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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.

vivo percutaneous absorption. The site of application of each formula on the skin was excised and fixed in 10% neutral carbonated-buffered formalin for at least 24 h before routine processing. Each section was rinsed with running water, dehydrated using a graded series of ethanol solution and embedded in paraffin wax and then frozen at −20 °C prior to sectioning. The tissues were cut into small sections (6 μm) and stained with hematoxylin and eosin for histological evaluation. All sections were examined by light microscopy.

The microscopic findings were graded into five levels of irritation, from no change (level 0) to a marked one (level 4) including the liquefaction of epidermis, edema of subepidermis, collagen fiber swelling and inflammatory cell infiltration in both the dermis and hypodermis, as well as degeneration of skin appendages (Takayama and Nagai, 1991). The total irritation score (TIS) was obtained by summation of each irritation score, and then used as an index of skin damage caused by the application of ketoprofen gel.

2.5. Data analysis

The penetration rate (PR) of ketoprofen was estimated from a two-compartment model based on the assumption that the rate of penetration of ketoprofen from the gel is constant after a lag time according to the following equation (Takayama and Nagai, 1991)

$$C = \frac{\mathrm{PR}}{V_1 k_{10}} \left\{ 1 + \frac{\beta - k_{10}}{\alpha - \beta} \mathrm{e}^{-\alpha(t-t_\mathrm{L})} + \frac{k_{10} - \alpha}{\alpha - \beta} \mathrm{e}^{-\beta(t-t_\mathrm{L})} \right\}$$

where C is the plasma concentration, PR the rate of penetration, t the time, t_L the lag time, V_1 the distribution volume of the central compartment, k_{10} the elimination rate constant from the central compartment and α and β are the hybrid first-order rate constant. The mean values of V_1, k_{10}, α and β, obtained from IV administration (Wu et al., 2001a), were used in this study to determine PR and t_L values.

In the response surface methodology (RSM) analysis, the responses: penetration rate (PR), lag time (LT) and total irritation score (TIS) of all model formulations were treated by Design-Expert® software. Statistical analysis including stepwise linear regression and response surface analysis were conducted. The model was tested for goodness of fit (R^2) and analysis of variance (ANOVA) was applied to verify the adequacy of the regression model in terms of a lack-of-fit test. The best-fitting mathematical model was selected based on the comparisons of several statistical parameters including the coefficient of variation (C.V.), the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2) and the predicted residual sum of square (PRESS) proved by Design-Expert software (Ho et al., 1998; Huang et al., 2005).

3. Results and discussion

In the development of transdermal dosage form, an important issue was to design an optimized pharmaceutical formulation with appropriate penetration rate in a short time period with minimum trials. Statistical methods such as response surface methodology (RSM) and artificial neural networks (ANN) have successfully been used in developing and optimizing pharmaceutical formulations (Takayama and Nagai, 1991; Takayama et al., 2000; Wu et al., 2001b; Huang et al., 2005). Hence, the RSM with central composite design was used in this study to evaluate the effects of combination of different enhancers including nonivamide, menthol and ethanol on drug permeation through rat skin, and to obtain optimal formulations with appropriate penetration rate, short lag time and acceptable skin irritation. The penetration rate (PR), lag time (LT) and total irritation score (TIS) of these systemically modeled formulations are shown in Table 1. There were significant differences in these parameters of these experimental formulations: PR from 14.33 to 693.17 μg/h, LT from 0.03 to 0.57 h and TIS from 5.7 to 14.0, respectively.

Table 2
Optimal regression equation for each response variable

Parameter	ln(PR)		LT		ln(TIS)	
	Estimate	p-Value	Estimate	p-Value	Estimate	p-Value
b_0	**5.21**	<0.0001	**0.57**	<0.0001	**2.43**	<0.0001
$b_1(X_1)$	**0.49**	0.0010	0.05	0.0706	0.06	0.4158
$b_2(X_2)$	**1.61**	<0.0001	**0.25**	<0.0001	**0.32**	<0.0001
$b_3(X_3)$	**−1.40**	<0.0001	**−0.13**	<0.0001	**−0.32**	<0.0001
$b_{12}(X_1X_1)$	**−1.45**	<0.0001	−0.11	0.0690	**−0.48**	0.0015
$b_{22}(X_2X_2)$	**−1.00**	0.0010	**−0.29**	<0.0001	−0.25	0.0784
$b_{33}(X_3X_3)$	−0.47	0.1027	**−0.20**	0.0013	**−0.29**	0.0461
$b_{12}(X_1X_2)$	0.21	0.5147	0.05	0.4438	−0.01	0.9481
$b_{13}(X_1X_3)$	**0.77**	0.0193	0.04	0.5684	0.24	0.1359
$b_{23}(X_2X_3)$	**−1.05**	0.0018	0.05	0.3988	−0.28	0.0782
p-Value of model	<0.0001		<0.0001		<0.0001	
p-Value of lack-of-fit	0.0701		0.9311		0.6241	
C.V.	11.75		25.58		12.06	
PRESS	16.07		0.66		3.91	
R^2	0.8891		0.7840		0.6207	
Adjusted R^2	0.8628		0.7328		0.5309	

PR, Penetration rate; LT, lag time; TIS, total irritation score; Significance of bold value, $p < 0.05$.

The wide variation indicated that different enhancer combinations could result in different effects on drug permeation. The code values of formulation variables and each response for all model formulations were treated by Design-Expert® software. The analysis parameters of model of each response are summarized in Table 2. The significant p-value (significance probability value) for model test, non-significant p-value for lack-of-fit test, smaller C.V. and PRESS and close value of adjusted and R^2 indicated that the assumed regression model was significant and valid for each considered response. The model describing the PR can be written as:

$$\ln(\mathrm{PR}) = 5.21 + 0.49X_1 + 1.61X_2 - 1.40X_3 - 1.45X_1X_1 - 1.00X_2X_2 + 0.77X_1X_3 - 1.05X_2X_3$$

The test of significant for regression coefficients was carried out by applying Student's t-test. A coefficient is significant if the calculated "t" value is greater than the critical value of "t", therefore it is included in the model. The values of the coefficients in the model are related to the effect of these variables on the response. A positive sign indicates a synergistic effect while a negative term indicates an antagonistic effect upon the response (Hamed and Sakr, 2001). This model showed that main effect of X_1 and X_2, and interaction effect of X_1X_3, had positive influence on the penetration absorption of ketoprofen; whereas, X_3, X_1X_1, X_2X_2 and X_2X_3, showed negative influence. Overall, ethanol (X_3) had a negative effect on the penetration rate, which was because the greater solubility of the drug in vehicle resulted in reduced thermodynamic activity of the drug, and consequently, a reduced rate of penetration. The response three-dimensional diagrams illustrating model equation and showing the effects of enhancers on individual response are depicted in Fig. 1 where level of ethanol was set at moderate (code = o). It can been seen that PR increased with in increase code of Nonivamide and menthol from −1.732 to 0.17 and −1.732 to 0.81, then slight decreased.

The model describing the lag time can be written as:

$$\mathrm{LT} = 0.57 + 0.25X_2 - 0.13X_3 - 0.29X_2X_2 - 0.20X_3X_3$$

Nonivamide was excluded in the model showed it had no effect on the LT. Ethanol had significant negative effect on the lag time, indicating the lag time can be shortened by addition of enhancers (Morimoto et al., 1993). The effect of menthol can be written as X_2 ($0.25-0.29X_2$) in this equation, indicating menthol had a positive effect on LT while X_2 was between 0 and 0.86 (Fig. 1).

The model describing the total irritation score can be written as:

$$\ln(\mathrm{TIS}) = 2.43 + 0.32X_2 - 0.32X_3 - 0.48X_1X_1 - 0.29X_3X_3$$

Menthol ($+0.32X_2$) had a positive effect, and nonivamide ($-0.48X_1X_1$) had negative effect on the skin irritation caused by topical application. When the level of X_3 was between 0 and −1.1, ethanol X_3 ($-0.32-0.29X_3$) showed a positive effect. Nonivamide is a synthetic analogue of capsaicin. The pharmacological and pungent profiles of nonivamide were found to be similar to those of capsaicin (Szolcsanyi and Jancso-Gabor, 1975; Hayes et al., 1984; Fang et al., 1995). In this model, nonivamide

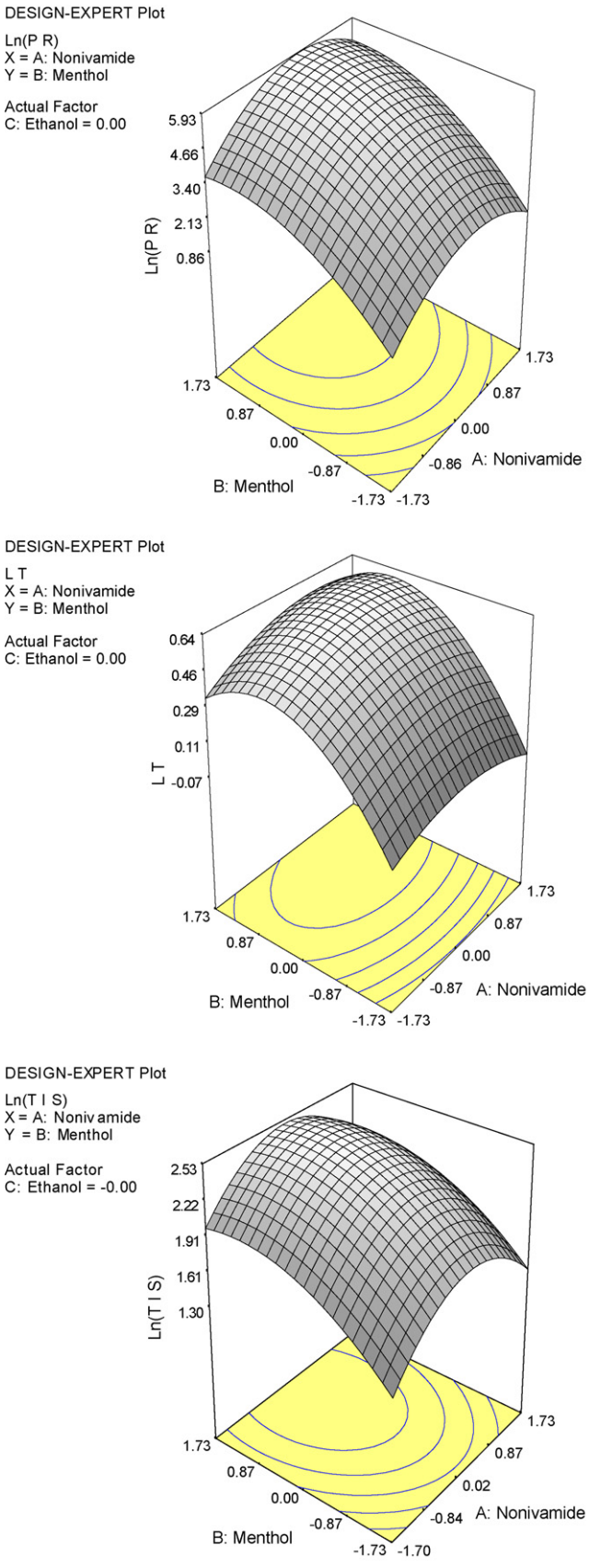

Fig. 1. Three-dimensional contour digrams illustrating the effect of nonivamide (X_1), menthol (X_2) and ethnaol (X_3) on the permeation parameters (penetration rate, PR; lag time, LT; total irritation score, TSI) of ketoprofen gel. The level of ethanol was set at medium level.

had a negative effect on TIS. A similar result was found in a previous study (Wu et al., 2001a), pointing out that nonivamide did not induce the irritation reaction of skin. It might be suggested that the combination of ketoprofen that possessed the inhibitory effect of erythema led to a decrease in irritation of skin evoked by nonivamide (Otterness et al., 1979; Wang et al., 2001).

In additional, a commercial product (Formax plus® gel containing 3% ketoprofen) was subjected to the in vivo permeation study for evaluating the possibility of clinical use of experimental formulations. The PR, LT and TIS of the commercial product were 462.2 ± 162.5 μg/h, 0.6 ± 0.1 h and 12.7 ± 0.6, respectively. Thus, it suggested that experimental formulation could be used in clinical when its PR was greater than 462.2 ± 162.5 μg/h, and LT and TIS were smaller than 0.6 ± 0.1 h and 12.7 ± 0.6, respectively. In comparing these parameters between the commercial product and model experimental formulations (Table 1), formulation F07 (PR, 556.3 ± 23.6 μg/h; LT, 0.6 ± 0.0 h; TIS, 14.0 ± 1.0); F11 (PR, 373.0 ± 41.3 μg/h; LT, 0.6 ± 0.0 h; TIS, 11.7 ± 0.6); F13 (PR, 693.2 ± 168.3 μg/h; LT, 0.5 ± 0.0 h; TIS, 13.0 ± 3.0); F14 (PR, 386.9 ± 53.6 μg/h; LT, 0.6 ± 0.0 h; TIS, 11.7 ± 4.0), their permeation parameters were similar to that of commercial product ($p > 0.05$, ANOVA test), demonstrating that these experimental formulations could possibly be used in clinical situations.

4. Conclusion

Experimental formulations such as F07 (code: −1/+1/−1), F11 (code: +1/+1/−1), F13 (code: 0/0/−1.732) and F14 (code: 0/+1.732/0) showed an appropriate PR, LT and TIS when compared a commercial product, indicated optimal ketoprofen gel could be obtained by using multi-enhancers.

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