The Thai Journal of Pharmaceutical Sciences

Volume 20
Issue 3 *1996*

Article 3

1-1-1996

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Thai J. Pharm. Sci., 1996, Vol. 20, No. 3, pp 159-169

Research Articles

Development of Skin Stripping Technique to Evaluate *In Vivo* Percutaneous Absorption of Diclofenac. I. Effects of Skin Stripping Sequence, Occlusion Time, and Sites of Application

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ABSTRACT: Several methods are available for the evaluation of percutaneous absorption of topical drug formulations. The most commonly used in vivo methods are the determination of drug level in plasma and measurements of pharmacological and/or therapeutic responses. However, these techniques are cost and time-consuming, may involve animal experiments, and often give variable results. Furthermore, measurement of plasma drug level may not necessarily represents the amount of drug that exerts its action in the subdermal tissues like joints and muscles. In vitro techniques, although more reproducible and easier to carry out, may not correlate with the vivo results. The aim of this study was therefore to develop a technique, based on the measurement of the drug content in the tape-stripped stratum corneum, to evaluate in vivo percutaneous absorption of diclofenac diethylammonium, a model NSAID, from a gel formulation. This report is the first part of the study in which the effects of skin stripping sequence, occlusion time and different application sites were investigated to determine the optimum experimental conditions. The optimum occlusion time for diclofenac gel was found to be 3 hr. Ten strippings per spot was adequate to remove most of the stratum corneum from the applied area, with the first two strips being discarded as they contained the residual, unabsorbed gel. Also, no significant difference was observed regarding the effect of minor variation in the application sites on the amount of diclofenac found in the tape-stripped stratum corneum. Analysis of the drug content in this skin layer thus directly yielded the amount of drug that has been released and penetrated the stratum corneum. In summary, the skin stripping procedure developed in this study was found to be simple, rapid, and may have potential as an alternative tool to evaluate percutaneous absorption of topical NSAID preparations like diclofenac diethylammonium. Further studies are being carried out to employ this technique to determine topical bioavailability of different diclofenac gels and to correlate the results with the in vitro experiments.

KEY WORDS: Skin stripping; diclofenac diethylammonium gel; percutaneous absorption; topical bioavailability; stratum corneum.

NTRODUCTION

Evaluation of percutaneous absorption of topical oducts applid to the skin is a difficult task. If the pro-cts are intended to produce systemic effect after skin plication, determination of the drug concentration in the stemic circulation may provide the most accurate method for evaluation of their in vivo permeation. However, this method is very cost- and time-consuming and it could be extremely difficult to determine the very low level of the transdermally absorbed drug in plasma. Evaluation of their pharmacological action or clinical effectiveness such as the skin blanching effect produced by topical corticosteroid can also be of value. Nevertheless, measurements of pharma-

cological activity can be highly subjective unless quantitative parameters are available (1). Assessment of clinical effectiveness of topical products in patients, although providing direct measurement of their therapeutic merits, is very difficult to control and may be even more expensive than the plasma drug analysis (2).

Alternative to the above in vivo techniques are the use of various membranes for the in vitro evaluation of drug release and percutaneous absorption. Theoretically, excised human skin is the best membrane for the in vitro permeation study (3). However, problems associated with human skin are difficulties in obtaining and handling the specimens as well as variation in the skin thickness and composition due to differences in race, body site, age and sex (4, 5). Several animal skins from different species such as rat, rabbit, pig and snake have also been used in place of the human skin (6, 7). Since these membranes are more readily available, less expensive and provide many characteristics similar to the human skin, they are often used in the in vitro permeation experiments and the results are then extrapolated to human (8).

However, there is as yet no animal skin that completely mimics the penetration characteristics of the human skin. For example, rat and pig skins were reported to be more permeable to many drugs than the human cadaver skin (9) whereas the shed snake skin is less permeable (7). Alternatively, artificial membranes can also be used in the in vitro diffusion study. They are convenient to handle, easy for use and give little variation. Nevertheless, their validity strongly depends on the correlation with the data from the animal or human skin (10).

Another technique to evaluate in vivo percutaneous absorption is to measure the drug content in the upper skin layer such as the stratum corneum (11). The technique has been applied to study the skin absorption of hydrocortisone (12) and betamethasone dipropionate (13) by measuring the steroid content in the tape-stripped stratum corneum following topical application of the cream or ointment to human subjects. The amount of steroids remaining in the upper skin layer was found to significantly correlate with the standard pharmacological response (skin blanching effect). The technique thus appears to have potential as an alternative method to evaluate topical bioavailability of the two steroids.

Despite the advantages of being simple, rapid and not involving plasma drug analysis, studies to apply this technique to other groups of topical drugs such as the non-steroidal anti-inflammatory drugs (NSAIDs) have been limited. Diclofenac diethylammonium was selected in this study as a model NSAID because of its popularity as a topical agent for the relief of common musculoskeletal injuries. Riess et al (14) have shown that topically applied diclofenac can penetrate into muscles and joints with only 6 percent of the dose being systemically absorbed. They also found that topical application of diclofenac over an inflamed joint resulted in the drug concentrations in the synovial fluid and synovial tissue greater than in the plasma. Thus, measurement of plasma diclofenac level may not be very relevant and other techniques which allow direct determination of the drug content in the target tissue or in the area close to the target (e.g. in the skin) may better characterize its in vivo percutaneous absorption.

The objectives of this study were therefore to develop an in vivo technique, based on the skin stripping procedure, to characterize the percutaneous absorption of diclofenac from the gel preparations and to apply this technique to compare the topical bioavailability among different formulations. This report is the first part of the study in which the effects of skin stripping sequence, occlusion time, and application sites on the drug content in the stratum corneum were investigated to determine the optimum experimental conditions.

MATERIALS AND METHODS

Chemicals

Diclofenac diethylammonium was a gift from Siam Pharmaceutical Co., Ltd. whereas phenylbutazone (used as the internal standard) was donated by S. Pharma Co., Ltd. A commercial diclofenac diethylammonium gel (Lot number 283; Manufacture date 30/12/93) was purchased from a local drugstore. Each 100 mg of the gel contains 1.16 mg diclofenac diethylammonium equivalent to 1 mg diclofenac. All other chemicals and reagents were analytical or HPLC grade.

Determination of Optimum Conditions for Skin Stripping Experiments

The purposes of this part of the study were to determine the optimum experimental conditions with respect to the appropriate skin stripping sequence and the optimum

occlusion time after drug application as well as the application sites. The protocol has been approved by the committee on graduate studies of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Determination of Appropriate Skin Stripping Sequence

Three healthy subjects, all female and aged between 25-30 years, volunteered to participate in this study. All of them did not take diclofenac or any other drugs, orally or topically, at least one week before entering the study. By means of a paper template, the area of their left mid-forearms was marked by pen into corresponding nine small squares, each having 1x1 cm^2 dimension. These squares are called the application spots on which the product was applied. The 3x3 arrangement of the nine application spots is shown in Figure 1, each vertical row being separated by a distance of 1.5 cm.

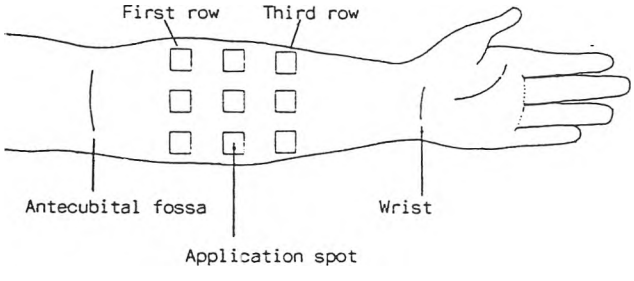

Figure 1 Diagram showing 3x3 arrangement of application spots. Each spot had a dimension of 1x1 cm^2 square. Each vertical row contained 3 spots and was separated by a distance of 1.5 cm.

The constant but excessive dose (100 mg) of the commercial diclofenac diethylammonium gel was subsequently smeared on each of the designated nine spots using a small spatula. Care was taken to make sure that the same amount of the gel was applied to each spot and that the gel contained within the spot boundary. All the spots were then occluded with Tegaderm^R tape (3M, St. Paul, Minnesota, USA) for different periods of time depending on their position in the 3x3 arrangement. Such the arrangement (Figure 1) allowed the application spots to be divided into three parallel rows, namely, upper, middle and lower rows, each containing 3 consecutive spots. The occlusion period after drug application was arbitrarily set at 1, 3 and 6 hr and was randomly assigned to each row. The purpose for the random assignment of the occlusion period was to balance out any difference due to the row effect so that the effect of the occlusion time, if existed, could be clearly seen. After the specified occlusion period, the Tegaderm^R coverings were removed from each row and any residual gel was gently wiped out three times from the surface of each spot using cotton buds. After removal of the excess gel, the skin stripping process was then started.

With the use of small forceps, ten pieces (or strips) of Transpore^R tape (3M, St. Paul, Minnesota, USA), cut to the same size as the application spots (1x1 cm^2), was consecutively placed on each of the application spots. After placing the first piece, a steady pressure was applied to the spot by means of a small roller. After a single roll, the tape was removed from the application spot and placed in a 13x100 mm culture tube. This would complete one stripping per spot. Each piece of tape was used to strip the skin only once. After the first stripping, the same procedure was repeated nine more times on each application spot. Previous experiments have shown that ten strippings per spot were adequate to remove most of the stratum corneum (14).

To determine the appropriate number of the sequential tape strips to be used in the actual drug analysis, the ten strips were separately analyzed for diclofenac content by pairing together the first and second strips, third and fourth, fifth and sixth, seventh and eighth, and the ninth and tenth strips, making a total of five pairs per spot. Therefore, during the skin stripping of each spot, five culture tubes were needed to keep the respective five pairs of tape strips. Analysis of the drug in each of the sequential pairs would allow one to determine the appropriate number of tape strips that would be combined for accurate analysis of diclofenac in the stratum corneum during the next phase of the study. An HPLC technique was used in all the skin stripping studies to analyze the amount of drug in the tape-stripped stratum corneum. Briefly, each pair of tape strips was immersed in 2.0 ml of the mobile phase containing 2 μg/ml of phenybutazone as the internal standard. After vortexing for one minute, the mixture was centrifuged at 3,000 rpm for 5 minutes. The clear supernatant was then injected onto the HPLC. Details of the analytical conditions have been described by Samitamarn (15).

The amount of diclofenac diethylammonium found in each pair (average of three spots in the same row) was then plotted against the sequential pair number (No. 1 to 5) for each subjest and at each occlusion time. The reason for making these plots was to see if there was any excess drug

that still remained on top of the stratum corneum. If there existed, analysis of the drug in the first few strips should give the drug content much higher than other subsequent strips. As a result, these initial strips should not be used for analysis since the drug found would not represent the amount of drug that has penetrated the stratum corneum.

Determination of Optimum Occlusion Time

After identifying the number of the first few strips that removed the unabsorbed drug from the skin surface, the amount of diclofenac in the remaining tape strips was then combined for each spot. Since each row represented different occlusion time (either 1, 3 or 6 hr) and each row also contained three identical spots, comparison of the average values among the three rows within an individual subject should give some idea about the effect of different occlusion times on the amount of drug found in the tape-stripped stratum corneum.

Effect of Difference in Application Sites

The next phase of study constituted the determination of the effect of different application sites on the amount of drug found in the stratum corneum, i.e. whether there were any differences due to the row effect within the same forearm or any differences between the left and right forearms of the same subject.

This study was carried out using one healthy volunteer who had not taken diclofenac or any other oral and/or topical drugs for at least one week before entering the study. Both the subject's left and right forearms were used for the gel application. However, the arrangement of the application spots in each forearm was slightly different from the previous experiment in that there were five parallel rows instead of three. Each row contained three spots and was separated by a distance of 1 cm as shown in Figure 2. Thus, a total of fifteen spots were marked on each left and right forearms. The first row was about 3 cm below the antecubital fossa and the fifth (lowest) row was about 3 cm above the wrist. Each square-shaped spot had a similar 1x1 cm^2 dimension. Marking of the application spots was also facilitated by means of a special paper template correspondingly cut to contain fifteen square-shaped holes in a 5x3 arrangement. About 100 mg of the commercial gel was carefully applied on each spot. When the application was completed, all the spots were occluded with Tegaderm^R tapes. Following occlusion for a fixed period of time (the exact occlusion time to be determined from the previc experiment), the surface of each spot was cleaned with c ton buds and all the spots were individually stripped w ten consecutive pieces of Transpore^R tapes. After disca ing the first few strips (the exact number to be discarc was also determined from the previous experiment), remaining strips were combined together for each spot subsequent HPLC analyses. By comparing the avera amount of diclofenac found in each of the five rows, effect of different rows within the same forearm could delineated following the analysis of variance (ANOVA 5% significance level. In addition, student's t-test, alsc 5% level, was applied to determine if there was any diff ence in the average amount of diclofenac content found the stratum corneum between the left and the right forear of this subject.

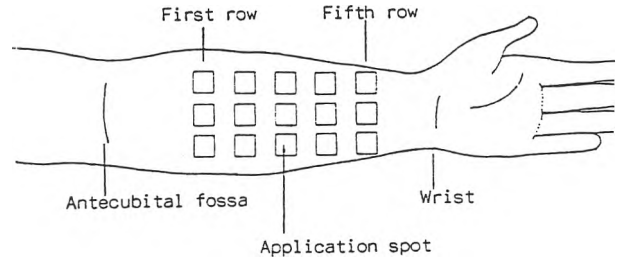

Figure 2 Diagram showing 5x3 arrangement of applica spots. Each spot has a dimension of 1x1 square. Each vertical row contains 3 spots is separated by a distance of 1 cm.

RESULTS AND DISCUSSION

Development of Skin Stripping Procedure Evaluate Percutaneous Absorption

There are many factors that need to be contrc during the in vivo skin stripping studies. Application is one factor which may greatly influence the extent of c release from the gel base as well as the amount pe taneously absorbed. In addition, since the application a were divided into several spots of the same dimension, ferences in the position (row) of these spots on the fore as well as variation in the pressure applied during strip may cause fluctuation in the amount of stratum corr being removed and the resultant differences in the am of diclofenac found in the tape strips. Therefore, the periments must be appropriately designed to balance these effects, if they did exist.

Determination of Appropriate Skin Stripping Sequence

Figures 3-5 depict the plots of average amount of diclofenac diethylammonium found in each pair of tape strips after 1, 3 and 6 hr application of the commercial gel product to the left forearms of the respective three volunteers. Representative numerical data are also shown in Table 1 for subject no.1. It can be seen from these figures that the highest amount of diclofenac was always detected in the first pair of tape strips irrespective of the subject and the occlusion time. This finding indicates that there was still some excess diclofenac gel remaining on top of the skin surface which could not be wiped off by the cotton buds. However, this unabsorbed, residual amount was substantially removed from the skin surface during the first two strippings. The data were in agreement with the results of Pershing et al (13) who reported that the first two strippings contained the excess amount of betamethasone dipropionate which did not pass into the stratum corneum. As a result, the drug found in the first pair of tape strips may not represent the amount of diclofenac actually penetrating the stratum corneum and was considered to be the unabsorbed drug which remained on the skin surface. Consequently, the first two strips were always discarded from the analysis. Only the remaining eight strips were combined for the total assay of diclofenac in the subsequent experiments.

Also, it can be noticed that the amount of diclofenac found in each pair of the later strips tended to decrease gradually, although not as sharply as observed between the first and second pairs (Figures 3-5). Caron et al (12) explained that this could be due to the less tightly packed nature of the upper layers of the stratum corneum which may have taken the drug to a greater extent than the more dense, deeper layers, resulting in the analysis of later strips giving lesser amount of drug.

Determination of Appropriate Occlusion Time

The same set of data were then reanalyzed by combining together the amounts of diclofenac found in strips number 3 to 10 for each of the spots in order to compare the effect of occlusion time. The data are shown in Table 2 and graphically represented in Figure 6 for each of the three subjects. It can be seen from this figure that the amount of diclofenac found in the stratum corneum of subjects no.1 and 3 was highest after 3 hr occlusion period. On the other hand, subject no.2 demonstrated highest amount of drug

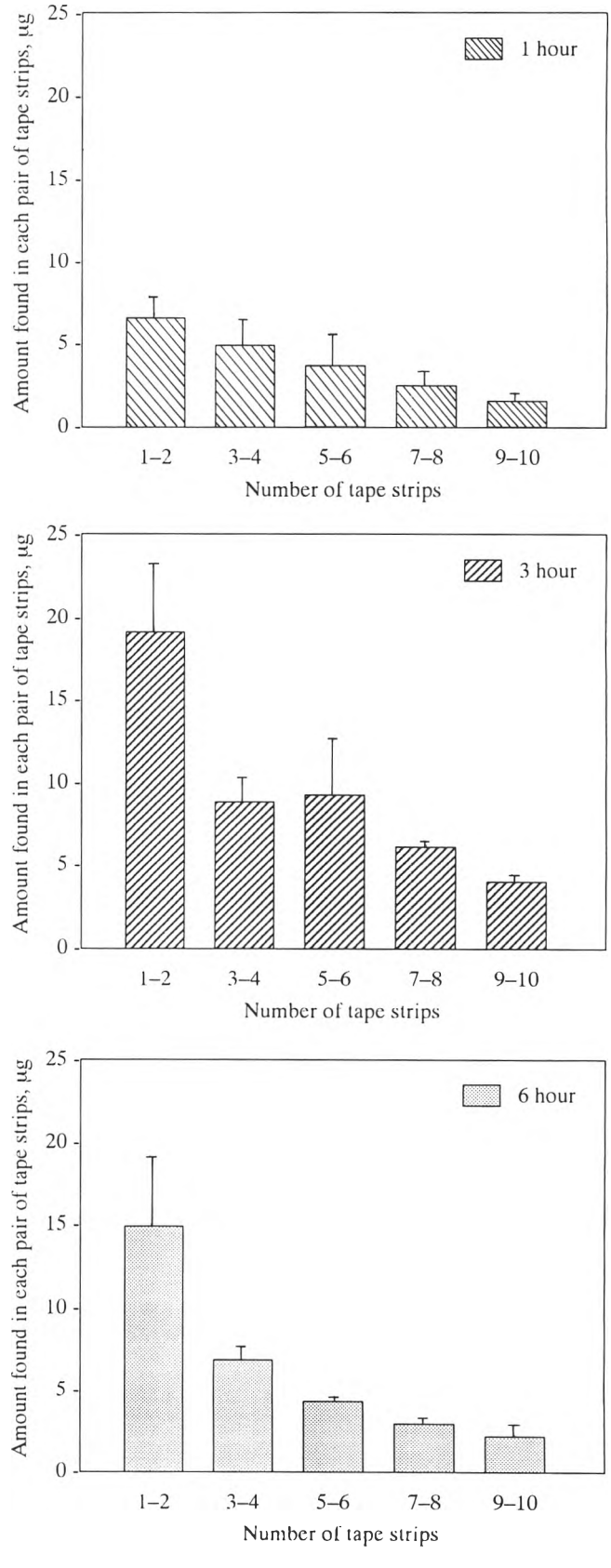

Figure 3 Preliminary data on the average amount of diclofenac diethylammonium found in each pair of tape strips after 1, 3 and 6 hr-occlusion: **Subject 1**

(Value = mean ± SD, n = 3 spots)

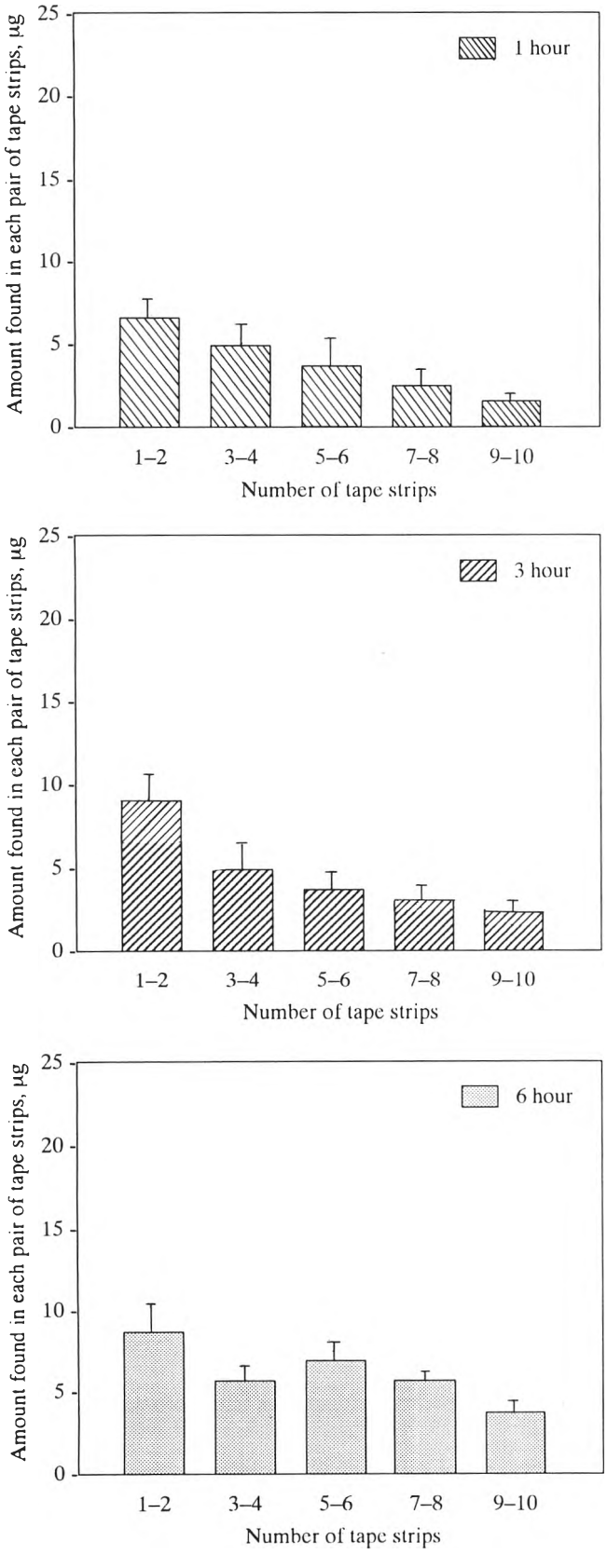

Figure 4 Preliminary data on the average amount of diclofenac diethylammonium found in each pair of tape strips after 1, 3 and 6 hr-occlusion : **Subject 2**

(Value = mean ± SD, n = 3 spots)

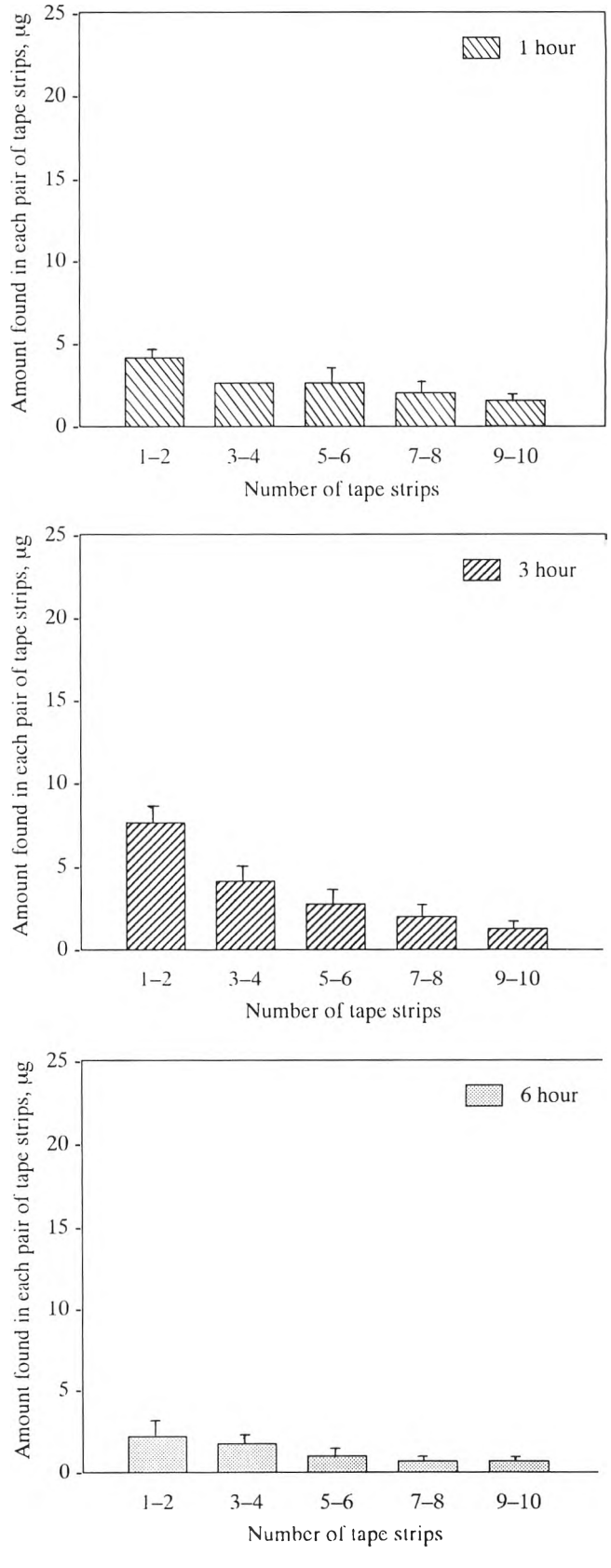

Figure 5 Preliminary data on the average amount c diclofenac diethylammonium found in each pa of tape strips after 1, 3 and 6 hr-occlusion **Subject 3**

(Value = mean ± SD, n = 3 spots)

Table 1 Preliminary results of skin stripping sequence (Amount of diclofenac found in each pair of tape strips in subject number 1).

Occlusion time = 1 hour

Strip Number / Spot Number	Strip 1+2 Amount (μg)	Strip 3+4 Amount (μg)	Strip 5+6 Amount (μg)	Strip 7+8 Amount (μg)	Strip 9+10 Amount (μg)
Spot 1	5.20	4.13	2.02	2.16	1.96
Spot 2	8.21	3.61	2.85	2.05	1.85
Spot 3	7.16	6.77	6.06	4.26	3.09
$\bar{X}$	6.86	4.84	3.64	2.82	2.30
SD	1.53	1.69	2.13	1.25	0.69

Occlusion time = 3 hours

Strip Number / Spot Number	Strip 1+2 Amount (μg)	Strip 3+4 Amount (μg)	Strip 5+6 Amount (μg)	Strip 7+8 Amount (μg)	Strip 9+10 Amount (μg)
Spot 1	18.22	7.15	5.62	4.63	3.03
Spot 2	15.53	8.94	8.98	5.52	4.06
Spot 3	23.65	10.36	12.89	5.05	3.28
$\bar{X}$	19.13	8.82	9.16	5.06	3.46
SD	4.14	1.61	3.64	0.45	0.54

Occlusion time = 6 hours

Strip Number / Spot Number	Strip 1+2 Amount (μg)	Strip 3+4 Amount (μg)	Strip 5+6 Amount (μg)	Strip 7+8 Amount (μg)	Strip 9+10 Amount (μg)
Spot 1	15.33	7.15	3.97	3.30	3.92
Spot 2	9.69	6.94	4.07	3.56	2.00
Spot 3	18.38	5.43	3.84	2.48	1.78
$\bar{X}$	14.47	6.51	3.96	3.11	2.57
SD	4.41	0.94	0.12	0.56	1.18

detected after 6 hr occlusion. Apparently, there should be an optimum application time for each drug which could result in the maximum drug release and penetration. Although the data in Table 2 show that subject no.2 gave maximum amount of diclofenac after occlusion for 6 hr (19.18±1.22 μg), the value at this time was not much higher than the value at 3 hr (15.68±4.25 μg). As a result, the occlusion time of 3 hr was chosen in all the subsequent experiments since it was considered to give the optimum amount of drug release and percutaneous absorption.

Effect of Difference in Application Sites

One volunteer participated in this study in order to see if there were any differences in the amount of diclofenac in the tape-stripped stratum corneum as a result of the difference in the application sites. Although the forearm was used as the application area in all the experiments, it was possible that different rows of spots even within the same forearm could have an influence on the amount of drug percutaneously absorbed. Likewise, the results obtained from the left and right forearms of the same subject may be different from each other. An experiment was thus designed to detect the significance of this minor variation in the application sites by means of statistical comparison of the diclofenac amount found in the tape-stripped stratum corneum from different spots, both within each forearm and between the left and right forearms of the same subject. Other factors such as the number of strippings per spot and the occlusion time were held constant by using the values obtained from the previous experiments, i.e. all the spots were occluded for 3 hr before stripping and each spot was stripped for 10 times, with the first two strips being discarded.

Table 3 shows the values of the amount of diclofenac found in the stratum coprneum removed from each of the fifteen spots located on the volunteer's right and left forearms. For the right forearm, the average amount found on each row (mean ± SD; n = 3 spots/row) ranged from 12.47±0.57 to 17.05±1.15 μg. Analysis of variance at 5% level revealed that there was no significant difference among the five rows with regard to the amount of diclofenac found in the stratum corneum (p-value > 0.05). The data from the left forearm of the same subject also demonstrated similar results (p-value > 0.05). This implies that, within the limited area of the forearm, application of the gel to and stripping the stratum corneum from the different rows of spots did not have a significant influence on the amount of

Table 2 Cumulative amount of diclofenac (strip 3-10) found in the tape-stripped stratum corneum in each of the three subjects for effect of occlusion time.

Subject 1

Occlusion time (hour) Spot No.	**1**	**3**	**6**
Spot 1	10.27	20.43	18.07
Spot 2	10.36	27.50	16.57
Spot 3	20.18	31.58	13.53
$\bar{X}$ (μg)	13.60	26.50	16.06
SD	5.70	5.64	2.31

Subject 2

Occlusion time (hour) Spot No.	**1**	**3**	**6**
Spot 1	12.48	12.85	17.81
Spot 2	12.39	13.61	20.15
Spot 3	13.26	20.57	19.58
$\bar{X}$ (μg)	12.71	15.68	19.18
SD	0.48	4.25	1.22

Subject 3

Occlusion time (hour) Spot No.	**1**	**3**	**6**
Spot 1	5.02	5.18	3.51
Spot 2	4.93	10.73	3.94
Spot 3	8.76	10.55	1.67
$\bar{X}$ (μg)	6.24	8.82	3.04
SD	2.19	3.15	1.21

diclofenac found in the tape strips, other factors being the same such as the occlusion time and the number of strippings per spot. Also, when the 15 spots were compared between the left and right forearms of this subject using Student's t test, there was no difference in the values of the diclofenac content in the tape-stripped stratum corneum (mean ± SD of right and left forearms = 14.72 ± 2.77 and 16.19 ± 3.36 μg; $p > 0.05$). It thus appears that, under similar experimental conditions, application of the gel to either the left or right forearm should yield the same results.

However, the results concluded here were obtained from only one subject. There was no guarantee that there would be no significant effect of application site in other subjects. To compensate for this uncertainty, particularly in the first experiment, the assignment of the occlusion time (1, 3 or 6 hr) to each row was always randomized so as to balance out the possible row effect. Furthermore, the pressure applied onto each spot may vary from stripping to stripping. This may cause fluctuation in the amount of the stratum corneum being removed after each stripping and thereby may result in the variation of the diclofenac content. The best way to correct for this variation is to normalize the amount of diclofenac found in each tape strip by dividing the weight of the drug with the weight of the stratum corneum removed from each stripping. This was not possible in our study, however, since it would need an ultrasensitive microbalance capable of detecting the weight difference as low as 0.1 μg. Furthermore, each tape strip must be weighed twice, i.e. before and after skin stripping, in order to obtain the weight of stratum corneum that has been removed from the skin. This would make the experiment extremely laborious. To compensate for this effect, a roller was always used to apply a steady pressure on the tape strip during each stripping. Also, since each spot had to be stripped for 10 times, the fluctuation in the amount of the stratum corneum being removed should somehow be balanced out over the entire stripping process.

The results from this part of experiments therefore provided an evidence that there was no difference in the percutaneous absorption of diclofenac due to minor differences in the application sites. Nevertheless, randomization of the application rows and the subject's forearm (left or right) during the drug application should be performed in all subsequent experiments to guarantee that, if there was any influence due to these factors, the effect would be randomly balanced.

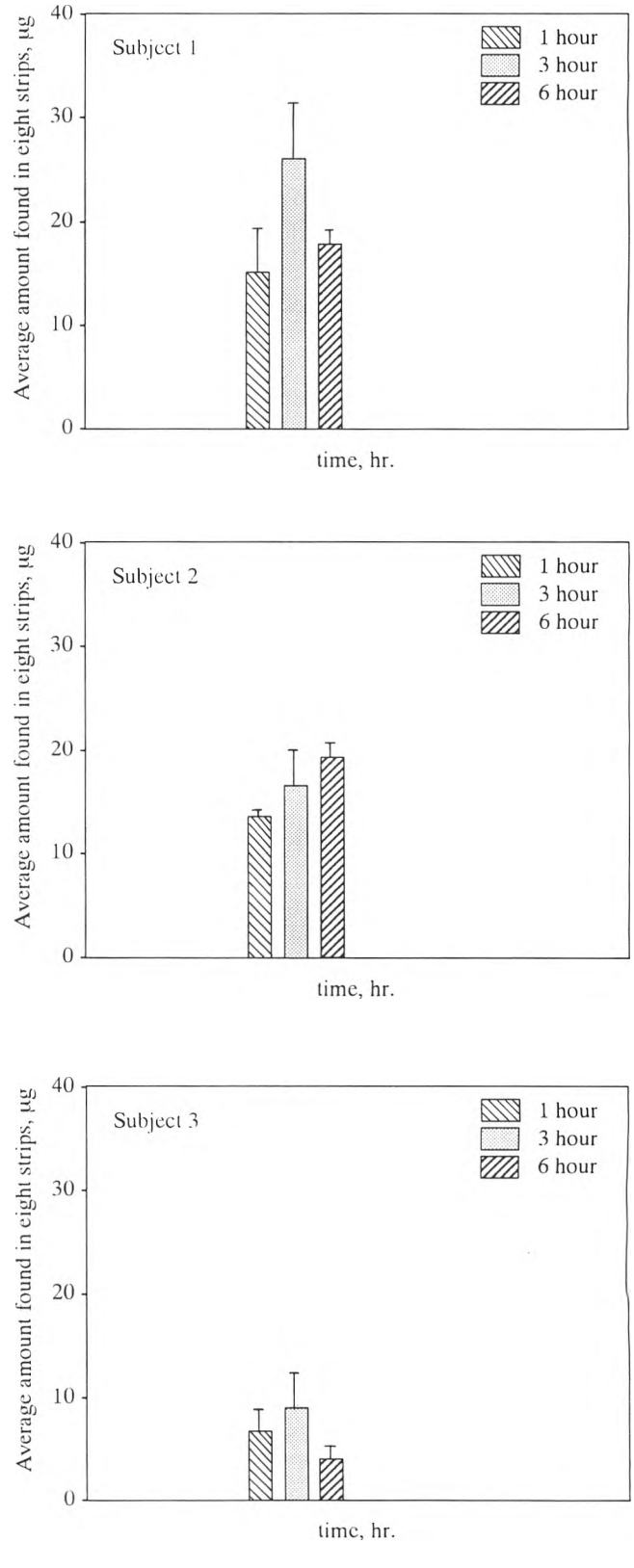

Figure 6 Effect of occlusion time on the amount of diclofenac found in the tape-stripped stratum corneum in each of three subjects
(Value = mean ± SD, n = 3 spots)

Table 3 Effects of application sites (row effect and left-vs-right forearm effect) on the amount of diclofenac diethylammonium (μg) found in the tape-stripped stratum corneum

Right forearm

Spot No.	Row 1 Amount (μg)	Row 2 Amount (μg)	Row 3 Amount (μg)	Row 4 Amount (μg)	Row 5 Amount (μg)
Spot 1	13.27	13.00	12.05	18.35	12.60
Spot 2	11.56	13.43	12.24	16.62	16.95
Spot 3	12.95	20.35	13.11	16.17	18.12
$\bar{X} \pm SD^a$	12.59 ± 0.91	15.59 ± 4.13	12.47 ± 0.57	17.05 ± 1.15	15.89 ± 2.91
Total mean[b] (n = 15)	14.72 ± 2.77 μg				

Left forearm

Spot No.	Row 1 Amount (μg)	Row 2 Amount (μg)	Row 3 Amount (μg)	Row 4 Amount (μg)	Row 5 Amount (μg)
Spot 1	19.74	19.94	20.31	15.94	12.94
Spot 2	19.00	12.40	10.83	12.85	12.81
Spot 3	20.63	18.02	17.79	14.10	15.60
$\bar{X} \pm SD^a$	19.79 ± 0.82	16.79 ± 3.92	16.31 ± 4.91	14.30 ± 1.55	13.78 ± 1.57
Total mean[b] (n = 15)	16.19 ± 3.36 μg				

a). There was no significant difference in the average amount of diclofenac diethylammonium found in the stratum corneum among the five application rows within the same forearm ($p > 0.05$).

b). There was no significant difference in the total average amount of diclofenac diethylammonium found in the stratum corneum between the right and left forearms of the same subject ($p > 0.05$).

CONCLUSION

In summary, the utilization of the skin stripping procedure appears to have a potential as an alternative tool to evaluate percutaneous absorption of topical NSAID preparations like diclofenac diethylammonium gel. The optimum occlusion time was found to be 3 hr for diclofenac and ten strippings per spot was adequate to remove most of the stratum corneum, with the first two strips being discarded as they contained the residual unabsorbed gel. Analysis of the drug in the tape-stripped stratum corneum could reflect the amount of drug that was released and permeated through the upper layers of the skin. Topical bioavilability thus can be estimated from the amount of drug found in the tape-stripped stratum corneum following specific period of drug application. The greater the amount of drug found in the stratum corneum, the higher the extent of drug release and penetration. This would, in turn, reflect the higher degree of percutaneous absorption and topical bioavailability. Further studies are being carried out to apply this technique to evaluate topical bioavailability of different diclofenac gel formulations and to correlate their results with the in vitro experiments.

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