MONITORING OF SKIN PENETRATION AND ABSORPTION WITH A NEW *IN VIVO* EXPERIMENTAL MODEL

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Abstract

The purpose of the study was to use a new type of *in vivo* animal model developed by our research group for the investigation of skin penetration, compared to the results of Franz cell measurements. The new *in vivo* experimental model provides possibility for the exact measurement of the quantity of the penetrated drug through the living mouse skin. Two gel formulations containing ibuprofen were investigated with or without penetration enhancer. The applied penetration enhancer was Transcutol®. The effect of the penetration enhancer was clearly detected in both models. The *in vitro* diffused amount of ibuprofen was significantly higher than the one permeated through the living skin. The presence of the active agent in the plasma was also determined after the observation period. It was possible to monitor the penetration and absorption of topical formulations simultaneously.

Rezumat

Obiectivul studiului a fost utilizarea unui nou tip de model animal *in vivo* realizat de grupul nostru de cercetare în scopul investigării penetrării prin piele, în comparaţie cu rezultatele măsurătorilor prin celula Franz. Noul model experimental *in vivo* oferă posibilitatea unei măsurări exacte a cantităţii de substanţă medicamentoasă care penetrează prin pielea de şoarece. Au fost investigate două formulări de tip gel cu ibuprofen, cu sau fără promotor de absorbţie. Promotorul de absorbţie utilizat a fost Transcutol®. Efectul promotorului de absorbţie a fost clar detectat în ambele modele. Cantitatea de ibuprofen difuzată *in vitro* a fost semnificativ mai mare decât cea difuzată prin pielea de şoarece. Prezenţa agentului terapeutic în plasmă a fost de asemenea determinată după perioada de observaţie. A fost posibilă monitorizarea simultană a penetrării şi absorbţiei ambelor formulări topice.

Keywords: skinfold model, Franz-cell, skin permeation, ibuprofen, Transcutol®

Introduction

There are several methods for modelling drug permeation through the skin. The Franz-type diffusion cell is an accepted and widely applied model for dermal and transdermal delivery. Basically, a donor and an acceptor compartment are separated by a synthetic membrane [1-2]. Generally, these membranes are more permeable than biological membranes and are non-discriminatory to the characteristics of the diffusant molecule and not suitable for the investigation of the effect of the penetration enhancer modifying the structure of *stratum corneum*. Nowadays, various biological membranes are used more extensively such as animal skin [3] or human epidermis [4] to study the drug interaction with the skin and the incidental reservoir function of the *stratum corneum*. The *in vivo* animal studies are based on monitoring of the effect and blood level of penetrating agents. Other models show the presence of the drug in the skin or demonstrate its impact on the structure or certain functions of the skin [5].

In our previous work a novel animal model was developed successfully [6]. Dorsal skin fold chamber was used, which has been an accepted experimental model to study the microcirculation, angiogenesis and wound healing for more than twenty years [7-8]. The modified version of this experimental setup seemed to provide effective means for the *in vivo* examination of transdermal permeation. In this modified skinfold model we can determine drug permeation kinetics through living mouse skin on the same animal in time to get information on the permeation of the studied agent. Moreover, the detection of local side effects, skin irritation or any other type of alteration in the skin becomes possible.

The aim of our work was to compare diffusion (Franz cell method, with synthetic membrane) and permeation (modified skinfold method) parameters using ibuprofen gels with or without penetration enhancer.

Materials and Methods

Materials

Ibuprofen (IBU) was obtained from Sigma, St Louis, USA. Transcutol® (Diethylene glycol monoethylether, (TR)) was from S & D Chemicals Ltd. Hungary, and Carbopol 971 was from BF Goodrich Co., USA. Polyethylene glycol 400 and trolamine were purchased from Molar Chemicals Kft, Hungary.

Preparation of the gels

First 3% w/w Carbopol 971 hydrogel was prepared. The pH was adjusted with the use of trolamine. The 5 % w/w IBU was dissolved in

polyethylene glycol 400 and was added to this gel (IBU gel). Similar composition was prepared by using 10 % w/w Transcutol® added to polyethylene glycol 400 before dissolution of active agent (IBU-TR gel).

In vitro study

The rate and extent of IBU release was measured by an autosampling system containing vertical Franz diffusion cells (Hanson Microette TM Topical & Transdermal Diffusion Cell System, Hanson Research Corporation, USA). The drug release profile was determined at 37 ± 0.5 °C using phosphate buffer of pH=7.4, porafil membrane filter (cellulose acetate, the pore diameter was 0.45 μm, Macherey-Nagel GmbH & Co. KG, Germany) was used. The effective diffusion surface area was 1.767 cm^2. Experiments were performed for 6 hours. Samples were taken from the acceptor phase every hour and replaced with fresh receiving medium. The drug content of the samples was tested using an HPLC method.

In vivo study

The *in vivo* studies were carried out using a modified skinfold model developed by our research group. In our previous article the modified skinfold chamber has been described for investigation of drug penetration through living animal skin [6]. In the schematic picture (Figure 1) it can be seen that the drug penetrates through the complete living skin to reach the acceptor phase (phosphate buffer of pH=7.4), where the presence of the drug can be detected.

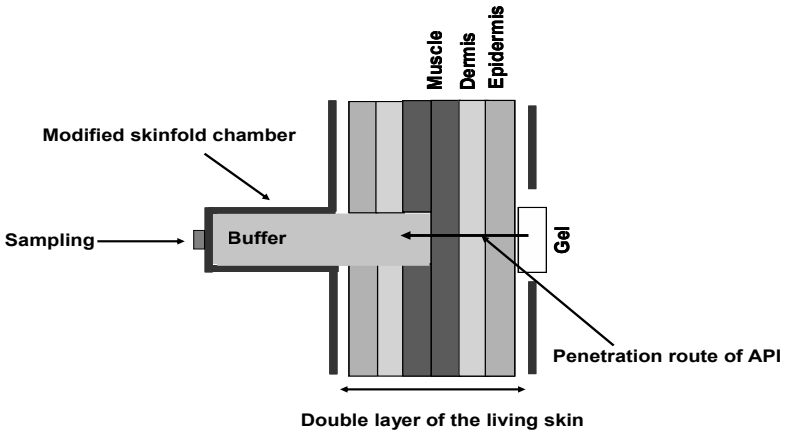

Figure 1.
Schematic picture of the model

The effective diffusion surface area was 1.539 cm^2. Experiments were performed for 6 hours. The total acceptor phase was changed every hour and the concentration of the drug was determined by an HPLC method.

At the end of the observation period a blood sample was taken from the inferior cava vein with a needle and syringe containing 250 IU of heparin. The blood was then centrifuged at 3500 g for 5 min in order to separate the cellular components. The plasma was collected and analysed by HPLC.

Data analysis

The diffusion and permeation parameters were calculated by the method described by Wagner et al. [9]. These parameters enable the comparison between different formulations of the same drug, though the influence of the vehicle can change the diffusion of the drug. The cumulative amount of IBU per area versus square time was plotted (Q). The steady state flux (J) was obtained as the slope of the plots. The lag-time (T_{lag}) was determined from the intercept of the plots with x-axis – symbolising the time of delay which describes the first contact of the drug with the skin surface until a steady state flux. The permeability coefficient (Kp) can be calculated dividing the flux by the initial concentration (Cd) of the donor phase.

$$Kp = J/Cd. \qquad \text{Eq. (1)}$$

Statistical analysis

Student's t-test was performed to see any significant difference in the diffused and permeated amount of IBU (Q) between the IBU and IBU-TR gel. Differences were regarded as significant, with $p < 0.05$.

HPLC method

Ibuprofen concentrations from buffer and plasma samples were determined by the method previously described by Eros et al. [6]. Based on the analysis of drug free serum, no interfering peaks of endogenous substances at the retention time of ibuprofen and I.S. indicated specificity of the method. The assay was linear in the range of 0.1 to 20 μg/mL and 0.5 to 15 μg/mL for buffer and plasma samples, respectively, with correlation coefficients of r=0.9993 ± 0.0031 (n=3) for buffer samples and r=0.9984 ± 0.0025 (n=3) for plasma samples. The limit of quantification was 0.2 and 0.5 μg/mL for buffer and plasma samples, respectively. The limit of detection was 0.06 μg/mL for buffer samples and 0.15 μg/mL for plasma samples. The intra- and inter-day accuracies for ibuprofen fell in the ranges 94.30–106.9% and 95.1–101.24%, and the intra- and inter-day precisions (CV%) were in the ranges 4.6–9.3% and 7.1–8.9%, respectively. Using this method the average recovery of ibuprofen from mice plasma was 98.2 ± 2.0%.

Results and Discussion

A modified skinfold animal model was studied for the investigation of skin penetration, compared to the result of Franz cell measurements. Two

gel formulations containing ibuprofen were investigated with or without penetration enhancer. The applied penetration enhancer was Transcutol®, which acts by increasing the solubility of the penetrant in the barrier. It works as a humectant, absorbing water, thus increasing the water content of the skin and the donor compartment. The change in the donor composition can influence the solubility and the thermodynamic activity of active pharmaceutical ingredient (API) [10]. However, Transcutol® has also been reported to increase the skin accumulation of topically applied compounds without a concomitant increase in transdermal permeation [11]. The results of diffusion and permeation studies are shown in Table I.

Table I.

The diffusion and permeation parameters of IBU. The table contains the means of six parallel measurements ± SD

		release time (h)	Cumulative amount of IBU (%)	Q (µg/cm^2)	J (µg/cm^2/h)	Kp*10^{-3} (cm/h)	T_{lag} (h)
IBU gel	synth. membr.	6	73.31±6.74	6276.33±291.95	2884.7	57.69	0.048
IBU-TR gel	synth. membr.	6	88.28±5.88	7504.85±249.66	3680.6	73.61	0.194
IBU gel	mouse model	6	2.13±0.09	68.17±3.1	73.577	1.47	1.548
IBU-TR gel	mouse model	6	5.81±0.85	189.03±27.74	172.82	3.45	1.369

The diffused amount of ibuprofen through the synthetic membrane was significantly higher than the one permeating through the living skin. The smaller quantity of the permeated drug, lower absorption rate and permeability coefficient and the longer lag time can be explained in terms of different factors in the present experiments. The drug has to cross the epidermis in order to enter the dermis, where ibuprofen is exposed to the barrier effect of the skin. Furthermore, ibuprofen can be taken up by the circulation. Only the proportion of the drug not eliminated by degradation or removal in the circulation can be delivered towards the deeper layer and the acceptor phase.

However, the effect of the penetration enhancer was clearly detected in both models. The cumulative amount of ibuprofen, the absorption rate and the permeability coefficient were significantly higher when applying the penetration enhancer containing IBU-TR gel. The effect of the penetration enhancer was more expressive in the animal model case. The changes of permeation parameters (Q, J, Kp) were more than twice higher. The lag time was shorter given the better permeation parameters.

In this study the presence of the active agent in the plasma was also determined after the observation period (Figure 2).

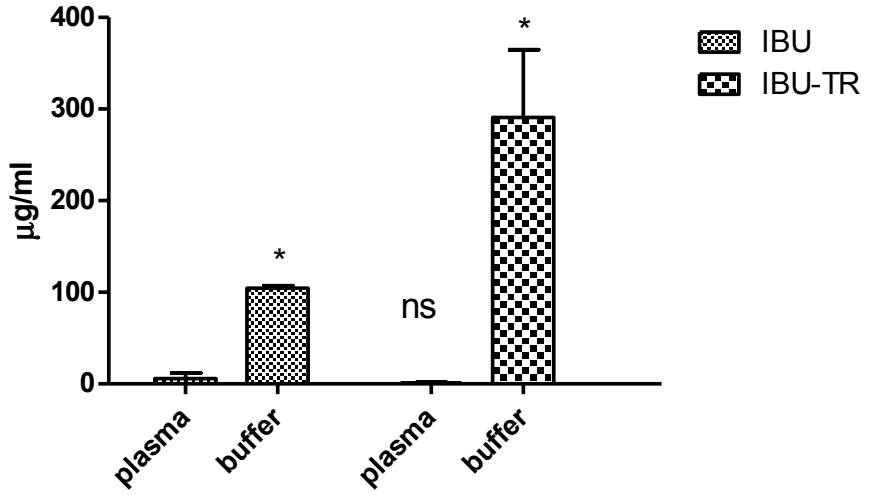

Figure 2.
Comparison of plasma and buffer concentration after 6 hours. The figure contains the means of six parallel measurements ± SD

The plasma and buffer concentrations were compared in case of IBU gel and IBU-TR gel. The buffer concentrations were significantly higher than plasma levels. The plasma levels were very low, 5.71 µg/mL in case of IBU gel and 1.03 µg/mL in case of IBU-TR gel. There was no significant difference in plasma levels when applying IBU gel or IBU-TR gel.

Conclusions

In this study it was possible to monitor the permeation of hydrogel containing ibuprofen through living skin compared to drug diffusion through synthetic membrane. The effect of penetration enhancer for diffusion and permeation could be detected in case of *in vitro* and new *in vivo* model too. Comparing the buffer and plasma levels, the buffer concentrations were significantly higher than plasma levels, which proves that the model is suitable and up-to-date for determining the quantity of drug permeation through living full-thickness skin.

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