique (ODT) (Naito and Tsai, 1981; Hsu et al., 1991). After both administrations, blood samples (500 μl) were taken via the jugular vein at 0.5, 1, 2, 3, 4, 5, 6, 8, 10 h. Each blood sample was centrifuged for 2 min at 14,000 × *g* and the plasma sample (100 μl) was mixed with methanol (300 μl) containing *p*-hydroxybenzoate–*n*-butyl ester (3 μl/ml) as internal standard. The mixture was centrifuged (14,000 × *g*, 2 min) again to precipitate the denatured proteins. Then the supernatant solution was analyzed by HPLC (Wu et al., 2001a). The coefficients of variation (CV%, $n = 6$) of the HPLC method were 7.5 and 1.1% for plasma concentration of 1 and 100 μg/ml in plasma, respectively. The limit of detection was 0.2 μg/ml.

2.4. Skin irritation evaluation by pathologic biopsy

Irritation evoked by experimental formulations on rat skin was microscopically judged after the end of experiments of in

Table 1
The composition and responses of ketoprofen model formulations arranged according to central composite design

	X_1		X_2		X_3		Penetration rate (μg/h)	Lag time (h)	TIS
	Code	Percentage	Code	Percentage	Code	Percentage			
F01	−1	0.02	−1	2.1	1	35.8	17.4 ± 0.9	0.1 ± 0.1	5.7 ± 2.1
F02	−1	0.02	1	7.9	1	35.8	34.4 ± 20.1	0.4 ± 0.1	6.7 ± 0.6
F03	1	0.08	1	7.9	1	35.8	102.1 ± 48.8	0.5 ± 0.1	7.0 ± 0.5
F04[a]	0	0.05	−1.73	0.0	0	30.0	14.3 ± 1.9	0.0 ± 0.1	7.3 ± 1.2
F05	−1	0.02	−1	2.1	−1	24.2	41.9 ± 23.7	0.3 ± 0.3	6.7 ± 0.6
F06	1	0.08	−1	2.1	−1	24.2	35.6 ± 11.4	0.3 ± 0.1	6.7 ± 1.2
F07	−1	0.02	1	7.9	−1	24.2	556.3 ± 23.6	0.6 ± 0.0	14.0 ± 1.0
F08[a]	0	0.05	0	5.0	1.73	40.0	23.9 ± 6.8	0.3 ± 0.1	6.0 ± 1.0
F09	1	0.08	−1	2.1	1	35.8	22.4 ± 6.4	0.1 ± 0.2	6.3 ± 0.6
F10[a]	0	0.05	0	5.0	0	30.0	169.4 ± 81.3	0.6 ± 0.0	10.0 ± 5.3
F11	1	0.08	1	7.9	−1	24.2	373.0 ± 41.3	0.6 ± 0.0	12.0 ± 0.6
F12[a]	−1.73	0.00	0	5.0	0	30.0	21.0 ± 4.4	0.4 ± 0.1	7.0 ± 2.0
F13[a]	0	0.05	0	5.0	−1.73	20.0	693.2 ± 168.3	0.5 ± 0.0	13.0 ± 3.0
F14[a]	0	0.05	1.73	10.0	0	30.0	386.9 ± 53.6	0.6 ± 0.0	11.7 ± 4.0
F15[a]	1.73	0.10	0	5.0	0	30.0	125.0 ± 82.1	0.6 ± 0.0	7.7 ± 1.2
F16	0	0.05	0	5.0	0	30.0	219.3 ± 46.5	0.6 ± 0.0	14.0 ± 1.0

[a]It was reported in our earlier study (Wu et al., 2001a). The permeation parameters represents the mean ± S.D. ($n = 3$). The amounts of ketoprofen, propylene glycol and HPC were fixed at 3, 40 and 3%, respectively. The level of variables nonivamide (X_1), menthol (X_2) and ethanol (X_3) were 0–0.1, 0–10 and 20–40%, respectively. TIS, Total irritation score.