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Research Article

Penetration and Distribution of Thiocolchicoside through Human Skin: Comparison Between a Commercial Foam (Miotens®) and a Drug Solution

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Abstract. Penetration and distribution of thiocolchicoside from a commercially available foam (Miotens® 0.25%, *w*/*v*) through human excised full-thickness skin were evaluated using two different *in vitro* apparatus: a Franz diffusion cell and a Saarbruecken penetration model-based cell. In order to evaluate the intrinsic capability of the drug to penetrate into the skin, a simple drug aqueous solution prepared at the same drug concentration as Miotens® was also tested. Results showed that both apparatus were suitable to study thiocolchicoside penetration into human skin. Penetrated drug amounts were comparable using the two apparatus, probably because skin acts as "sink" for the drug. Miotens® was found to significantly promote thiocolchicoside accumulation into full human skin thickness in comparison with the simple drug solution. The mixture of propylene glycol and propylene glycol diperlargonate contained into Miotens® foam has been proven to be effective to promote penetration of thiocolchicoside into human skin.

KEYWORDS: Franz cell; human full-thickness skin; Saarbruecken penetration model-based cell; thiocolchicoside; topical formulation.

INTRODUCTION

Thiocolchicoside (TCC; Fig. 1) is a semisynthetic derivative of colchicoside with GABA (γ-aminobutyric acid)-mimetic and glycinergic actions. In animals and humans under certain conditions, TCC can also manifest convulsant activity due to a potent competitive antagonism towards type A receptors of GABA (1).

GABA-mimetic activity in the central nervous system is responsible for muscle-relaxant, anti-inflammatory, and analgesic properties of TCC. This drug can be administered orally, intramuscularly, or topically for the symptomatic treatment of muscular spasms and rheumatologic disorders. Alternative routes, such as buccal (2) and transdermal (3), have also been investigated.

Topical formulations of TCC include ointments and creams. Recently, an innovative foam formulation has been marketed (Miotens®, Dompé, Italy), which has been developed to avoid contact with the afflicted areas during the spreading phase.

The physicochemical properties of TCC (relatively high MW, 563.3; low octanol/water partition coefficient: log $P = -2.71$) are not favorable to the permeation of the drug through the skin. Accordingly, chemical (transcutol and lauric acid) or physical (iontophoresis) penetration enhancers have been considered to promote permeation of TCC across full-thickness human skin and epidermis (3). Miotens® foam contains propylene glycol and propylene glycol diperlargonate as skin penetration enhancers. This formulation significantly increased the *in vitro* permeation of the drug through rat skin (4) and human excised skin (5) in comparison with another commercial product (Muscoril® ointment, Inverni della Beffa, Italy), which is enhancer-free and contains the same drug concentration (0.25%, *w*/*v*). Nevertheless, permeation studies were carried out using Franz diffusion cells, which maintain for a prolonged period of time the skin in direct contact with an aqueous phase. This could lead to an overhydration of the skin with a consequent change in its barrier properties. Another approach is the use of Saarbruecken penetration model-based cell (6–8). This cell has been reported to better mimic the *in vivo* conditions, since the skin acts as its own receptor medium, avoiding non-physiological hydration of the tissue. This permits the evaluation of drug penetration into the skin. Data regarding skin penetration of TCC from Miotens® are not yet available. Such information would be interesting from a biopharmaceutical point of view in order to assess the skin distribution pathway of TCC delivered from the foam and the influence of the formulation in the drug penetration. This can be useful in case of topical formulations. In fact, if drug deeply penetrates into the skin up to the dermis, it will be able to reach the underlying tissue, site of action, via capillary network.

Given these premises, the penetration and distribution of TCC from Miotens® into human excised skin were evaluated in this work. Results relative to a simple aqueous solution of TCC having the same concentration as in Miotens® were also

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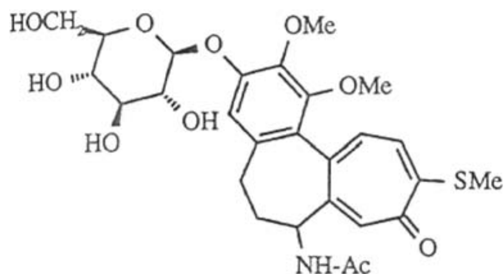

Fig. 1. Structural formula of TCC

obtained in order to determine the intrinsic penetration properties of the drug into the skin.

Experiments were done using two different *in vitro* apparatus: a Franz diffusion cell and a Saarbruecken penetration model-based cell (6). In order to evaluate the influence of the contact time on drug penetration, experiments were performed at different times: 1, 4, 7, and 24 h. In the case of Franz cell, in order to reduce overhydration of the tissue, the maximum time considered was 7 h. The methods employed for drug extraction from full-thickness skin and for drug assay were validated.

MATERIALS AND METHODS

Materials

TCC and Miotens® foam (0.25%, *w*/*v*) were supplied by Dompé S.p.A.

Methods

Skin Sample Preparation

Human skin specimens from Caucasian female donors were obtained from breast plastic surgery. After excision, the subcutaneous fat was removed and skin specimens of about 10×1.5 cm were cut. Skin pieces were wrapped in aluminum foil, frozen in polyethylene bags at −20°C, and used within 3 months. No significant changes in skin permeability were observed by freezing the tissue for this period of time (9,10).

For penetration experiments, skin specimens were thawed, cleaned with cotton soaked in an isotonic saline solution pH 7.4 (KH_2PO_4 1.90 g/l; Na_2HPO_4 8.10 g/l; NaCl 4.11 g/l), and cut into pieces of approximately 1×1.5 cm.

Penetration Measurements

Franz diffusion cell. Experiments were performed by means of Franz diffusion cells with 8-mm orifice diameter (diffusion area 0.5 cm^2) thermostated at 32±1°C. The skin was positioned with the stratum corneum facing the donor phase, between the donor and the receptor compartments of the Franz cell. The donor compartment was filled with 500 μl of Miotens® or with TCC aqueous solution 0.25% (*w*/*v*). The receptor compartment was filled with 3.3 ml of isotonic saline solution (pH 7.4) previously degassed under vacuum and continuously stirred by a magnetic bar. Skin was pre-hydrated with the receptor fluid for 30 min before starting experiments to increase reproducibility (6).

Five hundred microliters of the receptor solution was withdrawn for drug analysis at the end of the experiment. The donor phase (Miotens® or drug solution) was removed and the skin area in contact with the formulation (0.5 cm^2) was rinsed three times with isotonic saline solution, wrapped in aluminum foils, and immediately frozen in liquid nitrogen. The experiments were performed after contact times of 1, 4, and 7 h. It was not possible to prolong the experiments until 24 h due to the overhydration and the consequent swelling of the skin.

Saarbruecken penetration model-based cell. The Saarbruecken penetration model-based cell is based on the Saarbruecken penetration model (6–8), and it is reported in Fig. 2. The skin was placed, with the stratum corneum facing up, into the cavity of a Teflon block (A). A filter paper soaked with isotonic saline solution (pH 7.4) was used to support the tissue and to avoid skin dehydration. A Teflon cylinder (B) was filled with 500 μl of Miotens® foam or drug aqueous solution 0.25% (*w*/*v*). The area of the cylinder cavity was 0.5 cm^2.

Block A and cylinder B were put in contact and fixed by means of a spring closing device. The assembled system was closed in a polyethylene bag and placed (the Teflon cylinder standing in the upper part) in a water bath at 32±1°C.

At the end of the experiment, drug formulation/solution was removed and the skin area in contact with the drug

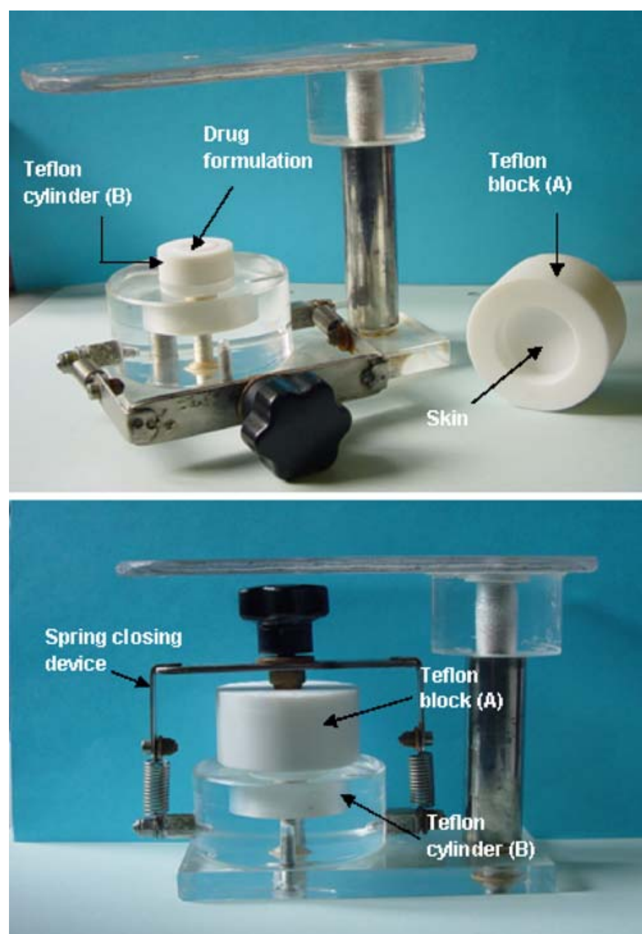

Fig. 2. Saarbruecken penetration model-based cell: single parts (*up*) and assembled system (*down*)

Table I. Analytical and Suitability Parameters of HPLC Method in the Medium Used for TCC Extraction from Skin and in pH 7.4 Buffer

Parameter		Medium	
		Extraction ($H_2O/HClO_4$ 1:1)	Isotonic buffered solution (pH 7.4)
Log–log slope		0.97 (0.70)	1.02 (2.03)
LD (μg/ml)		0.4	0.4
LQ (μg/ml)		0.6	0.6
Repeatability (S_R, %)	Peak area (*A*)	1.07–6.18	5.65–7.31
	t_R	0.59–1.20	0.43–3.46
Column efficiency (*N*)		≅5,500	≅5,500
Tailing factor (*T*)		1.14	1.12

Values in parentheses are variation coefficients (%)

(0.5 cm^2) was rinsed three times with isotonic saline solution, wrapped in aluminum foils, and immediately frozen in liquid nitrogen. Experiments were performed for 1, 4, 7, and 24 h.

Drug extraction from skin samples. After experiments, the frozen skin portions were included in an embedding medium for tissues (Jung Tissue Freezing Medium, Leica Microsystems Nussloch GmbH, Germany) and transferred into a cryostat (Kryostat 1720, Leitz Wetzlar, Germany) at −20°C. Horizontal slices 20 μm in thickness were cut, collected in Eppendorf microtubes, and stored at −20°C before analysis. Ten slices (total thickness, 200 μm) were collected for each tube.

TCC was extracted from skin slices at room temperature for 60 min with 200 μl of distilled water (3). The content of each tube was mixed during extraction in a vortex every 15 min for 10 s. Two hundred microliters of 1 N $HClO_4$ was then added to precipitate residual proteins, and the mixture was centrifuged at 5,000 rpm for 30 min. The supernatant was filtered through a 0.45-μm membrane (Millipore®, Italy) and analyzed via high-performance liquid chromatography (HPLC) as described in “Drug assay”.

For the validation of TCC extraction from the skin, blank skin slices (which had not been in contact with TCC), from different donors and taken at different depths, were used for selectivity and recovery measurements (11). For selectivity, blanks were submitted to the extraction procedure in order to compare the retention time of endogenous compounds to that of TCC. Recovery was evaluated by putting in contact for 3 h known volumes (5 and 10 μl) of TCC aqueous solution (2 mg/ml) with the blank skin slices before submitting them to drug extraction and analysis. Extraction recovery was calculated as the ratio of the amount of TCC extracted from the skin and the amount of TCC applied onto the skin.

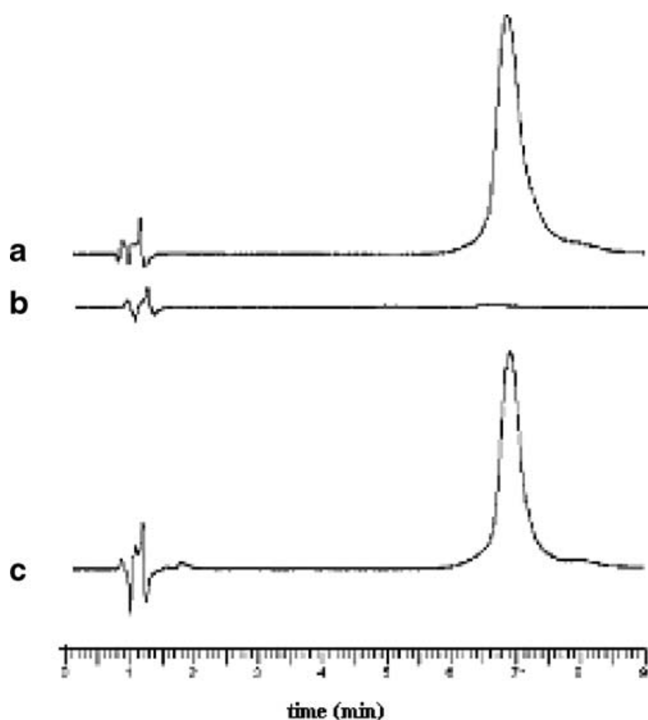

Fig. 3. Typical chromatograms of **a** TCC standard solution (0.025 mg/ml) in the extraction medium; **b** skin slices; **c** skin slices put in contact with TCC standard solution

Drug Assay

Drug assay was performed by means of an HPLC method opportunely modified from (12) and validated for the new purpose to characterize drug penetration and permeation into/across full-thickness skin. Perkin Elmer series 200B/250 HPLC pump equipped with a series 200 LC autosampler, and a series 200 UV–vis spectrophotometer (Perkin Elmer, Italy) was used. The column was a Spherisorb® $C_{18,}$ 5 μm, 125×4.6 mm (C.P.S. Analitica, Italy), and the mobile phase was a mixture of KH_2PO_4 5 g/l (adjusted to pH 4.5 with phosphoric acid) and CH_3CN. The flow rate was set at 1 ml/min, the injection volume at 20 μl, and the detector wavelength at 370 nm. Analytical data were recorded and processed using TotalChrom WS 6.2 software package (Perkin Elmer).

The analytical method was validated by determining analytical (linearity, detection limit, quantification limit, repeatability) and suitability (column efficiency, tailing factor) parameters according to the indications of ICH and USP, respectively.

Statistical Evaluation

Statistical differences were determined using one-way analysis of variance (ANOVA) and the *post hoc* Sheffe test for multiple comparisons (Siphar, Creteil, France). Differences between groups were considered to be significant at $P<0.05$.

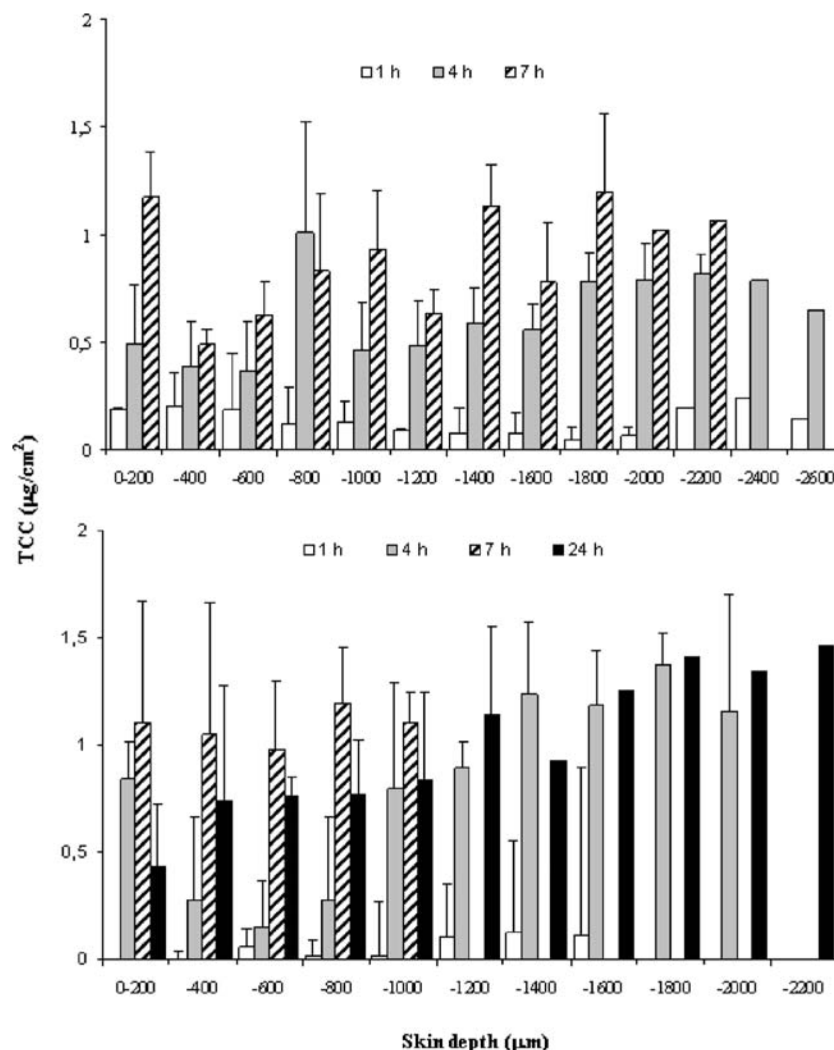

Fig. 4. Average amounts of TCC extracted from human full-thickness skin obtained for Miotens® foam after different incubation times in Franz cell (*up*) and in Saarbruecken penetration model-based cell (*down*, mean values±SD; n=2–5). No error bars were reported for data corresponding to a single replicate

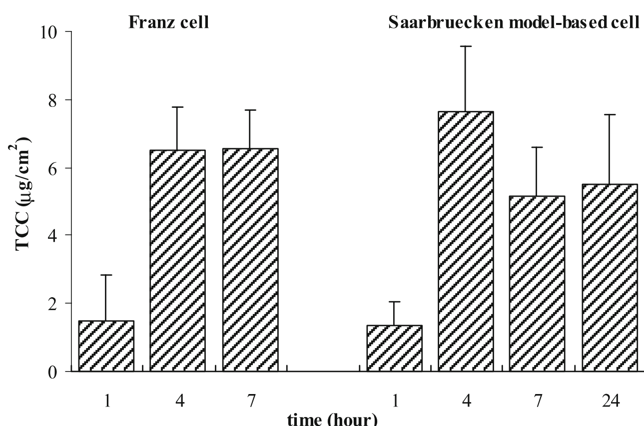

Fig. 5. Total amount of TCC (Miotens®) penetrated into the skin after different incubation times in the two cells (mean values±SD; n=2–5)

RESULTS AND DISCUSSION

Drug Assay

Retention time of TCC in the extraction medium from skin ($H_2O/HClO_4$ 50:50, *v/v*) and in saline isotonic solution (pH 7.4) was 7 min. The detector response was linear in the range 0.001–0.08 mg/ml in both media (five data points, replicated three times, have been considered). Related linear equations were y=37,136×−1.295 (R^2=1.0000) and y= 37,473×+18.902 (R^2=0.9992) in the extraction medium and buffer solution pH 7.4, respectively.

Analytical and suitability parameters, calculated according to ICH and USP recommendations, are given in Table I. The slope of the curve obtained by plotting the log of drug concentration *vs* the log of peak area (log–log slope) fulfilled ICH recommendations (0.95–1.05 accepted interval) in both

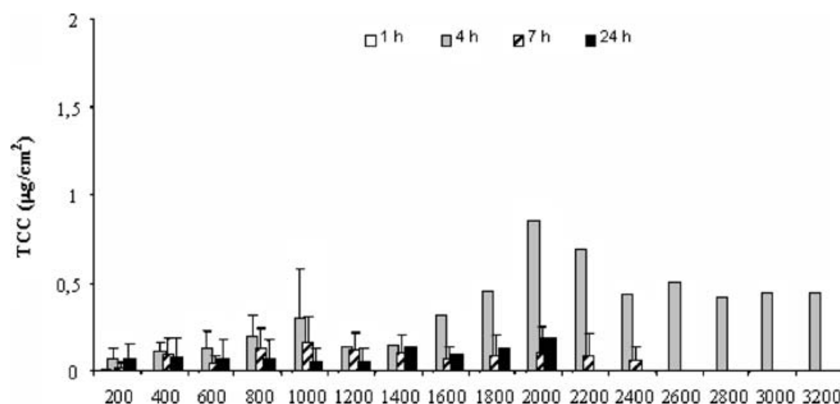

Fig. 6. Average amounts of TCC extracted from human full-thickness skin obtained for TCC solution at different incubation times in Saarbruecken penetration model-based cell (mean values±SD; n=2–5). No error bars were reported for data corresponding to a single replicate

media, with a variation coefficient (S_R) ≤2%. The limits of detection (LD) and quantification (LQ), determined from the signal-to-noise ratio, were <1 µg/ml in both media and allowed the assay of <400 ng of TCC in each Eppendorf microtube (skin thickness, 200 µm). Repeatability (variation within-day) of peak area (A) and retention time (t_R), expressed as relative standard deviation (S_R), were determined on the basis of nine analysis (three concentrations/three replicates each) of TCC standard solutions covering the linearity range in both media. Repeatability was between 0.43% and 3.46% for retention time and between 1.07% and 7.31% for peak area, respectively. Column efficiency, expressed as the number of theoretical plates (N), was about 5,500 both in extraction fluid and pH 7.4 buffer. Tailing factor (T) was about 1.1, indicating that suitable peak symmetry has been reached in both media.

Chromatograms obtained for blank skin specimens (samples used for validation of drug extraction from skin) and for TCC standard solution 0.025 mg/ml are compared in Fig. 3. Using the optimized analytical conditions, no peaks of endogenous compounds were found at the detector wavelength (370 nm) despite the complexity of the biological matrix; drug response, as well, was not affected by the presence of skin. Extraction recoveries were satisfactory and greater than 90% (ranging from 93.3% to 99.6%) for all samples tested.

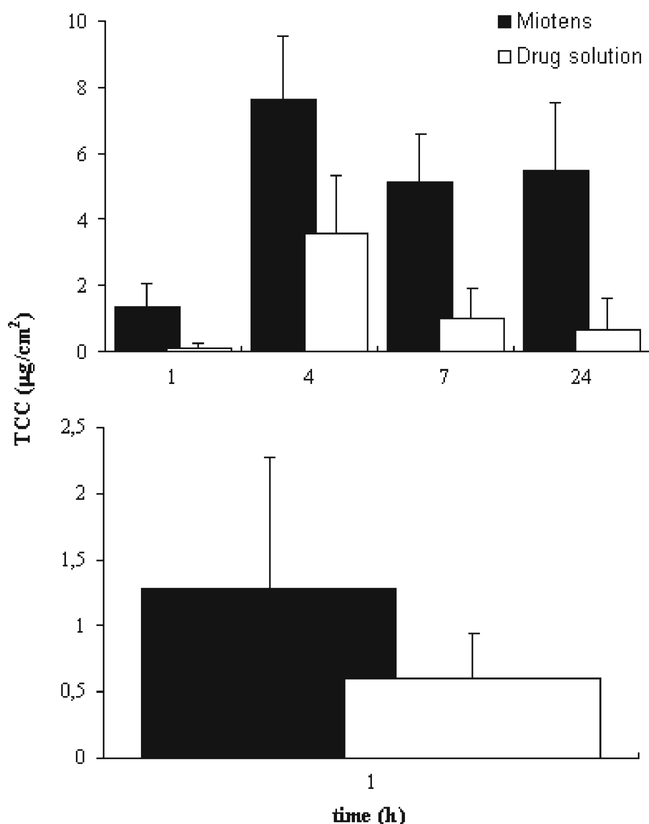

Fig. 7. Total amount of TCC penetrated into the skin from Miotens® foam and drug aqueous solution. Penetration cell (*up*) and Franz cell (*down*) (mean values±SD; n=2–5)

Penetration and Permeation Properties

Figure 4 shows TCC amounts penetrated into full-thickness skin as a function of layer depth when Miotens® foam was used in Franz diffusion (up) and Saarbruecken penetration model-based (down) cells, respectively. At each time, TCC penetrated higher skin depths when Franz diffusion cell was used. This result could be explained by the skin overhydration promoted by the contact with the receptor solution, and it is in line with data obtained by other authors with different drugs (6). They reported that using Franz cells, water was taken up by the tissue determining a higher thickness.

For both cells, Franz (Fig. 4, up) and Saarbruecken (Fig. 4, down), the amount of TCC detected into the skin after 1 h was very low (<1 µg/cm²); higher amounts of drug (between 1 and 1.5 µg/cm²) were found increasing the incubation time, especially at higher skin depths (P<0.001, one-way ANOVA).

In the majority of the experiments, the drug is found in the deepest layer analyzed, indicating its ability to reach dermis. The amount of TCC (Miotens® foam) permeated across full-thickness skin and assayed into Franz cell receptor solution (isotonic pH 7.4 buffer) was very variable: no drug (<LQ) was found in the receptor medium after 1 h of incubation; after 4 h and 7 h, the TCC amounts were 2.42±2.10 µg (one replicate resulting in no drug detectable) and 1.92±1.62 µg (two replicates resulting in no drug detectable), respectively. The high variability obtained is probably due to

the prolonged contact between skin and receptor phase, resulting in possible overhydration and alteration of the barrier properties of the tissue. Other authors found similar results in Franz cell experiments using flufenamic acid as a model drug; although relatively high amounts of drug were detected into skin after long incubation times, no drug was found in the receptor solution, suggesting the skin acted as a sink (6, 8). Even if TCC is characterized by a lower lipophilicity with respect to flufenamic acid, this result could be explained by skin overhydration. However, the high variability of the data does not permit drawing firm conclusions.

In Fig. 5, the total amounts of TCC (Miotens® foam) extracted from skin samples are shown. No significant differences were found using the two models of cell (one-way ANOVA; post hoc Sheffe test). Moreover, for both systems, the amount of drug penetrated increased when the time of incubation was longer than 1 h: with Franz cell, the total amount of TCC detected into the skin after 4 h was about 6.5 $\mu g/cm^2$, and no further increase was observed after longer incubation time (7 h). The same trend was observed using Saarbruecken model-based cell: Fig. 6 reports that the TCC amounts penetrated into full-thickness skin as a function of layer depth when TCC solution (0.25%, *w*/*v*) was used. Given the good correlation of the results obtained for Miotens® foam using Franz and Saarbruecken penetration model-based cells and the skin overhydration occurring at prolonged experimental times with Franz cells, the intrinsic TCC penetration properties were evaluated using only the Saarbruecken penetration model-based cell.

The amount of TCC detected into skin after 1 h was very low (<0.2 $\mu g/cm^2$); higher drug amounts were found on increasing incubation time (Fig. 6). However, for all the contact times considered, the average amounts of drug penetrated are lower than 0.3 $\mu g/cm^2$; only for one replicate performed at 4 h were higher amounts detected.

The comparison between the results obtained for Miotens® formulation and TCC solution (Figs. 4 down and 6) evidenced that significantly higher amounts of TCC ($P<$ 0.001, one-way ANOVA) penetrated into skin from Miotens® in comparison with drug solution due to the peculiar properties of the formulation.

In Fig. 7 (up), the total amounts of TCC penetrated into the skin from the foam formulation and the drug aqueous solution (0.25%, *w*/*v*) using the Saarbruecken model-based cell are compared. At each incubation time, significantly higher amounts of drug were detected for the foam formulation ($P<$ 0.001). No significant differences were observed in Franz diffusion cells at earlier (1 h) time of incubation (Fig. 7, down).

CONCLUSIONS

From the results obtained, it can be concluded that Miotens® foam induced higher accumulation of TCC into full human skin thickness (stratum corneum, epidermis, and dermis) in comparison to the simple drug aqueous solution. Both the *in vitro* apparatus (Franz and Saarbruecken model-based cells) were suitable for biopharmaceutical studies of TCC penetration into skin. No statistical differences in the amount of drug penetrated were found using the two cells. This is probably because the skin acts as “sink” for the drug. The drug absence in the receptor phase, observed in the majority of the experiments performed with Franz cells, confirms this hypothesis. However, in Franz cells, the presence of the receptor solution determined a non-physiological hydration of the tissue (overhydration), resulting in increased skin thickness (swelling) compared to Saarbruecken model-based cell especially after prolonged incubation time.

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