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IN VITRO AND *IN VIVO* PERCUTANEOUS ABSORPTION STUDIES OF KETOTIFEN PATCHES

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ABSTRACT

The purpose of the study is to develop a suitable transdermal delivery system for ketotifen. The physicochemical properties and skin penetration of ketotifen were evaluated for the optimization of the method of patch preparation. The distribution coefficients of ketotifen were determined in octanol/phosphate buffer systems, the partition coefficient and pka value of ketotifen were obtained as 120 and 6.73, respectively. *In vitro* skin penetration of ketotifen solution was determined at 35 °C in the Valia-Chien diffusion cell by using the abdominal skin of nude mouse. The results showed that ketotifen had an optimal skin permeability at pH 7.5. In addition, ketotifen had higher rate of penetration through stripped skin, it was suggested that the main barrier for percutaneous absorption of ketotifen is stratum corneum. Ketotifen patch was fabricated in a stainless mold containing Eudragit S-100 and PEG 400. Other components, tween, span and fatty acids were also incorporated into the patch as penetration enhancers. For animal study, a patch with area of 30 cm^2 was applied on the dorsal skin of rabbit. The plasma level, after 10 hrs administration were reached 60 ng/mL and maintained a constant level. The results proposed that ketotifen was successfully absorbed through the skin from the applied patch.

INTRODUCTION

Asthma is a chronic disease, the routine activity of patient was usually disturbed by the disease. In serious asthmatic condition, the disease also caused fatality. The incidence of asthma is about 1-2% for overall population, however for children, the incidence increases to 2.3-4.8% which is about 2 or 3 times that of adult (1,2). The preparations of antiasthmatic agents are frequently used as oral dosage form or inhalation. Although these

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preparations are administrated easily for adults and elder children but which are used difficultly for younger children. As a result, enough dose to control the asthmatic condition might not be obtained due to bad compliance. Thereby, developing a suitable drug delivery system for asthmatic patients, especially for young kids would possibly improve the therapeutic effect in the treatment of children's asthma.

Recently, ketotifen has been used orally to prevent the allergic asthma by directly blocking the release of allergic intermediator from the mast cell (3). The pharmacokinetics of ketotifen has been studied. For daily dose 1 mg of ketotifen, the oral bioavailability is only 0.5, most of the ketotifen is eliminated in liver as metabolites, and only 1% of the intact drug is excreted from the kidney (4). Therefore, ketotifen might be designed as a suitable delivery system with long-term effect and bypass the liver, such as transdermal delivery system, the delivery system may have constant drug delivery rate to the circulation system and convenient use for children.

The study was to evaluate the chemical properties of ketotifen, and *in vitro* percutaneous absorption of ketotifen, consequently, some matrix types of methylmethacrylate of ketotifen patches were prepared. Rates of drug released and amount of drug absorbed through skin were tested for these patches. The purpose of the study is to develop a transdermal delivery system of ketotifen which may be used in the treatment of asthma.

MATERIALS AND METHODS

Materials

Ketotifen fumarate (Betchmann, USA) is used as received. Eudragit S-100 (Rohm Pharm, Germany), propylene glycol (Wako, Japan), dibutyl phthalate (Wako, Japan), triacetin (Nakarai, Japan) and polyethylene glycol 400 (Wako, Japan) were used to prepare the patch of ketotifen. Other chemicals were used either reagent or chromatographic grades.

Determination of Partition Coefficient

Various pHs of ketotifen fumarate solutions in 0.02 M phosphate buffer were prepared with concentration 500 μg/mL. The drug solution 2 mL was added in a partition determinator (MIXXOR 5 mL, Cole-Parmer, USA), then 2 mL of octanol presaturated with phosphate buffer was also added, after 20 times mixing, then the mixture was equilibrated in a water bath at 35 °C. The aqueous phase in lower layer was withdrawn and centrifuged at 1500xg for 5 min, and determined by the HPLC method.

Percutaneous Absorption of Ketotifen Solution

In the study, Valia-Chien diffusion cell was used to determine the skin permeability of ketotifen. Ketotifen fumarate was prepared in different pHs of phosphate buffer solutions with pH 4.6, 6.1, 6.9, 7.2 and 7.5. After nude mouse (Animal Care Unit, Triservice General Hospital, Taiwan) was sacrificed by dislocating the spinal cord, the abdominal skin was carefully excised and mounted in the diffusion cell, the donor site was filled 3 mL of

drug solution, the receptor site contained 3 mL of normal saline, the temperature was controlled at 35 °C. During the experiment, 0.2 mL of aliquot was withdrawn at suitable time intervals, then equal volume of normal saline was added to the receptor site, following, the sample was added 0.2 mL of 6-ethoxyzolamide (Sigma, USA) and assayed by the HPLC method.

In order to determine the resistance of stratum corneum of the skin permeability of ketotifen, the whole mouse skin was stripped 23 times by the Scotch tape to remove the stratum corneum (5), then the stripped skin was conducted percutaneous study in Valia-Chien diffusion cell, the drug solution in the donor site with three different pHs, 6.0, 7.0 and 8.0, the experimental procedure was the same as stated before.

Preparation of Ketotifen Patches

After 6.5 g of Eudragit S-100 had been dissolved in 30 mL of alcohol, 650 mg of ketotifen fumarate was also dissolved in the alcoholic soluiton. Following, depending on the formulation, some plasticizers, glycerin, dibutyl phthalate, triacetin were incorporated into the mixture. Then, the mixture was poured into a stainless mold with area 125 cm^2 and dried at 40 °C for 24 hrs, finally a thin patch with thickness 0.6 mm was obtained. The components of ketotifen patches were tabulated in Table 1.

Drug Release and Mechanical Properties of Patches

Drug release studies were conducted in Franz diffusion cells, the patch was placed on the top of the diffusion cell, the receptor site contained 5.5 mL of normal saline. At suitable time intervals, 0.3 mL of sample was withdrawn from the receptor site. The release of drug from ketotifen patch was tested for 30 hrs and determined in 6 sets of diffusion cells.

The elongation and tensile strength of patch were determined by a tensile strength tester (Vertical Tensile Tester, Hung Ta Instrument Co., Taichung, Taiwan). The experimental condition was controlled at 35 $\pm$ 0.5 °C and 70% relative humidity. The film of patch was cut into 10 x 10 mm strip which was run with an elongate speed of 2.0 cm/min.

Effect of Aliphatic Acid and Surfactant on Percutaneous Absorption of Patches

For a series of aliphatic acids, including hexanoic acid, octanoic acid, lauric acid, myristic acid and oleic acid were incorporated into the patches. The patch was composed of 4.5% aliphatic acid, 30% polyethylene glycol, 5% ketotifen fumarate, 15% triethanolamine and 45.5% Eudragit S-100. For a series of surfactants, 4.5% tween and span 20, 40, 60, and 80, were also incorporated into the patch to substitute aliphatic acid. These patches were studied to determine the release rate and *in vitro* percutaneous absorption of ketotifen.

In Vivo Study

New Zealand white rabbits either sex, weight 2-3 kg, were used in the study. After the head of rabbit was restrained rightly in a cage, the central ear artery of rabbit was cannulated by a retained PE tube with an aid of #21 needle. Rabbit was injected intravenously from the marginal ear vein with a dose of 400 μg/kg

TABLE 1

Components of various ketotifen patches

Formula[a]	Glycerin	Triacetin	Propylene Glycol	Dibutyl Phthalate	Sorbitol 70%
			(g)		
1	-	-	-	-	-
2	0.65	-	-	-	-
3	1.3	-	-	-	-
4	2.6	-	-	-	-
5	-	0.65	-	-	-
6	-	1.3	-	-	-
7	-	2.6	-	-	-
8	-	-	0.65	-	-
9	-	-	1.3	-	-
10	-	-	2.6	-	-
11	-	-	-	0.65	-
12	-	-	-	1.3	-
13	-	-	-	2.6	-
14	-	-	-	-	1.3

[a]Contents in 125 cm^2 of patch, other components including: Eudragit S-100 6.5 g, ketotifen fumarate 0.65 g, and PEG 400 3.9 g.

of ketotifen fumarate. About 3 mL of blood was collected in a PE vial containing 0.1 mL of 20 unit/mL heparin at 15, 30, 60, 120, 180 and 240 min. After the blood was centrifuged at 1500xg for 15 min, the plasma was taken and analyzed by the HPLC method.

In addition, 5 rabbits after the back hair was carefully cut and shaved, a patch of ketotifen was sticked on the back skin of each rabbit (6). Then, at suitable time intervals, the blood was taken and determined by the HPLC method.

Drug Analysis

In vitro samples of drug release and skin penetraition study were directly assayed by the HPLC method. The HPLC system equipped a pump (880-30, Jasco), an integrator (Waters, 740), an autosampler with 20 μL sample loop (Model 23, SIC) and an UV detector (Model 875, Jasco). A C_{18} column (15 cm x 4.6 mm I.D., Vercopak) was used to analyze the ketotifen. The mobile phase was composed of methanol: phosphate buffer (0.02 M, pH 3.6): tetrahydrofuran with ratio 50:48:2 (V/V). The flow rate was set to 1 mL/min and operated in ambient.

For the determination of drug concentrations in plasma samples, 5 μL of 6-ethoxyzolamide was added to 0.5 mL of plasma as internal standard, then added 1.5 mL of methanol, after 2 min vortex, the mixture was centrifuged at 1500xg for 15 min, the supernatant was used in the HPLC analysis. The mobile phase of the HPLC system was composed of methanol: phosphate buffer (0.02 M, pH 3.6): tetrahydrofuran with ratio 40:58:2 (V/V), other analytical conditions were the same as the samples of *in vitro* studies.

RESULTS

HPLC Profiles

The HPLC profiles of ketotifen for *in vitro* and *in vivo* studies are shown in Fig. 1. For *in vitro* studies, ketotifen and internal standard 6-ethoxyzolamide had retention times 4.5 and 8.7 min, respectively. For *in vivo* studies, the mobile phase was changed to decrease the ratio of methanol, thus the retention times were increased to minimize the interference of plasma components.

Determination of Distribution Coefficient

The distribution coefficients of ketotifen were determined in five different pHs, 4.4, 5.8, 6.7, 7.0 and 7.2. The partition coefficient (PC), distribution coefficient (DC) and Ka had following relationship:

$$\frac{1}{DC} = \frac{1}{PC} + \frac{[H^+]}{PC\ Ka} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Thus, plotting 1/DC versus $[H^+]$, the intercept of y axis is equal to the reciprocal of PC, and slope is equal to the reciprocal of PC.Ka. According to eq. 1, PC and pKa could be determined which were 120 and 6.7 respectively.

Permeability Coefficient of Percutaneous Absorption

The skin penetration profile for different pHs of ketotifen solutions are shown in Fig. 2. The skin permeability coefficients were determined from the slope by plotting the cumulative amount of drug versus time then divided by drug concentration and the active surface area of diffusion study. The lag time of percutaneous absorption study was also obtained from the intercept of ordinate. These results are listed in Table 2.

The skin after de-stratum corneaum had higher percutaneous absorption of ketotifen in three different pHs but shorter lag time, the results are shown in Table 3.

Effect of Plasticizers on Drug Release and Mechanical Properties

Drug release from the patch was described as matrix-controlled type and fit by the Higuchi equation as following:

$$Q = K_r\ t^{1/2}$$

where Q represents the cumulative amount of drug release, t is time, K_r is a release constant with unit $\mu g/hr^{1/2}$. The release results for different plasticizers with various concentrations are shown in Fig. 3. In addition, the tensile strength and elongation of patches with different plasticizers are shown in Table 4.

Percutaneous Absorption of Ketotifen Patches

The components of patches containing tween, span and aliphatic acid had different effects on the skin penetration. The skin permeability coefficients and lag times are listed in Table 5. According to these results, span series could not enhance the skin penetration , but tween 80 of tween series improved the percutaneous absorption of ketotifen.

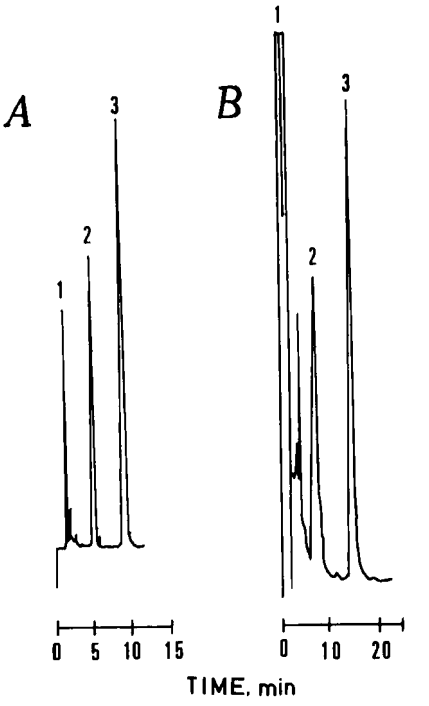

FIGURE 1

HPLC profiles of ketotifen, (A) *in vitro* samples and (B) *in vivo* samples. Key: (1) solvent front; (2) ketotifen and (3) 6-ethoxyzolamide.

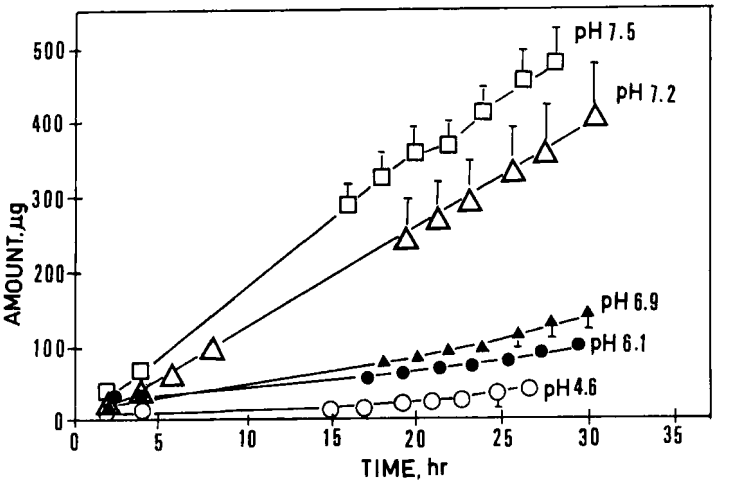

FIGURE 2

Skin penetration profiles for different pHs of ketotifen solutions. (n=4-6, ± 1 SD)

TABLE 2

Permeability coefficients and lag times for different pHs of ketotifen solution penetrating through the intact nude mouse skin

pH	Permeability Coefficient (10^{-7} cm/sec)	Lag Time (hr)
4.6	6.9 ± 3.0	11.7 ± 0.2
6.1	11.0 ± 3.0	9.3 ± 1.9
6.9	14.5 ± 4.2	5.5 ± 1.9
7.2	38.6 ± 8.1	4.6 ± 1.3
7.5	56 ± 14	0.4 ± 0.2

TABLE 3

Permeability coefficient of ketotifen for drug penetrating through de-stratum corneum skin

pH	Permeability Coefficient (10^{-7} cm/sec)
6.0	116.2 ± 4.8
7.0	103.9 ± 4.5
8.0	106.9 ± 2.5

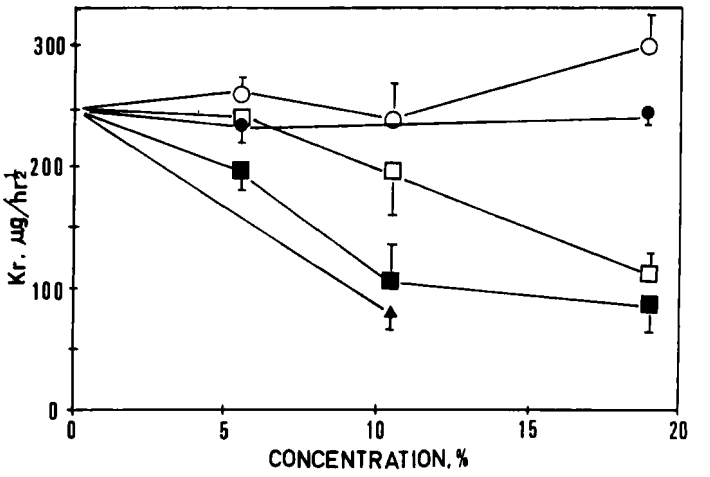

FIGURE 3

Release constants of ketotifen for patches with different concentrations of various plasticizers. Key: (●) glycerin; (▲) 70% sorbitol; (○) propylene glycol; (■) dibutyl phthalate and (□) triacetin.

TABLE 4

Tensile strengths and elongations of ketotifen patches

Plasticizers[a] Components	Amount (g)	Tensile Strength (kg/cm^2)	Elongation (%)
--	--	37.5 ± 2.0	267 ± 29
Triacetin	0.65	34.3 ± 1.5	295 ± 26
	1.3	29.3 ± 1.9	330 ± 36
	2.6	25.0 ± 2.8	364 ± 29
Dibutyl Phthalate	0.65	33.7 ± 1.2	303 ± 37
	1.3	31.5 ± 3.0	372 ± 34
	2.6	23.0 ± 2.7	380 ± 42
Propylene Glycol	0.65	36.1 ± 2.0	284 ± 11
	1.3	34.3 ± 1.8	325 ± 25
	2.6	33.1 ± 1.3	326 ± 23
Glycerin	0.65	44.5 ± 3.7	258 ± 16
	1.3	51.4 ± 3.9	202 ± 23
Sorbitol 70%	1.3	42.1 ± 2.3	236 ± 34

[a]Contents of 125 cm^2 patch, other components including: Eudragit S-100 6.5 g, ketotifen fumarate 0.65 g and PEG 400 3.9 g.

TABLE 5

Permeability coefficients and lag times of ketotifen patches with tween, span and aliphatic acid as enhencers

Components[a]		Permeability Coefficient (10^{-9} cm/sec)	Lag Time (hr)
--		6.2 ± 0.4	7.7 ± 1.2
Tween	80	11.9 ± 0.7	3.4 ± 0.6
	60	6.9 ± 0.1	6.7 ± 1.2
	40	6.3 ± 0.6	4.0 ± 1.1
	20	6.4 ± 0.7	6.1 ± 1.9
Span	80	6.1 ± 1.2	5.0 ± 1.3
	60	4.6 ± 0.1	1.2 ± 0.4
	40	3.2 ± 0.2	3.6 ± 1.5
	20	3.6 ± 0.3	2.1 ± 1.0
Hexanoic Acid		8.6 ± 1.0	3.8 ± 1.1
Octanoic Acid		9.1 ± 1.2	5.2 ± 1.7
Lauric Acid		10.3 ± 0.2	5.4 ± 0.8
Myristic Acid		10.1 ± 0.9	4.9 ± 1.3
Oleic Acid		11.9 ± 3.5	9.6 ± 1.2

[a]Contents of 125 cm^2 patch, other components including: Eudragit S-100 6.5 g, ketotifen fumarate 0.65 g, PEG 400 3.9 g and triethanolamine 2.0 g.

TABLE 6

Permeability coefficients and release constants of ketotifen patches with different drug loading

Drug Loading[a] (%)	Permeability Coefficient (10^{-9} cm/sec)	Release constant K_r (ug/hr$^{1/2}$)
5	6.2 ± 0.4	127.3 ± 4.4
9.2	5.1 ± 0.7	189.4 ± 6.7
12.8	5.2 ± 0.5	274.6 ± 5.1
14.4	5.5 ± 0.8	358.8 ± 6.9
15.9	6.5 ± 0.6	368.9 ± 8.7

[a]Contents of 125 cm^2 patch, other components including: Eudrgit S-100 6.5 g, PEG 400 3.9 g and triethanolamine to neutrilize the pH.

Drug Loading and Percutaneous Absorption

For patches containing various amount of ketotifen were also evaluated, the release constants and skin permeability coefficients were determined which were listed in Table 6. The results demonstrated that K_r increased with the loading dose. Although the skin permeability coefficient for different loading dose of patches were not much different but the cumulative amount of ketotifen penetrated through the skin had a trend of increasing for increasing loading dose (Fig. 4).

In Vivo Animal Studies

The time courses of ketotifen in plasma for intravenous bolus administration of 0.4 mg/kg ketotifen and ketotifen patch (30 cm^2) were shown in Fig. 5. The areas under the plasma level with time for intravenous administration and transdermal delivery were 4300 ng.hr/mL and 2100 ng.hr/mL, respectively. The terminal half-life of ketotifen was estimated from the intraveous administration which was 3.3 hrs.

DISCUSSION

The skin penetration of ketotifen in aqueous solution was highly depend on the pH, especially for pH near the pKa of ketotifen. Another, we found that distribution coefficient was related to the skin permeability. It could be that the skin permeability combined the partition coefficient of drug between aqueous solution and skin, the thickness of skin, and diffusion coefficient (7). For the percutaneous studies of ketotifen in various pHs, the effect of thickness of skin and diffusion coefficient were about equal, however, different pHs of ketotifen solutions had unequal distribution coefficient which was related to the partition coefficient of drug in skin and aqueous solution. The distribution coefficient was increased as pH increased, as a result, the skin permeability was also improved.

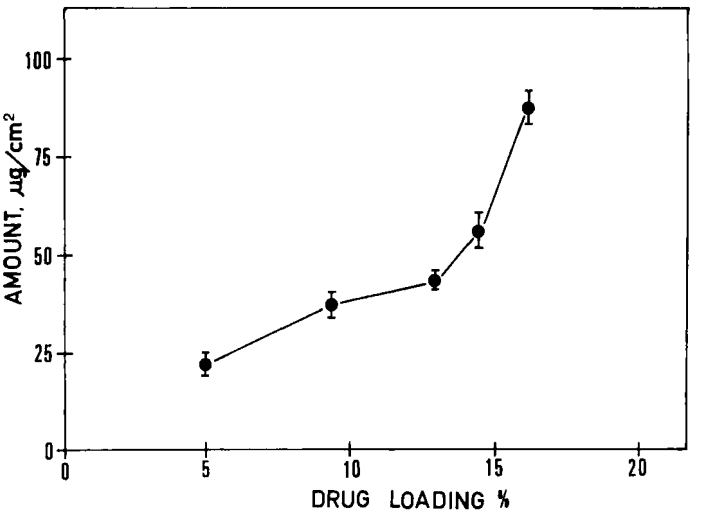

FIGURE 4

The percutaneous absorption of drug at 28 hrs for different loading concentrations of ketotifen patches. (n=5, ± 1 SD).

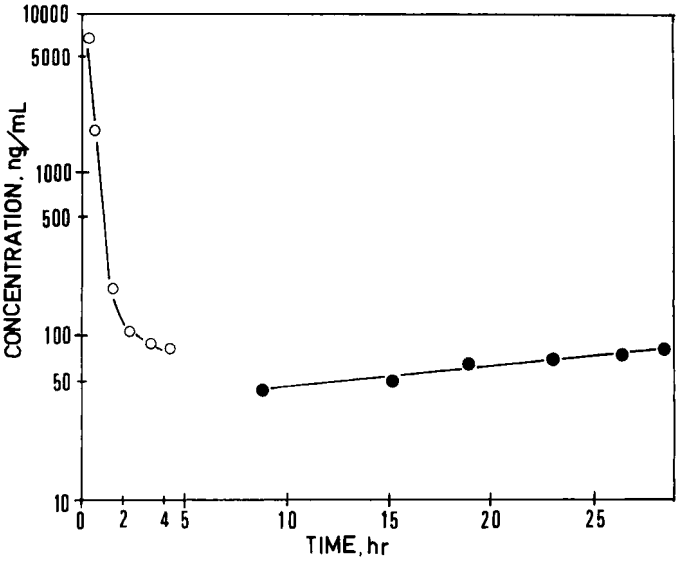

FIGURE 5

The average plasma profiles of ketotifen after administering two dosage forms of ketotifen fumarate in 5 rabbits. Key: (○) intravenous administration of 0.4 mg/kg, and (●) patch with area of 30 cm^2.

In addition, as pH increased, the lag time of skin penetration also decreased (8), it proposed that the nonionized form of ketotifen was dramatically increased for pH higher than the pKa of ketotifen (pKa=6.73) (9), the nonionized form of ketotifen penetrated through the whole skin less resistance in comparison with the ionized form (8).

The stratum corneum was regarded as the major barrier for skin penetration of ketotifen (10), while the stratum corneum was stripped out, the skin permeability of ketotifen was increased which was also independent on the pH of drug solution in the donor site. It suggested that the stripped skin had similar resistant between ionized form and nonionized form of ketotifen.

Lin et al. had been reported the mechanical properties of Eudragit film. They suggest that some Eudragit E with suitable plasticizers are possibly used as transdermal delivery system (11). However, their study used plain Eudragit without drug. In the study, we fabricated the ketotifen patch using Eudragit S-100 to instead of Eudragit E. From the animal study, the patch was applied on the rabbit, the amount of drug absorption was estimated from the AUC of intravenous administration, about 0.5 mg of ketotifen fumarate was absorbed through the skin to reach the circulation system, although the available fraction of dose is small for the study but it might obtain the therapeutic effect, due to the daily oral dose of ketotifen fumarate is 1 mg, with 0.5 bioavailiablity. Thus, the delivery system of ketotifen patch may have a potential for further development.

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