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The Effect of Mixed-Solvent and Terpenes on Percutaneous Absorption of Meloxicam Gel

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The purpose of the present study was to develop the meloxicam transdermal dosage form. The response surface methodology was used to obtain an appropriate mixed-solvent system of pH-7.4 buffer and ethanol for preparing meloxicam hydrogel. The enhancement effects of terpenes on drug precautious absorption were evaluated via in vitro and in vivo study. The result showed that the solubility of meloxicam was dependent on the pH value of buffer solution. The mixed-solvent system of pH-7.4 buffer and ethanol had a synergistic effect on the increase of drug solubility. The highest solubility was obtained in the ratio of 50/50 pH 7.4 buffer/ethanol. A series of terpenes were used as enhancer for improving the penetration rate of meloxicam. The penetration rates were significantly increased by about 70–593 fold and the lag times were shortened from 7.92 to 0.17 hr by enhancer incorporation. Among these terpenes, menthol showed the greatest effect. In vivo penetration study, the AUC_{48h} was increased by about 1.7 fold by the addition of 5% menthol as enhancer.

Keywords meloxicam; solubility; terpenes; transdermal enhancer; in vivo penetration study

INTRODUCTION

Meloxicam (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide) is a potent nonsteroidal anti-inflammatory drug and has been used in treatment of rheumatoid arthritis, osteoarthritis and degenerative joint disease. It preferentially inhibits COX-2 (cyclooxgenease-2) over COX-1, but here is still has a 10–20% of incidence of gastrointestinal side effect (Engelhardt, 1996; Noble & Balfour, 1996; Stei et al., 1996; Busch et al., 1998; Kaplan-Machlis & Klostermeyer, 1999; Parfitt, 1999). In view of the characteristics of meloxicam including small oral dose (7.5–15 mg/day), low molecular weight (354.1), higher lipid solubility (log $p = 0.32$) and excellent tissue tolerability (Parfitt, 1999; Chang et al., 2006), it seems that there is potential for investigating the meloxicam transdermal dosage as an additional route for meloxicam administration (Stei et al., 1996; Gupta et al., 2002; Chang et al., 2006).

In the development of the transdermal dosage form, the most difficult aspect of transdermal delivery system is to overcome the barrier of stratum corneum against foreign substances. It is well known that the penetration rate of drugs through the stratum corneum can be increased with appropriate vehicles and transdermal penetration enhancers, due to their ability to increase the solubility of drug and/or enhancers in pharmaceutical formulations and their ability to change the structure of lipophilic and/or keratinized domains in stratum corneum (Lambert et al., 1989; Ashton et al, 1992; Morimoto et al., 1993; Kabayashi et al., 1994; Wu et al., 2001). In this study, the mixed-solvent system and different types of penetration enhancers were used to enhance the penetration rate and decrease the lag time of meloxicam from hydroxypropyl cellulose (HPC) gel through the excised rat skin. The pharmacokinetic parameters of the experimental meloxicam gel were also determined by the occlusion dressing technique in rat.

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MATERIALS AND METHODS

Materials

The following reagents were used: meloxicam, piroxicam, limonene, cineole, carvone, thymol, menthone, cineole (Sigma-Aldrich), hydroxypropyl cellulose (HPC 1000-4000), azone, menthol and sodium lauryl sulfate (sodium dodecyl sulfate, SDS), (Tokyo Chemical Industry, Japan). All other chemicals and solvents were of analytical reagent grade.

Solubility Study

The solubility of meloxicam in different pH values of McIlvaine buffer solution (0.2 M di-sodium hydrogen phosphate and 0.1 M citric acid), cosolvent and mixed-solvents of buffer were determined. An excess of meloxicam sodium was placed in contact with 2 mL of solvent in sealed glass tubes. The tubes were shaken for 24 hr at room temperature. The saturated solution was centrifuged and the supernatant was filtered through a 0.45 μm membrane. The concentration of drug in the saturated solution was determined after appropriate dilution with the selected solvents by HPLC as described in our previous study (Chang et al., 2006). A HPLC equipped with a Hitachi model L-7100 pump, a Hitachi model L-4000H detector, a Spark Holland basic Marathon autosampler and Merck Lichrocart® C18 column (55 × 4 mm I.D., particle size 3 μm) was performed. Piroxicam was prepared as the internal standard. The mobile phase was a mixture of 0.05M di-ammonium phosphate (adjust to pH 6.5 by phosphoric acid) and methanol in the ratio of 55:45, at the flow rate of 1 mL/min.

Preparation of Meloxicam Gel

The HPC gel formulations contained meloxicam (0.1–1% w/w) and menthol (1–5%). Meloxicam and menthol were dissolved in mixed solvent of pH 7.4 McIlvaine buffer solution and ethanol, and then hydroxypropyl cellulose was added and maintained at room temperature overnight. After they had been completely hydrated, all components were mixed well and the resulting hydrogels were stored in air-tight containers at room temperature prior to use.

In Vitro Skin Penetration Experiments

The extent and rate of skin permeation of meloxicam from gel formulations were determined using a modified glass diffusion cell fitted with excised rat skin. The skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. The donor cell was filled with 1 mL of meloxicam solution with or without terpenes or 3 g of meloxicam gels and occluded by paraffin. The receptor compartment was filled with 20 mL of pH 7.4 phosphate buffer containing 20% ethanol and 20% PG and its temperature was maintained at 37 ± 0.5°C by thermostatic water pump during the experiment. The effective diffusion area was 3.46 cm^2. Approximately 0.5 mL of the receptor medium was withdrawn at determined intervals and replaced immediately with an equal volume of receptor solution to maintain a constant volume. This dilution of the receiver content was taken into account when evaluating the penetration data. The sample withdrawn from the receptor compartment was then analyzed by HPLC. Each data point represents the average of three determinations.

In Vivo Study

Male Wistar rats weighting about 200–300 g were used in the pharmacokinetics study accordance with a protocol approved by the Institutional Review Board-Use and Care of Animals at Kaohsiung Medical University. Rats were anesthetized with urethane (0.75 g/kg) by intraperitoneal injection. Meloxicam gel (1.0 g) was administered via shaven abdomen by the occlusive dressing technique. Blood samples of 0.3 mL were drawn from the jugular vein at appropriate intervals. Each blood sample was centrifuged for 10 min at 3000 rpm. The plasma sample 0.1 mL was placed into a tube containing 3 mL dichlormethane, 0.05 mL 1N HCl and 0.05 mL piroxicam. The mixture was horizontally shaken for 10 min and centrifuged at 3000 rpm for 10 min. The organic layer was transferred to another tube and evaporated to dryness by vacuum pump. The dry residue was reconstituted in 0.15 mL of mobile solution and the clear supernatant was injected into the HPLC system.

Data Analysis

From in vitro study, the cumulative amount of the drug penetration through rat skin was plotted as a function of time and a linear regression analysis was used to determine the flux and lag time of the drug. Statistical analysis was performed by using the ANOVA test, and the significance level adopted was $p < 0.05$.

RESULTS AND DISCUSSION

Solubility in Ethanol-pH 7.4 Buffer System

As listed in Figure 1, the solubility of meloxicam significantly increased with increase in the pH value of medium. Because meloxicam is an acidic drug (pK_a 1.1 and 4.2), the percentage of drug ionized and thus the solubility increased with an increase in the pH value (Seedher & Bhatia, 2003). The solubilities of meloxicam in ethanol, methanol and 20% glycerin were 424.3 ± 3.3, 540.4 ± 10.3, and 534.9 ± 6.8 mg/L, respectively. The drug solubility of these solvents and buffer solution alone was lower than that required for preparing 1% meloxicam gel. Numerous studies (Valvani et al., 1981; Li et al., 1999) indicated that mixed-solvent is an effective technique for enhancement the solubility poorly soluble drug. The pH value of skin is 4.5–6.5 (Walker 1961); hence, the pH 7.4

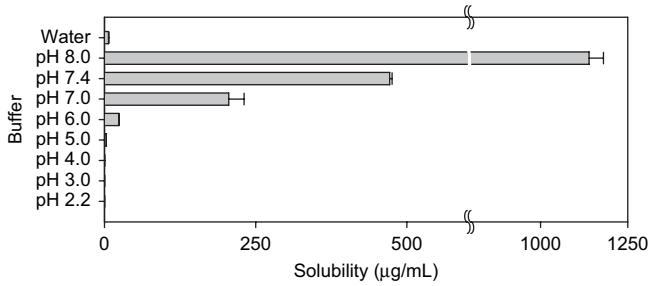

FIGURE 1. Solubility of meloxicam in buffer at different pH values.

buffer pH was used in the thereinafter experiment. Ethanol is generally considered to be a safe solvent for transdermal system. Therefore, the mixed-solvent system of pH 7.4 buffer ethanol was used to increase the solubility in this study.

In order to obtain the optimal mixed solvent for meloxicam transdermal dosage form speedily, response surface methodology (Huang et al., 2004) with a two factors mixture design was used. The composition of independent variables and response (solubility) are listed on Table 1. It can be seen that the solubility of meloxicam in mixed solvent of pH 7.4 phosphate buffer-ethanol was higher than that in pure solvents. To quantify the influence of each independent variable and their possible interactions, the responses of these model formulations were treated with Design-Expert® software using the response surface method including a multiple regression analysis (Huang et al., 2005). The polynomial equation obtained for describing the response surface model is:

$$\text{Ln (Solubility)} = 6.19 \times (\text{pH 7.4 buffer}) + 5.46 \times (\text{Ethanol}) + 14.95 \times (\text{pH 7.4 buffer}) \times (\text{Ethanol})$$

The significance *p*-value (significance probability value), R-square, adjusted R-square and c.v. of this model were <0.0001, 0.9945, 0.9923, and 2.13, respectively, indicating that the assumed regression model was significant and valid for each considered response. 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 & Sakr, 2001). From this equation, it is clear that the interaction of pH 7.4 buffer and ethanol had the greatest potential influence on solubility of meloxicam. The response surface and the contour plot for solubility is shown in Figure 2. The increase in content of ethanol in mixed solvent from 0 to 50% increased the solubility of meloxicam, while a further increase, from 50 to 100%, results in a decrease in the solubility. The highest solubility was observed at the ration of 50/50 of pH 7.4 buffer-ethanol. For evaluation of the reliability of the response surface model, the set of runs P1-P3 were conducted (Table 2) and comparing the predicted values. As shown in Table 2, the predicted error was less than 15%, indicated an acceptable agreement between the predicted values and the experimental data.

TABLE 1
The Composition and Responses of Meloxicam Model Formulations

Run	pH 7.4 Buffer (%)	Ethanol (%)	Response Solubility (μg/mL)
1	50	50	12287.40 ± 256.94
2	0	100	240.67 ± 0.96
3	25	75	4788.73 ± 22.90
4	75	25	8912.09 ± 148.39
5	0	100	236.17 ± 4.09
6	100	0	459.57 ± 11.47
7	50	50	12502.06 ± 135.91
8	100	0	462.73 ± 3.47

In Vitro Skin Penetration Experiments

Terpenes are a series of naturally occurring compounds which consist of isoprene (C5H8) units and were reported to show an enhancement effect on permeation absorption of hydrophilic and hydrophobic drugs such as propranolol, tamoxifen, indomethacin and ketoprofen (Kunta et al., 1997; Gao & Singh, 1998; Levang et al., 1999; Zhao & Singh, 1999; Katayama et al., 2001). In this study, different terpenes including menthone, menthol, cineole, limonene, thymol, and carvone were used to enhance the permeation rate and reduce the lag time of meloxicam. The cumulative amounts of drug penetration through the skin were plotted against the time as shown

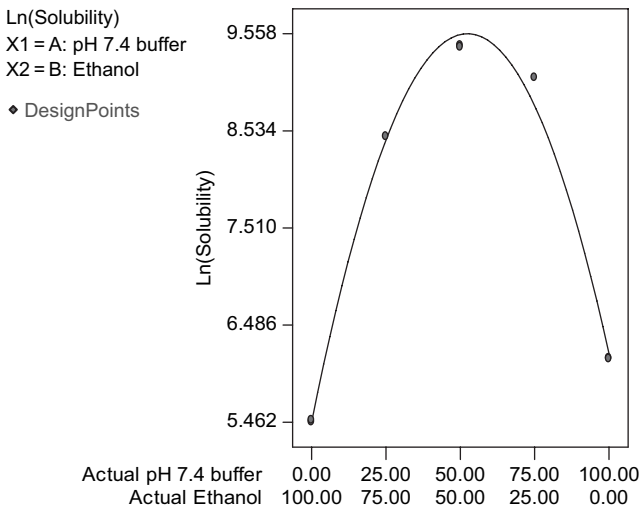

FIGURE 2. Response surface plots showing the effect of independent variables on the drug solubility (response).

TABLE 2
The Predicted and Experimental Data of Solubility for Several Formulations Used to Evaluate the Reliability of the Response Surface Methodology

Run	pH 7.4 Buffer (%)	Ethanol (%)	Solubility (μg/mL)		Predicted Error (%)
			Predicted	Experimental	
P1	35	65	9006.71	8705.13 ± 1004.33	9.70 ± 0.34
P2	65	35	11193.35	11836.58 ± 856.36	–7.66 ± 4.47
P3	45	55	13045.19	11184.16 ± 293.97	14.27 ± 2.25

Predicted Error = (Predicted value – Experimental value) / Predicted value × 100%.

in Figure 3, by which a linear relationship was obtained for each formulation ($R^2 > 0.881$), showing that the penetration of ketoprofen from the gel was well described by the zero-order kinetics. The flux and lag time of meloxicam in mixed solvent of pH 7.4 buffer/ ethanol (50/50) with and without 5% enhancers are listed in Table 3. The flux significantly increased about 70.6–769.2 fold and the lag times were shortened from 7.92 to 0.17 hr by terpenes incorporation. The rank order of enhancement effect for drug penetration rate was menthol > thymol > menthone > cineole > limonene > carvone. The terpenes with a hydroxyl group such as menthol and thymol were found to be most effective on the transport of meloxicam ion in mixed solvent of pH 7.4 phosphate buffer/ ethanol. This result was consistent with previous studies (Cornwell & Barry, 1994; Kunta et al., 1997), which reported that enhancers containing functional groups with hydrogen-bonding ability are effective in promotion of the skin transport of hydrophilic drug. The mechanisms of enhancing activity of terpenes were attributed to increase the drug partition into the SC, lipid extraction and perturbation of the macroscope barrier properties of the skin (Williams & Barry, 1991; Kunta et al., 1997; Zhao & Singh, 1999).

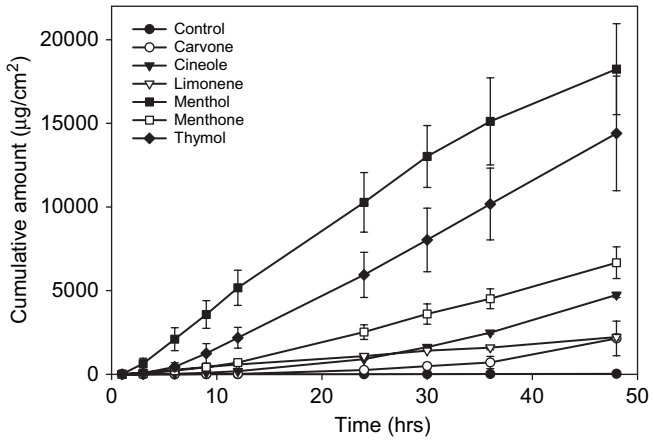

FIGURE 3. In vitro penetration-time profile of meloxicam in mixed solvent of pH 7.4 phosphate buffer/ethanol (50/50) with and without 5% different type of transdermal penetration enhancers.

For the decrease effect of lag time, the menthol and limonene showed the greatest effect, followed by thymol, menthone and cineole. Except for menthol and thymol, the hydrophobic terpene, limonene without a hydroxyl group also showed a significant effect on shortening lag times. However, meloxicam with menthol shows the highest flux and the shortest lag time.

For clinical utility, meloxicam gel containing different concentrations of drug and menthol was prepared and evaluated.

TABLE 3
Flux and Lag Time of Meloxicam in Mixed Solvent of pH 7.4 Phosphate Buffer/Ethanol (50/50) with and without 5% Different Type of Transdermal Penetration Enhancers

Enhancer	Flux (μg/cm²/hr)	ER	Lag Time (hr)	Zero Order R^2
Control	0.53 ± 0.23	1.0	7.92 ± 0.48	0.893 ± 0.028
Carvone	37.42 ± 17.94*	70.6	7.96 ± 0.22	0.881 ± 0.009
Cineole	92.83 ± 2.60*	175.2	6.73 ± 0.12*	0.947 ± 0.003
Limonene	46.14 ± 3.64*	87.1	0.17 ± 0.62*	0.999 ± 0.001
Menthol	407.65 ± 59.14*	769.2	0.24 ± 0.76*	0.995 ± 0.001
Menthone	144.55 ± 21.42*	272.7	4.41 ± 0.22	0.992 ± 0.001
Thymol	314.44 ± 70.38*	593.3	3.79 ± 0.38*	0.996 ± 0.001

*significant difference $p < 0.05$ compared with control group.

TABLE 4
Flux and Lag Time of Meloxicam Gel Formulations with Different Concentrations of Drug and Menthol

	Meloxicam (%)	Menthol (%)	Flux (μg/cm^2/hr)	Lag Time (hr)	Zero Order R^2
F1	0.1	5	12.77 ± 1.04	0.70 ± 1.22	0.966 ± 0.020
F2	0.5	5	62.08 ± 5.36	0.55 ± 0.96	0.972 ± 0.012
F3	1	5	129.69 ± 1.66	0.44 ± 0.77	0.965 ± 0.023
F4	1	3	142.85 ± 6.45	5.60 ± 0.24	0.980 ± 0.003
F5	1	1	0.97 ± 0.19	6.20 ± 0.84	0.935 ± 0.027

Comparison the effect of drug level: for flux – there were significant differences ($p < 0.05$) among F1, F2, and F3; for-lag time – there were non-l significant differences ($p > 0.05$) among F1, F2, and F3.

Comparison the effect of enhancer level: for Flux – there were significant differences ($p < 0.05$) among F3, F4, and F5; for lag time – F3 showed significant lower than F4 and F5 ($p < 0.05$).

The flux and lag time of these meloxicam gel are given in Table 4. With an increase in the amount of meloxicam from 0.1% to 1% in hydrogel, the flux was increased from 12 to 129 μg/cm^2/hr with a linear relationship ($r > 0.9996$) and the lag time was decreased from 0.70 to 0.44 hr. In comparison, with addition of menthol, it can be seen that the highest enhancement penetration effect could be obtained at the level of 3%. The lag time decreased significantly with an increase in the concentration of menthol from 1% to 5%, even though flux has reached the maximum value at the 3% level. In addition, the required flux of meloxicam gel to maintain a therapeutic concentration was about 400 μg/cm^2/hr in accordance with the previously studies (Busch et al., 1998) which reported that after oral administration 7.5 mg meloxicam in human, the C_{max} and Clearance are 0.88 mg/L and 0.42–0.48 L/hr, respectively. Therefore, the formulation containing 1% meloxicam and 5% menthol with higher flux (129.69 μg/cm^2/hr) and lowest lag time (0.44 hr) was chosen for in vivo penetration absorption evaluation.

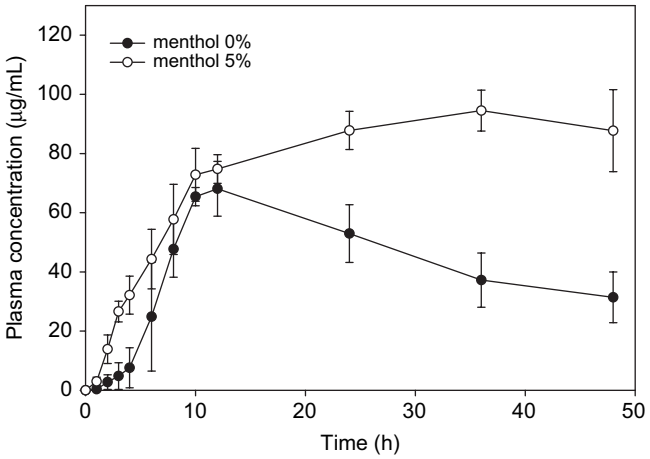

FIGURE 4. The plasma concentration-time profile of meloxicam after topical administration of 1% meloxicam gel (10 mg/2.3 cm^2) with and without 5% menthol.

In Vivo Study

The plasma concentration-time profiles after topical administration of meloxicam gel with and without 5% menthol (10 mg/2.3 cm^2) are shown in Figure 4. The permeation absorption rate of meloxicam with 5% menthol was higher than that of meloxicam without enhancer. In the case of meloxicam gel without enhancer, the plasma concentration slowly increased with time in the first 4 hr, then quickly increased to reach a maximum concentration at about 10–12 hr, after which the plasma concentration decrease accompanied the increase of elimination rate. Comparably, using meloxicam gel containing 5% menthol, the plasma concentration quickly increased with time to reach a maximum concentration at about 10–12 hr, then the high concentration was maintained for a period. The $AUC_{48\ hr}$ and C_{max} were 2414.3 ± 377.9 mg·hr/L and 68.1 ± 9.27 mg/L for meloxicam without enhancer, and 4130.2 ± 379.9 mg·hr/L and 74.78 ± 4.84 mg/L for meloxicam with 5% menthol. The Cmax slightly increased without any significant difference ($p > 0.05$) and the $AUC_{48\ hr}$ increased obviously by about 1.7-fold with by enhancer incorporation.

CONCLUSION

The solubility of meloxicam was pH dependent. The solubility of meloxicam in the mixed-solvent of pH 7.4 buffer and ethanol was higher than that of in pure solvent alone. The response surface methodology (RSM) was successfully used to obtained the optimal mixed-solvent system for preparing meloxicam formulation. Using terpenes as transdermal enhancer, menthol had greatest effect on enhancement of flux and decrement of lag time of meloxicam gel through rat skin. The $AUC_{48\ hr}$ was increased about by 1.7-fold when enhancer was incorporated into to the in vivo penetration study.

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