Drug Development and Industrial Pharmacy, 24(3), 213–217 (1998)

RESEARCH PAPER

Therapeutic Effect and Pharmacokinetics of Ketotifen Transdermal Delivery System

Chiao-Hsi Chiang,[1,*] Jiin-Long Chen,[1] Yu-Tien Liu,[2] and Da-Peng Wang[1]

[1]*School of Pharmacy and* [2]*Department of Microbiology and Immunology National Defense Medical Center, Taipei, Taiwan*

ABSTRACT

Ketotifen transdermal delivery systems were prepared using polyisobutylene, liquid paraffin, and fatty acid. In vitro skin penetration studies were conducted in Franz diffusion cells using excised porcine skin to determine the skin permeation rates of ketotifen patches. A trend of increased skin penetration of ketotifen was observed as the amount of liquid paraffin in the patch was increased. In addition, we found that lauric acid was a suitable enhancer for percutaneous absorption of ketotifen. Challenge tests were performed in guinea pigs to determine the therapeutic effect of the delivery systems for the inhibition of anaphylactic shock using varied concentrations of chicken ovum albumin as sensitizer. Our results showed that compared with the treatment of intramuscular administration, the skin patch was more effective and produced higher survival rates. The pharmacokinetics of the ketotifen patch were determined by applying the skin patch to the dorsal skin of rabbits. The plasma levels were maintained constant (42.5–36.4 ng/ml) from 9 to 30 hr. From our study, the prepared ketotifen patch may further be developed for the treatment or prevention of allergic asthma.

*To whom correspondence should be addressed.

INTRODUCTION

The incidence of asthma is about 1–2% for the overall population. However, the incidence for children increases to 2.3–4.8%, which is about two or three times that of adults (1,2). In the treatment of asthmatic diseases, oral dosage forms and inhalations of asthmatic agents are frequently used. In spite of that, the duration of traditional antiasthmatic preparations is usually less than 1 day, which may not be suitable for the prevention of occurrence of nocturnal asthma. The transdermal delivery systems have been developed to deliver a constant rate of drugs into the circulation and are convenient for use in children. The therapeutic effect may be improved by increasing the compliance and the duration of drug effect. Ketotifen has been used orally to prevent allergic asthma by stabilizing the membrane of mast cells (3,4). The daily dose of ketotifen is 1–2 mg which is significantly less than other regular asthmatic drugs such as salbutmal and terbutaline (4,5). Therefore, ketotifen might be a good candidate to be designed as a transdermal delivery system with long-term effect in the treatment of asthma. In the previous study, we fabricated a ketotifen patch using Eudragit S-100 and other components including glycerin, triacetin, propylene glycol, dibutyl phthalate, and sorbitol (6). The ketotifen patch maintained a constant plasma level of ketotifen after being applied to the dorsal skin of rabbits. In this study, we report another method to prepare the ketotifen patch using polyisobutylene adhesive. The purpose of the study is to develop a suitable transdermal delivery system of ketotifen. The pharmacokinetics of in vivo drug percutaneous absorption and antiallergic effect of the developed ketotifen patch were investigated to evaluate the potential therapeutic effects.

MATERIALS AND METHODS

Ketotifen fumarate (Betchmann, Sifavitor, Italy) was ordered and converted into base for patch preparation. Two types of polyisobutylene including high and low molecular weights were gifts from Symphon Chemical Company (Taipei, Taiwan). Oleic acid and lauric acid were purchased from E. Merck (Darmstadt, Germany). Other chemicals used were either regent or chromatographic grade.

Ketotifen Base

After ketotifen fumarate was dissolved in water, twice molar equivalents of sodium bicarbonate were added. The precipitate of ketotifen was collected and washed with water. The wet mass of ketotifen was dried in a vacuum oven. The yield of ketotifen base was 95% of the theoretical value.

Patch Preparation

High and low molecular weights of polyisobutylene were separately dispersed in toluene and in chloroform with concentrations of 6 and 1.5%, respectively. Two solutions of polyisobutylene were mixed and combined with liquid paraffin, oleic acid, or lauric acid and the drug. The formulations are shown in Table 1. The mixture was vacuum dried in 35°C to remove most of the organic solvents, then the viscous liquid was dispersed on a backing membrane (heat-sealable polyester, film type 1009, 3M, St. Paul, MN). After solvent was completely removed, a releasing line (low adhesion polyester film type 1022, 3M) was covered on the surface of the

Table 1

Components of Ketotifen Patches and the Total Penetrating Percentages of Ketotifen in In Vitro Percutaneous Absorption of Ketotifen Determined by Franz Diffusion Cell Using Porcine Skin

	Components				
		Polyisobutylene Solution (g)			
Formulation	Fatty Acid (g)	High MW	Low MW	Liquid Paraffin (g)	Total Penetration in 24 hr (%)
I	Lauric acid 0.16	25.9	8.6	2.8	43.6
II	Lauric acid 0.16	25.9	8.6	3.2	52.5
III	Oleic acid 0.23	25.9	8.6	2.8	33.1
IV	Oleic acid 0.23	25.9	8.6	3.2	35.9

prepared patch as a protective layer. The patch was stored at 5°C and protected from light.

Challenge Test

In the study, 24 guinea pigs, either sex, with body weight of 250–350 g, were divided into four groups which included blank control (without injection of chicken ovum albumin), control, treatment with intramuscular injection of ketotifen (0.32 mg/day), and treatment with ketotifen patch (patch size 3.2 cm^2, containing 3.2 mg of ketotifen). Prior to 2 weeks, except blank control, the other three groups were injected intraperitoneally with 1 ml of 1% chicken ovum albumin. During the test, guinea pigs of the two control groups received no treatment. Two treated groups were administered either ketotifen injection or skin patch for 1 week. For guinea pigs treated with the skin patch, the ketotifen patch was applied on the dorsal skin after the back hair was carefully cut and shaved. The skin patch was changed every 2 days. The challenge test was conducted on all four groups of guinea pigs. After the guinea pigs were anesthetized with intramuscular injection of 7.5 mg of ketamine and 1.8 mg of xylazine, they were intravenously injected with the chicken ovum albumin solution into the cephalic vein of the front limbs and observed for 24 hr. The mortalities of the four groups were recorded and used to compare the therapeutic effects of both treatments.

In Vitro Percutaneous Absorption

For in vitro skin penetration, the skin was obtained from the ventral side of a hog. The skin was prepared with a thickness of 0.7 mm by a dermatome after the skin hair was carefully shaved. The percutaneous absorption study was performed in the Franz diffusion cell. After the ketotifen patch was mounted on the donor compartment of the diffusion cell with an active surface area of 0.65 cm^2, the receptor compartment was filled with 3 ml of normal saline and maintained at 37°C by a circulating water bath. During the experiment, a 0.2-ml aliquot was withdrawn at certain time intervals, then an equal volume of normal saline was added to maintain the constant volume of the receptor compartment.

In Vivo Study

New Zealand white rabbits, either sex, weighing 2.5–3.5 kg, were used in the study. After the head of the rabbit was restrained in a wooden cage, the central ear artery was cannulated by a retained polyethylene (PE) tube with the aid of a no. 21 needle. The back hair of the rabbit was carefully cut and shaved, then a 10-cm^2 ketotifen patch (formulation II) was stuck on the skin of the dorsal side and further fixed by an elastic bandage. Then, at suitable intervals, 3-ml blood samples were collected in PE vials containing 0.1 ml of 20 unit/ml heparin. After centrifugation of blood samples, plasma samples were obtained and analyzed by an HPLC method.

Drug Analysis

Samples from the in vitro skin penetration study were directly determined by an HPLC method. The analytic method was according to the previous report and is summarized as follows (6). A C18 column (Vercopack, 150 × 4.6 mm i.d., 5 μm) was used to analyze the ketotifen. The mobile phase was composed of methanol:sodium phosphate buffer (0.02 M, pH 3.61):tetrahydrofuran with a ratio of 50:48:2 (v/v). The flow rate was set to 1 ml/min and the instrument was operated in ambient temperature.

For the determination of the drug concentration in plasma samples, 5 μl of 6-ethoxyzolamide was added to 0.5 ml of plasma as the internal standard, then 1.5 ml of methanol was also added. After a 2-min vortex, the mixture was centrifuged at 1500 × *g* for 15 min and the supernatant was concentrated to 0.25 ml using a centrifugal vaporizer (CVE-200 D, Tokyo Rikakikai Co., Japan) and used in the HPLC analysis. The analytical conditions were the same as those in the sample from in vitro studies, except the ratio of mobile phase was changed to 40:58:2 (v/v).

RESULTS AND DISCUSSION

Figure 1 shows the skin penetration profiles of ketotifen patches. In the study, four formulations of ketotifen patches were prepared. The percentages of cumulative penetration of ketotifen in 24 hr were 43.6, 52.5, 33.1, and 35.9 for the four formulations, I–IV, respectively. It seems that increasing the amount of liquid paraffin in the patches also enhances the skin penetration rate. In the study, we incorporated equimolar fatty acids, either lauric acid or oleic acid, with ketotifen in the patch. The patches with lauric acid (C14) obtained higher skin penetration rates than those with oleic acid (C18). This percutaneous result is similar to the report

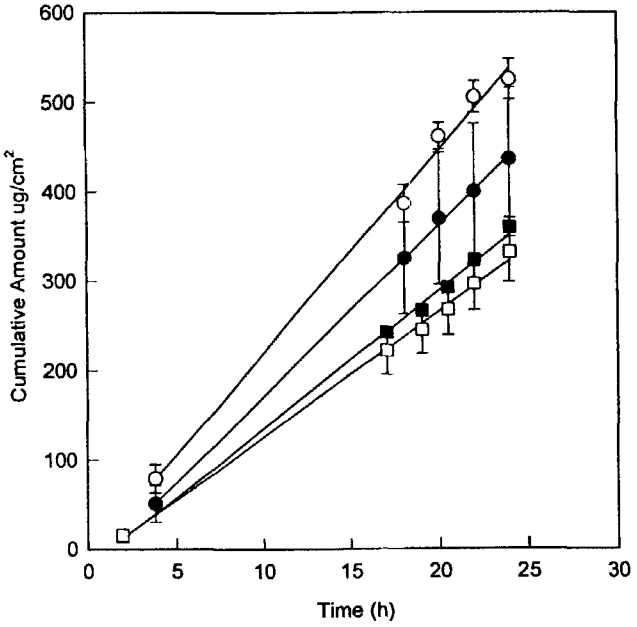

Figure 1. Skin penetration profiles for four formulations of ketotifen patches (n = 3, ±1 SD. Key: (○): II; (●): II; (■): IV; and (□): III.

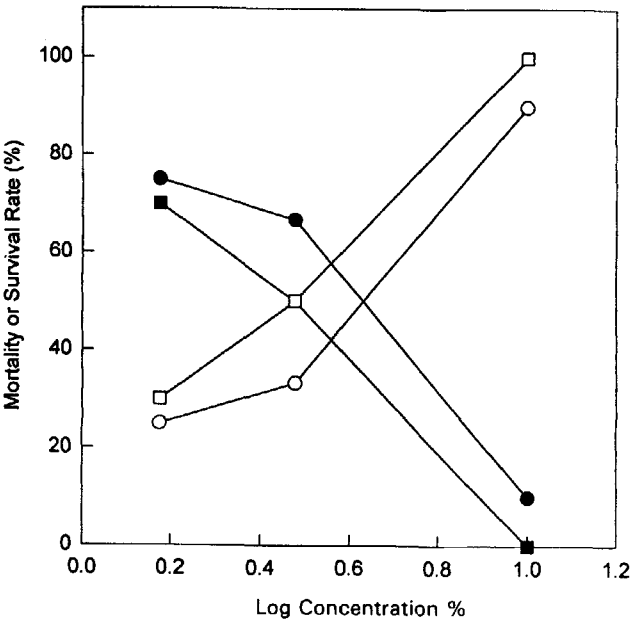

Figure 2. Mortalities (open symbols) and survival rates (solid symbols) for comparison of two treatments either with skin patch (circle) or intramuscular injection (square) of ketotifen in the challenge test of guinea pigs.

in which a series of fatty acids were used as penetrative enhancers in the penetration study: the combination with lauric acid obtains the most enhancing effect in percutaneous absorption (7). The fatty acid and ketotifen might form a complex by an electron donor–acceptor mechanism between the carboxyl group of fatty acid and the nitrogen atom of ketotifen base (8). The complex increased the loading dose of drug in the polyisobutylene matrix. We found that ketotifen patch without fatty acid could be loaded with only one-half the amount of drug.

Challenge Test

The guinea pigs from the blank control group were all alive (no pretreatment with chicken ovum albumin) after the challenge test. However, those of the control group (no treatment) were all dead after being injected with chicken ovum albumin. This result suggested that anaphylactic shock of the challenge test animals was induced by pretreating with chicken ovum albumin (9). In comparison with two treatments, intramuscular injection and skin patch of ketotifen, we found that the mortalities of the two methods increased as the concentrations of chicken ovum albumin in the challenge test increased (Fig. 2). The results demonstrated that the protective effect of ketotifen became noticeable as the concentration of chicken ovum albumin in the challenge test was reduced. The treatment of the ketotifen skin patch seemed more effective than that of intramuscular injection for maintaining a high survival rate.

Pharmacokinetic Studies

The time course of ketotifen concentration in plasma after the ketotifen patch is applied in the dorsal skin of the rabbits is shown in Fig. 3. The maximum concentration of ketotifen was obtained at 6 hr (55.2 ng/ml). Then, the plasma level was quickly decreased to 42.5 ng/ml at 9 hr. The ketotifen concentrations were maintained at an almost constant level (42.5–36.4 ng/ml) from 9 to 30 hr, then a fast disappearance of plasma level was noticed. The terminal elimination rate constant was estimated from the last three points, 48, 54, and 72 hr, to be 0.025 hr^{-1} with a half-life of 27.7 hr. The area under the curve ($AUC_{0-\infty}$) for plasma profile with time axis was also determined (2560 ng/ml · hr), the AUC_{0-74} was determined from the trapezoidal method, and the $AUC_{74-\infty}$ was determined from the ketotifen concentration of last point divided by the elimination

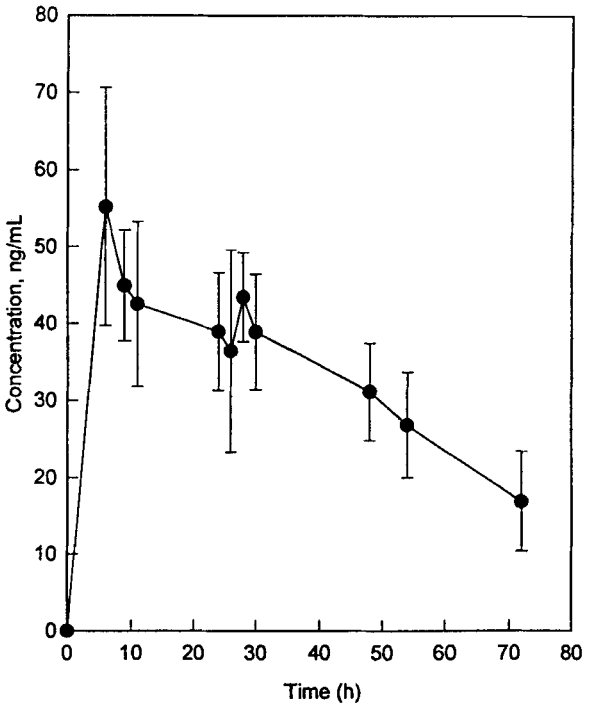

Figure 3. The average plasma profile of ketotifen after applying the ketotifen patch (10 cm^2) in the dorsal skin of the rabbits (n = 6, $\pm$1 SD).

rate constant (10). The prepared patch had a higher skin penetration rate initially due to the first sampling point with the peak plasma concentration, then a slow release rate followed which was possibly controlled by the matrix of polyisobutylene. The bioavailability of the ketotifen patch was estimated from the ratio of AUC for skin patch and intravenous injection: about 0.6 mg of ketotifen was absorbed into the systemic circulation (6). The absorbed amount of drug was similar to our previous study for the preparation of skin patch using Eudragit as matrix, but the patch size in this study was significantly reduced to only one-third of that used before.

In conclusion, we fabricated a ketotifen patch using polyisobutylene with suitable fatty acids. The patch demonstrated a good protective effect to decrease the mortality of anaphylactic shock in guinea pigs induced by chicken ovum albumin. Furthermore, from the pharmacokinetic study, the ketotifen patch could maintain a constant plasma level for 3 days, which might further be developed in the treatment or prevention of allergic asthma.

ACKNOWLEDGMENT

The authors are deeply indebted to the Kun-Po Soo Medicine Research Foundation for the financial support of this study.

REFERENCES

1. E. T. Herfindal and J. I. Hirschaman, *Clinical Pharmacy and Therapeutics*, 3rd ed., Williams, Baltimore, 1984, pp. 476–491.
2. A. B. Kay, K. F. Austen, and L. M. Litchtenstein, *Asthma, 3rd International Symposium,* Academic Press Inc., London, 1984.
3. J. F. L. Bigol, J. M. Begue, J. R. Kiechel, and A. Gullouzo, Life Sci., 40, 883 (1987).
4. S. M. Grant, K. L. Goa, A. Fitton, and E. M. Sorkin, Drugs, 40, 413 (1990).
5. G. R. Kennedy and J. M. Sherriff, Respiration, 41, 264 (1981).
6. Y. L. Lee, C. H. Chiang, and J. L. Chen, Drug Dev. Ind. Pharm., 20, 2965 (1994).
7. P. T. Kao and C. H. Chiang, J. Med. Sci., 15, 141 (1994).
8. A. Martin, P. Bustamante, and A. H. C. Chun, *Physical Pharmacy*, 4th ed., Lea & Febiger, Philadelphia, 1993, pp. 254–257.
9. J. Kuby, *Immunology*, W. H. Freeman and Company, New York, 1992, pp. 358–381.
10. M. Gibaldi and D. Perrier, *Pharmacokinetics*, 2nd ed., Marcel Dekker, New York, 1982, pp. 445–449.