DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 17(16), 2157-2172 (1991)

AQUEOUS-LIPID TRANSDERMAL FORMULATION OF ANTI-INFLAMMATORY AGENTS, PREPARED WITH HYDROGENATED SOYA PHOSPHOLIPID

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ABSTRACT

The in vitro transport of various anti-inflammatory agents were examined using rat dorsal skin and shed snake skin as models of the stratum corneum for the evaluation of transdermal formulation. The aqueous-lipid vehicle formulations prepared with hydrogenated soya phospholipid increased the transport of ketoprofen and sodium diclofenac, and an addition of tetradecanol to the formulation increased the transport of both drugs more markedly. The amounts of drug (%) transported during 12 h with rat dorsal skin and during 24 h with shed snake skin was about 50% for ketoprofen and about 30 % for sodium diclofenac. The aqueous-lipid formulation including tetradecanol also increased the transport of indomethacin, but only about 10 %. It is suggested that the small effect of the aqueous-lipid vehicle formulation

on indomethacin transport is due to the low solubility of indomethacin in the vehicle.

INTRODUCTION

There have been many approaches taken to improve the transdermal absorption of drugs. These include: (i) formulation modification (1, 2), (ii) development of agents to enhance skin permeability (3, 4, 5), and (iii) prodrug synthesis (6).

We have reported that an aqueous-lipid formulation prepared with hydrogenated soya phospholipid increased transport of sodium diclofenac in rats and humans (7, 8). This formulation may be considered in both the categories of (i) and (ii) described above. The enhancing mechanism of this formulation might involve an increase in water content in the stratum corneum (9). In the human study (8), plasma diclofenac concentration was 10 to 50 ng/ml during experimental period of 8 h. Appearance of these plasma diclofenac concentrations after transdermal application may also suggest effectiveness for topical treatment near the application area, because the plasma concentration of diclofenac after application on the rat dorsal skin increased with a significant increase in diclofenac accumulation in the subcutaneous tissue under the skin to which the formulation was applied (7). However, the bioavailability of diclofenac was less than 10% when calculated from the area under the curve of plasma diclofenac concentrations in humans.

It would be interest to determine how much this aqueous-lipid vehicle formulation prepared with phospholipid increases the transport of other anti-inflammatory agents, for the optimization of formulation compositions. In the present study, we have prepared

TABLE 1

The codes for and constituents of the aqueous gel formulations.

Constituents	Amount, % w/w, in the Formulations			
	I	II	III	IV
Drug	1.0	1.0	1.0	1.0
Phospholipid	6.0	6.0	6.0	0
Urea	0	0	3.0	0
Triglyceride	6.0	3.0	3.0	0
Tetradecanol	0	3.0	3.0	0
Ethanol	5.0	5.0	5.0	35.0
Water	82.0	82.0	79.0	64.0
TOTAL	100.0	100.0	100.0	100.0

aqueous-lipid vehicle formulation with phospholipid, including other additive(s) such as tetradecanol and urea, and evaluated the transport of three anti-inflammatory agents using rat dorsal skin and shed snake skin as models of the stratum corneum.

MATERIALS AND METHODS

Materials: Indomethacin, sodium diclofenac, ketoprofen, tetradecanol, and urea were obtained from Sigma Chemicals (St. Louis, USA). Hydrogenated soya phospholipid (phospholipid) which was supplied by Nihon Surfactant Co. Ltd (Tokyo, Japan) contains 70% w/w phosphatidyl-ethanolamine and 30% w/w phosphatidylcholine with an iodine value of 3%. Triglyceride (Witepsol H-15) was supplied by Dynamit Nobel Chemicals (NJ, USA). Other reagents used were of analytical grade.

Preparation of the aqueous-lipid vehicle: Three kind of aqueous lipid vehicles were prepared according to the method described previously (8). Codes and compositions

are listed in Table 1. Briefly, water (80°C) was added to other ingredients and agitated vigorously by a propeller for 5 min at 80°C. The mixture was cooled to room temperature with continued agitation to obtain the viscous vehicle.

To investigate the transport of each drug from solution across rat dorsal skin and shed snake skin, a buffered solution (0.05 M isotonic phosphate buffer at pH 7.2) or an ethanol-water solution of each drug was prepared.

In vitro transport study: Male wistar rats, 200 to 250 g, were used. The dorsal hair of the rats was shaved with electric clipper. The dorsal akin was excised just before an experiment as described previously, except that the excised skin included subcutaneous tissue (7).

Shed snake skin of a black rat snake which was supplied by the Animal Care Unit of The University of Kansas, was used as a model stratum corneum. The snake skin was stored at -20°C prior to use. Before experiments were conducted, the shed snake skin was rinsed with deionized water at 40°C for 30 min and then sonicated for 3 seconds.

In the transport study, Franz type diffusion cells which were described previously (10) were employed. Isotonic phosphate buffer (0.05 M, pH 7.2) was used as the receptor medium. The volume was 10 ml. During experiments, the receptor medium was maintained at 35°C with a water jacket. Following the application of each formulation on the donor side, 50 ul of the medium in the receptor were collected at regular intervals and 50 ul of the buffer was added to the receptor immediately after each collection.

Solubility determination: The apparent solubilities of the drugs in aqueous solution were determined at 25°C by the following method: 10 ml of solution (described in

TABLE 2

The solubility (mg/ml) of the anti-inflammatory agents in various solvent system at 25°C.

Solvent	Ketoprofen	Sodium Diclofenac	Indomethacin
		mg/ml	
0.1 M phosphate buffer (pH 7.2)	1.32	8.01	0.67
distilled water	0.14	7.82	0.012
+ 25% w/w ethanol	5.92	56.74	1.10
+ 35% w/w ethanol	26.21	159.25	6.10
+ 1% w/w phospholipid	0.82	20.24	0.18

Table 2) was added to the test tube containing 3.0 g of sodium diclofenac or 1.5 g of other drugs. At 36 h, the sample solution was obtained through millipore filter (pore size of 0.20 um) after centrifugation at 3,000 rpm for 10 min, and the concentration of drug in the solution was determined. When a solution containing phospholipid was used, the solution was prepared as follows: the mixture of the phospholipid and distilled water was agitated for 10 min at 80°C, and was cooled to room temperature gradually with agitation.

Assays: The assays of the drug were carried out by a HPLC method as follows: The separation column was 4.6 mm i.d. x 15 cm in length and contained a reverse-phase column material (RP-18). The mobile phase used was a mixture of acetonitrile and 0.05 M phosphate buffer (pH 3.0) at a volume ratio of 45:55 for ketoprofen, and 60:40 for indomethacin and diclofenac. The flow rate was 0.8 ml/min. Ketoprofen and indomethacin were measured using a UV detector at 265 nm, and diclofenac was detected at 276 nm. Retention time was 3.6 min for ketoprofen, 4.1 min for diclofenac, and 4.8 min for indomethacin.

TABLE 3

Parameters for in vitro transport of drug across rat dorsal skin (including subcutaneous tissue) and across shed snake skin when 2 ml of the drug solution at a concentration of 200 ug/ml was placed on the donor side of the membrane.

	Rat dorsal skin		Shed snake skin	
	Le min	dQ/dt ($x10^{-3}$) µg/ml	Le min	dQ/dt ($x10^{-3}$) µg/ml
Ketoprofen				
buffer[a]	127±29	9.33±1.94	220±18	3.89±0.83[d]
25% ethanol	97±42	19.42±2.61[c]	99±24	7.27±1.02[c,d]
Sodium Diclofenac				
buffer[a]	217±36	3.25±0.82	404±66	0.76±0.21[d]
25% ethanol	221±42	3.45±0.41	429±52	0.92±0.20[d]
Indomethacin				
buffer[a]	209±27	3.25±0.61	396±41	0.71±0.26[d]
25% ethanol	212±36	6.67±0.99	251±27	2.87±0.32[d]

[a]donor solution was prepared with 0.05 M isotonic phosphate buffer; [b]donor solution was prepared with 25% ethanol solution diluted with distilled water; [c]$p<0.01$ versus results for buffer; [d]$p<0.01$ versus results of rat dorsal skin. Each value represents the mean ± S.D. (n=4 to 5).

Statistical analyses: Statistical analyses were carried out by using a Student's t-test.

RESULTS AND DISCUSSION

Transport of the anti-inflammatory agents from solution across rat dorsal skin and shed snake skin.

The transport of the three anti-inflammatory agents from a donor solution across rat dorsal skin or shed snake skin was examined using either buffer or 25% ethanol in distilled water solution containing drug at a concentration of 200 ug/ml in 2 ml of donor solution.

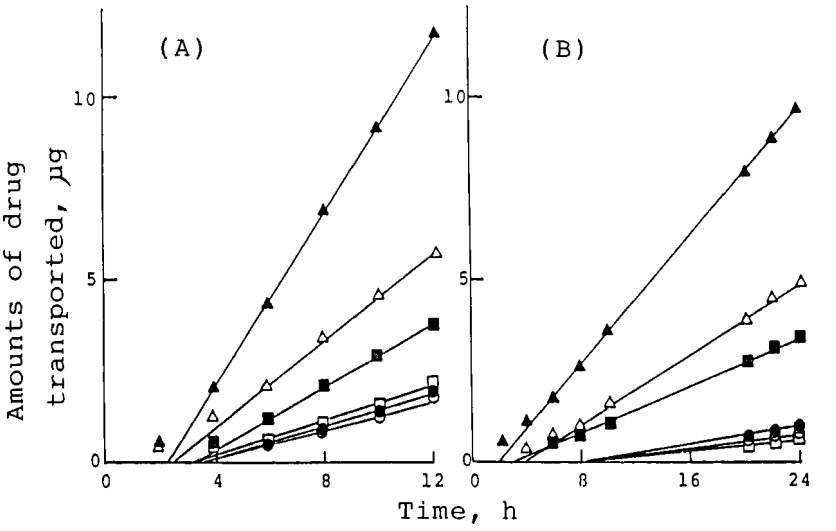

FIG. 1

Transport profile of various anti-inflammatory agents across (A) rat dorsal skin and (B) shed snake skin after the application of 2 ml of the donor solution containing 200 ug of drug/ml.

Keys: open symbols for the buffered solution and closed symbols for the 25% ethanol solution; and for ketoprofen; and for sodium diclofenac; and for indomethacin. Each value represents the mean (n=4 to 5).

The hydro-alcoholic solutions were used because of the low solubility of ketoprofen and indomethacin in distilled water, as shown in Table 2. After a lag time (Le, min in Table 3), steady state transport of the drugs were observed following the application of either the buffer or 25% ethanol solution (Fig. 1). The transport rate (dQ/dt in Table 3) of the drugs at steady state was in the following order: ketoprofen>indomethacin>sodium diclofenac.

The transport rate of drugs across rat dorsal skin compared with that across shed snake skin was about 2.5

times greater for ketoprofen about four times for sodium diclofenac and about two times (ethanol solution) or five times (buffered solution) for indomethacin (Table 3). Thus, in terms of the permeability of the membrane for the three anti-inflammatory agents used, shed snake skin exhibited a greater barrier function than did rat dorsal skin. The apparent greater permeability of rat dorsal skin to these drugs may be due to the presence of follicles as well as differences in the thickness and/or composition of the stratum corneum.

The transport rates of ketoprofen and indomethacin from the 25% ethanol solution was two times or more greater than the transport rates from the buffer solution. However, no significant differences were observed for the transport rate of sodium diclofenac between 25% ethanol solution and the buffer (Table 3). The differences in the transport rates of ketoprofen or indomethacin seem to reflect differences in the transport rate on the unionized form and the ionized form, because either drug is presence almost completely in the ionized form in the buffer. No significant change in lag time, Le, was observed for any of drugs examined when the ethanol solution was applied (Fig. 1 and Table 3). However, as mentioned below, a significant reduction in the lag time for the transport of these drugs was observed when 35% ethanol solution were applied.

Since the thickness of shed snake skin which was used in the presence study, has been investigated (11), effective skin permeability, Pe cm/min, which is presented in equation 1 and 2, was calculated.

$$(1) \qquad Pe = (dQ/dt)/(A\ Cd(0)) \qquad (2) \qquad Pe = (K\ D)/h \qquad (3) \qquad Pe = h^2/(6\ D)$$

Where A = the surface area exposed to receptor solution, 1.8 cm^2); Cd(0) = the initial drug concentration in the

TABLE 4

Parameters for drug transport across shed snake skin.

Drug	D[a] ($\times 10^{-9}$, cm^2/min)	Pe[b] ($\times 10^{-5}$, cm/m)	K[c]
ketoprofen			
buffer	7.09±1.36	1.08±0.24	3.0
25% ethanol	6.37±1.61	2.02±0.29	6.3
Sodium Diclofenac			
buffer	1.65±0.12	0.21±0.06	2.5
25% ethanol	1.55±0.44	0.25±0.08	3.2
Indomethacin			
buffer	1.68±0.29	0.20±0.07	2.3
25% ethanol	2.66±0.28	0.80±0.09	6.0

[a]Diffusion coefficient; [b]Effective permeability; [c]Donor solution to snake skin partition coefficient. Each value represents the mean ± S.D. (n=5).

donor solution, ug/cm^3; dQ/dt = the appearance rate of the drug in the receptor solution at steady state; D = the diffusion coefficient; K = the partition coefficient to snake skin tissue; h = the thickness of snake skin (0.0015 to 0.002 cm, reference (11); 0.002 cm was used for the calculation in the present study). Pe and D were calculated from equation 1 and 3, respectively, and the K value was calculated from equation 2. Pe, D, and K for each drugs are summarized in Table 4.

Among the drugs examined, ketoprofen showed the greatest value of both D and Pe (Table 4). In the present study of drug transport from buffer solution, the Pe value for ketoprofen was about 5 times greater than those for sodium diclofenac and indomethacin. For the study with 25% w/w ethanol solution, Pe for ketoprofen was 10 times greater than that for diclofenac. The Pe value of ketoprofen from the buffer solution across shed snake skin was 10 times smaller than the recently obtained value for ethylparaben (Nishihata et al., unpublished

data). It seems to be that the transport of these anti-inflammatory agents is very small in comparison to that of ethylparaben. Thus, it is necessary to make formulation modification to increase the transport of these drugs for practical use.

Effect of aqueous-lipid vehicles on drug transport across rat dorsal skin and shed snake skin.

The aqueous-lipid vehicle formulations listed in Table 1 were examined as practical transdermal formulations. The formulation listed as Code-I includes sodium diclofenac and has been reported previously (8). Each drug was dissolved in a solution once during preparation on the formulation by warming to 80oC for 5 min with strong agitation. It is clear that the presence of phospholipid in the formulation increased the solubility of each of the drugs tested at room temperature (25°C) (Table 2), but it is not clear how much of drug precipitates after reaching room temperature. Only sodium diclofenac can one expect complete dissolution in the formulations based on the results in Table 2. Thus, in the present study, all formulations generally were examined at seven days after the preparation of the vehicle. Further, for the formulation of sodium diclofenac (Code-III), the evaluation was performed at one week intervals for six weeks after the preparation. As a control formulation, the 35% w/w ethanol solution containing 1% w/w of drug was used (Code-IV).

As shown in Fig. 2A to 2D, substantial transport of ketoprofen and sodium diclofenac from each formulation across either rat dorsal skin and shed snake skin was observed when formulation Code-I was applied. Greater transport of both dugs across rat dorsal skin was

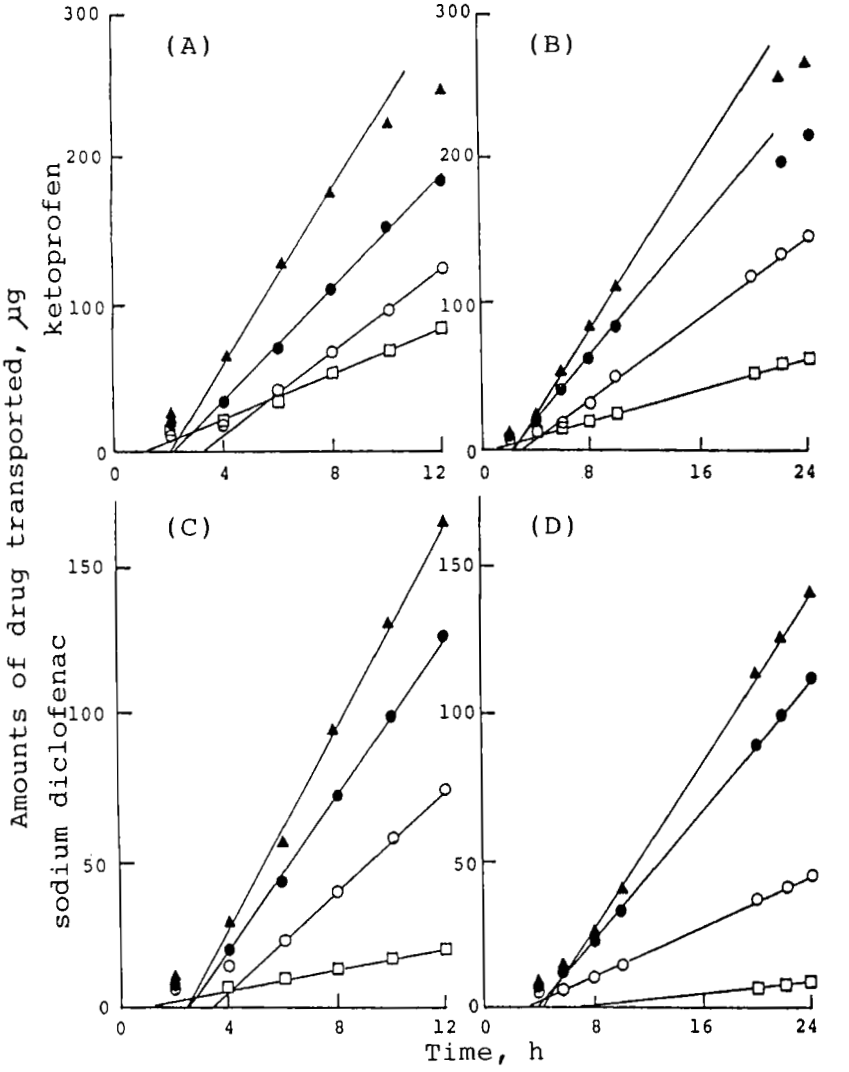

FIG. 2.

Transport profiles of ketoprofen (A and B) and sodium diclofenac (C and D) from various vehicles across (A and C) rat dorsal skin and (Band D) shed snake skin. Dose of vehicle was 50 mg containing 500 µg of sodium diclofenac. for Code-I; for Code-II; for Code-III; for Code-IV. Each value represents the mean (n= 4 to 5).

observed than that across shed snake skin. Among the formulations, Code-IV resulted in a smallest value of Le.

The transport of sodium diclofenac and ketoprofen from Code-I was markedly greater than from Code-IV (Table 5). The presence of tetradecanol in the formulation (Code-II) increased the transport of three drugs, compared to Code-I and -IV. The further addition of urea (Code-III) accelerated the transport of all of the drugs. About 50% transport of ketoprofen was observed within 12 h for rat dorsal skin and within 24 h for shed snake skin (Table 5).

With respect to the transport of sodium diclofenac, about 30% transport was observed within the above periods. However, less transport of indomethacin from Code-I was observed compared to that of ketoprofen and sodium diclofenac (Fig. 3 and Table 5). The transport of indomethacin from Code-II and -III were greater than that from Code-I across either rat dorsal skin and shed snake skin, but the amount transported was only about 10%. For shed snake skin, the increase in dQ/dt from Code-III was about two times for the indomethacin, about four times for ketoprofen and more than ten times for sodium diclofenac. This small increase in indomethacin transport may be due to the relatively low solubility of indomethacin in the formulation, as mentioned earlier (Table 2). Thus, the effect of concentration in the Code-I formulation was investigated. For this purpose, the transport of indomethacin from formulation Code-I containing various amounts of indomethacin across shed snake skin was compared to that of sodium diclofenac, because complete dissolution of sodium diclofenac is expected in the formulation as described above.

With respect to sodium diclofenac, concentration changes from 0.1% w/w to 1.0% w/w in the formulation of Code-1 did not cause a change in the lag time (Le), the

TABLE 5

Transport parameters of drugs from the aqueous-lipid vehicle containing 500 μg of each drug[a] across either rat dorsal skin or shed snake skin.

Code	Le	dQ/dt ($x10^{-2}$, μg/min)	Percent transported[b]
[1] Rat dorsal skin			
Ketoprofen			
I	227±35[c]	22.2±3.7[d]	23.8±3.1
II	130±26[d]	32.4±4.1[c]	36.4±4.4[c]
III	122±42	48.6±4.2[c]	49.6±5.9[c]
IV	64±12	12.9±1.8	18.1±4.2
Sodium Diclofenac			
I	204±32[c]	13.7±2.4[d]	14.7±3.6[d]
II	141±31[d]	23.2±3.1[c]	26.2±3.1[c]
III	138±27[d]	30.6±2.9[c]	34.1±5.3[c]
IV	49±24	6.1±1.2	7.7±1.0
Indomethacin			
I	231±29[c]	5.7±1.1	6.7±1.3
II	164±29[d]	9.4±1.4[d]	9.5±0.8[d]
III	172±32[d]	10.1±1.9[d]	11.8±1.1[d]
IV	69±17	5.1±1.2	6.5±0/7
[2] Shed snake skin			
Ketoprofen			
I	174±29[c]	11.3±1.6[d]	29.1±3.7[c]
II	185±42[d]	19.2±1.6[c]	44.9±6.2[c]
III	151±27[c]	24.9±3.2[c]	54.1±4.6[c]
IV	81±24	5.1±1.2	13.2±1.7
Sodium Diclofenac			
I	238±36[c]	3.5±0.7[c]	8.4±1.2[c]
II	222±31[c]	8.7±1.6[c]	21.9±3.1[c]
III	251±47[c]	11.6±1.2[c]	27.7±4.6[c]
IV	81±24	0.8±0.2	1.5±0.2
Indomethacin			
I	209±42[d]	2.0±0.7	4.1±0.8
II	267±64[c]	4.0±0.5[d]	9.6±1.2[d]
III	251±47[c]	4.2±0.7[d]	10.1±1.1[d]
IV	108±19	2.7±0.4	6.1±0.7

[a]50 mg of formulation was applied on the donor side of the membrane; [b]percent transported at 12 h for rat dorsal skin and at 24 h for shed snake skin. Each value represents the mean ± S.D. (n=4 to 5). [c]$p<0.01$ versus Code-IV; [d]$p<0.05$ versus Code-IV.

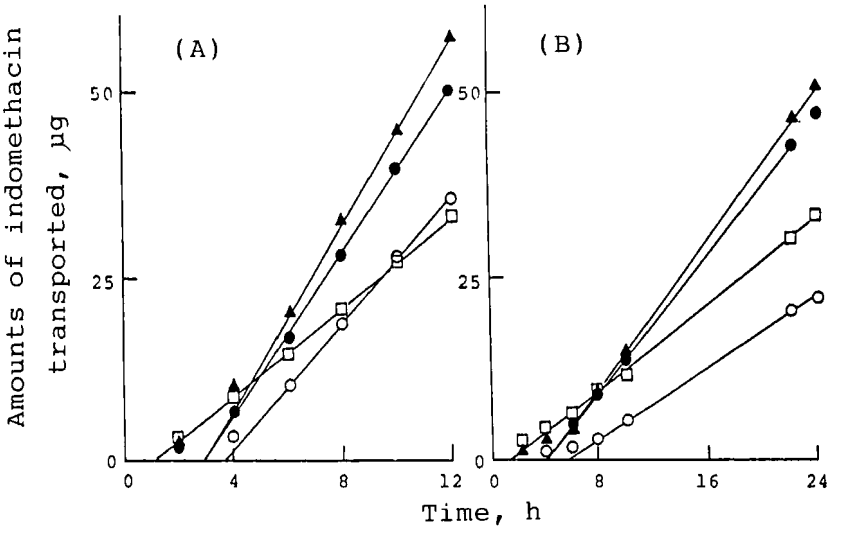

FIG. 3.

Transport profiles of indomethacin from various vehicles across (A) rat dorsal skin and (B) shed snake skin. Dose of vehicle was 50 mg containing 500 µg of indomethacin. for Code-I; for Code-II; for Code-III; for Code-IV.
Each value represents the mean (n=4 to 5).

TABLE 6

The effect of the concentration of indomethacin or sodium diclofenac in formulations of Code-I[a] on the transport across shed snake skin.

Drug content in formulation %w/w	Le min	dQ/dt $x10^{-2}$ug/min	dQ/dt content $x10^{-4}$/min	Percent transported at 24 h
Sodium DIclofenac				
0.1	226±42	0.32±0.06	0.64	8.0±1.6
0.5	224±37	1.73±0.21	0.67	8.3±1.4
1.0	238±36	3.56±0.72	0.71	8.4±1.2
Indomethacin				
0.1	272±64	0.71±0.21	1.42	18.1±3.6
0.5	285±56	1.60±0.42	0.64	7.9±1.1
1.0	309±42	2.01±0.31	0.40	4.1±0.8

Each value represents the mean ± S.D. (n=4).

ratio of dQ/dt/content, or percent of drug transported at 24 h in the study using shed snake skin (Table 6). However, a decrease in the content of indomethacin significantly increased the ratio of dQ/dt/content and the percent of drug transported after 24 h (Table 6). These results suggest that solubility of the drug in the formulation is an important factor. Thus, effect of tetradecanol in the formulation on the transport of indomethacin may involve an increase in indomethacin solubility in the Code-II and -III formulations in comparison to that in Code-I.

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