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Prediction of the skin permeability of topical drugs using *in silico* and *in vitro* models

Cristina Alonso[1*], Víctor Carrer[1], Sonia Espinosa[2], Miriam Zanuy[2], Mònica Córdoba[2], Bernat Vidal[2], María Domínguez[2], Núria Godessart[2], Luisa Coderch[1] and Mercè Pont[2]

[1]*Institute of Advanced Chemistry of Catalonia, 18-26 Jordi Girona St, 08034, Barcelona, Spain*

[2]*Almirall R&D Center, Ctra. Laureà Miró, 408-410, 08980, Sant Feliu de Llobregat, Barcelona, Spain*

[*]*Cristina Alonso is the corresponding author*

Additional contact information authors:

Bernat Vidal: bernat.vidal@almirall.com

Cristina Alonso: cristina.alonso@iqac.csic.es

Luisa Coderch: luisa.coderch@iqac.csic.es

María Domínguez: m.dominguezblanco@gmail.com

Mercè Pont: merce.pont@almirall.com

Miriam Zanuy: miriam.zanuy@almirall.com

Mònica Córdoba: monica.cordoba@almirall.com

Núria Godessart: nuria.godessart@almirall.com

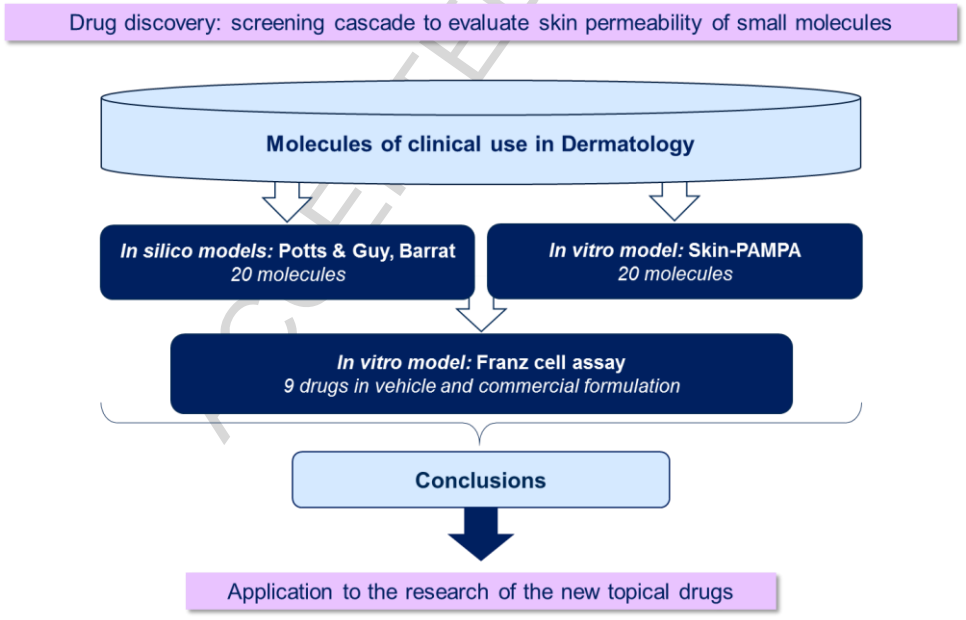
Sonia Espinosa: sonia.espinosa@almirall.com

Victor Carrer: lcnesl@iqac.csic.es

Abstract

The main challenge of topically applied drugs is to overcome the skin barrier to reach the site of action at the concentration needed for efficacy. In the research of new topical drugs, design of molecules with optimized properties for skin penetration is a key factor and assays for its characterization are needed. A group of 20 representative topical molecules of clinical use were studied in two *in silico* models (Potts & Guy and Barratt), and an *in vitro* assay with artificial membrane (Skin-PAMPA). A subset of 9 drugs were also evaluated in the Franz cells assay, formulated in a solvent and in a marketed formulation. Each assay allowed us to grade compounds according to their permeability value. Globally good alignments were found for the studied compounds when comparing models, although discrepancies for some compounds such as tazarotene, tacrolimus, ketoconazole and metronidazole were observed. Overall, the studied *in silico* and the *in vitro* models are useful tools to support selection and characterization of research compounds in terms of skin permeability.

Graphical abstract



Keywords: Skin-PAMPA, Franz cell, skin, drug, dermatology, permeability

1. Introduction

Topical therapies remain the mainstay treatment of a variety of skin conditions (Hanifin et al, 2004; Wollina, 2014; Stein Gold, 2014). In chronic diseases, such as psoriasis and atopic dermatitis, they are the first line therapy for mild to moderate forms of the disease. For the most severe forms, generally involving a higher body surface area, they may be used in combination with oral and biological treatments (Beck et al, 2014; Gollnick et al., 2002). The topical route has an obvious advantage in terms of safety because it minimizes blood exposure and reduces systemic side effects (Hwa et al., 2011). However, reaching the site of action (epidermis or dermis) at the concentration needed for efficacy and with the optimal residence time are the main challenges. This is in part due to the highly organized structure of the skin with an external layer, the *stratum corneum*, that acts as an efficient barrier against the penetration of exogenous elements and the loss of water (Matsui and Amagai, 2015).

In the topical drug discovery process, newly synthesized compounds are tested in assays of increasing complexity to select those with the properties in terms of molecular weight (MW), lipophility, solubility and skin retention, suitable for topical administration. Several mathematical *in silico* and *in vitro* experimental tools can be used to predict and measure, respectively, the penetration of molecules through the skin. Mathematical tools consist on equations to predict skin permeability based on physicochemical properties such as MW and the octanol-water partition coefficient (logKow) among others (Barratt, 1995; Mitragotri, 2002; Potts and Guy, 1992; Wilschut et al., 1995). The Skin-PAMPA assay evaluates experimentally the permeability of a molecule through an artificial membrane which mimics the composition of the *stratum corneum* (Avdeef, 2005; Sinko et al., 2012). The *in vitro* Franz cells using human or pig whole skin samples have traditionally been used to determine the percentage of compound that penetrates the *stratum corneum,* distributes between dermis and epidermis and reaches the receptor fluid. The degree of penetration of a compound has to be put in context with its potency. In the Franz cell assay the amount of compound moving through an area of skin during a given period of time (flux) can be compared with the IC_{50} of the compound in a relevant cellular assay to estimate if the penetrated compound is enough to efficiently exert a pharmacological activity (Walters and Roberts, 2007; Cordero et al, 2001). Several references disclose correlations

between *in silico* models and *in vitro* skin penetration models (e.g. Lee et al, 2010; Karadzovska and Riviere, 2013).

In vivo efficacy testing poses an additional layer of complexity, due to species differences between the skin of laboratory animals and humans (Monteiro-Riviere et al., 1990). Minipigs offer a good alternative to rodents although the number of experimental models in this species are limited. In conclusion, there is no single model able to predict bona fide skin penetration and efficacy following topical administration.

In this work, 20 compounds were chosen because of their demonstrated efficacy in humans by topical route in different dermatological diseases. Two *in silico* (Potts & Guy and Barratt) and one *in vitro* model of skin penetration (Skin-PAMPA), were applied. A subset of compounds were subsequently tested in the Franz cells system formulated both in propylene glycol (PG) and commercial formulations. Our objective was to test the predictability of those models to be applied to the screening of novel topical drugs.

2. Materials and methods

2.1. Materials

Compounds, CAS number and provider are indicated as follows: adapalene (106685-40-9), azelaic acid (123-99-9), betamethasone dipropionate (5593-20-4), diclofenac sodium (15307-79-6), dithranol (480-22-8), fluorouracil (51-21-8), flurandrenolide (1524-88-5), lidocaine (137-58-6), salicylic acid (69-72-7) and tazarotene (118292-40-3) were purchased from Sigma-Aldrich; clobetasol propionate (25122-46-7) was supplied by AK Scientific; bexarotene (153559-49-0) and terbinafine (91161-71-6) were supplied by Selleck; calcipotriol monohydrate (147657-22-5) was provided from MatTek; clindamycin (18323-44-9) was provided from Axon Medchem; dapsone (80-08-0) and metronidazole (443-48-1) were provided from Fluka; diphenhydramine (58-73-1) was provided from Pacific; ketoconazole (65277-42-1) was provided from Intex Quimica and tacrolimus monohydrate (109581-93-3) was provided from LC Laboratories. PG, dimethyl sulfoxide (DMSO), Dulbecco's phosphate buffer, gentamicin sulfate salt and bovine serum albumin, were purchased at Sigma-Aldrich.

The commercial formulations evaluated in the Franz cell assay are indicated as follows: betamethasone dipropionate 0.5 mg/g cream (Diproderm, Merck Sharp & Dohme), clobetasol propionate 0.5 mg/g cream (Clovate, IFC), fluorouracil 50 mg/g cream (Efudix, Meda Pharmaceuticals), flurandrenolide 0.5 mg/g cream (Cordran, Aqua Pharmaceuticals), ketoconazole 20 mg/g cream (Fungarest, Janssen), lidocaine 20 mg/g cream (Dermovagisil, Laleham Health and Beauty), metronidazole 7.5 mg/g gel (Rozex, Galderma), tacrolimus monohydrate 1 mg/g ointment (Protopic, Astellas) and tazarotene 1 mg/g gel (Zorac, Pierre Fabre).

2.2. In silico properties and skin permeability determination

Physicochemical properties were calculated for all compounds using two different software platforms: ChemAxon (Chemaxon, Hungary) software was used to calculate logKow and MW; and BIOVIA Pipeline Pilot (Accelrys, USA) software was used to calculate molecular volume (MV).

The melting point (MP) was measured in a Büchi melting point apparatus, Model B-540, ranging from 32ºC to 345ºC, with an increment of temperature of 1ºC/min. The MP was calculated as the medium value of the temperature at which the compound starts melting and the final temperature when the transition is completed.

The skin permeability coefficients (logKp) were calculated with the Potts & Guy and the Barrat models:

Potts & Guy model: $\log Kp = 0.71 \log Kow - 0.0061 MW - 6.3$

Barrat model: $\log Kp = 0.82 \log Kow - 0.0093 MV - 0.039 MP - 2.36$

2.3. Skin-PAMPA assay

The Skin-PAMPA assay was performed following manufacturer instructions (Pion INC) also described in Sinkó et al., 2012 . Briefly, the bottom (donor) plate was filled with 200 µL of 20 µM test compounds solution of Pion buffer solution at pH 5.5 containing 2% (v/v) DMSO. The acceptor plate was filled with 200 µL of fresh Pion Prisma HT buffer at pH 7.4 and 2% (v/v) DMSO. After 5 h of incubation at room temperature, compound concentration was determined in the samples from donor and acceptor

compartments by Ultra Performance Liquid Chromatography-tandem Mass Spectrometer (UPLC-MS/MS) as detailed in the Analytical Conditions section.

Effective permeability coefficient (P_e , cm/s) was calculated using the following formula detailed elsewhere (Chen et al., 2008):

$$\mathrm{Pe} = \frac{-\ln[1 - \frac{\mathrm{CA(t)}}{\mathrm{Cequilibrium}}]}{\mathrm{A} * \left(\frac{1}{\mathrm{VD}} + \frac{1}{\mathrm{VA}}\right) * \mathrm{t}}$$

where A=effective filter area (=f×0.3 cm2, where f=apparent porosity of the filter), VD is the donor well volume (0.2 ml), VA is the acceptor well volume (0.2 ml), t is the incubation time (s), CA(t) is the compound concentration in acceptor well at time t, CD(t) is thecompound concentration in donor well at time t, and at equilibrium (Cequilibrium) is calculated as follows:

$$\mathrm{Cequilibrium} = [\mathrm{CD(t)} * \mathrm{VD} + \mathrm{CA(t)} * \mathrm{VA}] / (\mathrm{VD} + \mathrm{VA})$$

2.4. Static Franz diffusion cell assay

The *in vitro* permeation profile of all compounds was studied using pig skin in a static Franz diffusion cell system (Lara-Spiral, France). Unboiled porcine skin was provided from the Department of Cardiology from the Hospital Clínic of Barcelona. Animal handling was approved by the Institutional Review Board and Ethics Committee of Institut d'Investigacions Biomèdiques Agustí Pi i Sunyer and the management of the animals conforms to the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 2011). Back skin from Landrace large white pigs weighing 30-40 kg was collected as previously described (Carrer et al., 2018). Briefly, hair-clipped skin was dermatomed to a thickness of 500 ± 50 µm and stored frozen until use considering the OECD guidelines.

The Franz cell diffusion assay was conducted as described (Carrer et al., 2018). The skin effective diffusion area was of 1.86 cm^2. Receptor chamber was filled with 3 mL of receptor fluid consisted on Dulbecco's phosphate-buffered saline at pH 7.4 supplemented with 0.04% (w/v) gentamicin sulfate salt and 5% (w/v) bovine serum albumin. Sink conditions were maintained through all the experiment. A volume of 20 µL of formulation or compound PG solution was applied onto the skin under a finite

regimen administration. After 24 h, the skin surface was washed thoroughly and *stratum corneum* removed. Remaining epidermis, dermis and receptor fluid were collected. Following an extraction protocol with acetonitrile and trifluoroacetic acid, compound levels were quantified by UPLC-MS/MS.

All samples of the test system were analyzed and mass balance (or recovery of compound *versus* applied dose) was determined. Permeation values were calculated as the sum of compound present in *stratum corneum*-removed epidermis, dermis and receptor fluid. Franz cell assays in the present work showed a mass balance in the 75-125% range. In the assays where compound levels in the receptor fluid were higher than 30% of applied dose, skin permeation was considered as >30% since the diffusion process through the skin is limited and percutaneous absorption cannot be accurately determined.

The evaluation of compounds in PG and in formulation was performed in the same assay with skin from 2 donors (3 replicates per donor) or 3 donors (2 replicates per donor).

Kruskal-Wallis test was applied to detect significant differences between permeation of actives in PG and in formulations. The Statgraphics plus 5 software (Statgraphics. Net, Spain) was used for statistical analysis. Significant differences in the mean values were evaluated by the F test, a p value ≤ 0.05 was considered significant.

2.5. Analytical conditions

The chromatographic separations were performed in a Waters Acquity system coupled to a Waters Xevo TQS mass spectrometer detector, using a Waters Acquity UPLC BEH C18 (1.7 µm, 2.1x50 mm) column at 40ºC. The autosampler temperature was 8ºC. The mobile phases consisted of water containing 0.05% of formic acid and 2.5 mM of ammonia (solvent A) and acetonitrile (solvent B) at a flow rate of 400 µL/min.

3. Results and discussion

The objective of this study was to study 20 compounds (Table 1) used in the clinics, which show different mechanisms of action and physicochemical properties to evaluate skin permeability using different methodologies. We expected that their different physicochemical properties would translate into a wide range of skin permeability, allowing us to make conclusions regarding the link between the physicochemical properties of the actives and the permeability prediction obtained on the *in silico* and *in vitro* models. A subset of 9 compounds were additionally evaluated in the Franz cells assay in two different forms: dissolved in PG and as marketed formulations. Results can be applied to define a screening cascade for new molecules in dermatology research.

3.1. Skin permeability prediction by in silico models

Mathematical models predict skin permeability based on physicochemical properties of the molecules. They have been used for the risk assessment of chemicals that may be in contact with the skin either accidentally or by design (Chen et al., 2013). Among different available models, we selected Potts & Guy (Potts and Guy, 1992) and Barrat (Barrat, 1995) models to be used in this work. Potts & Guy model was defined considering the existing skin permeability data from a variety of sources, that include compounds with MW ranging from 180 to over 750 Da, logKow values between -3 and +6 and encompass broad therapeutic and structural classes (Mitragotri et al., 2011). It assumes that the lipophilic *stratum corneum* is the rate-limiting factor of skin permeation, therefore lipophilicity becomes very relevant in the calculation and this model significantly underpredicts skin permeability of many hydrophilic compounds. Barrat (Barrat, 1995) reported an improved model for the calculation of permeability coefficients. It considers an additional parameter, the MP as an independent variable in addition to the logKow and MV. The MP describes the solubility of the chemicals in both polar and non-polar solvent. The accuracy in the prediction of the permeability with this model was demonstrated with 60 molecules including small molecules and steroids (Barrat, 1995).

We calculated the skin permeability coefficient (logKp) of the selected compounds using the two mathematical models (Table 2). For data analysis, the physicochemical properties of the studied molecules are shown. Lipophilicity is considered as the logKow at pH 5.5, corresponding to the average pH of *stratum corneum* (Fluhr and Elias, 2002). Compounds were ranked according to the calculated logKp in each model (Figure 1).

In the Potts & Guy model, the compound with the lowest estimated permeability was clindamycin, with a logKow value of -1. In contrast, the compounds with the highest predicted permeability value in this model were the most lipophilic ones, such as bexarotene, tazarotene and adapalene, with logKow values around 5 and MW around 400.

In the Barratt model, compounds with the lowest predicted permeability turned out to be fluorouracil and adapalene. The MP of these compounds was above 280ºC and the lipophilicity and the MV varied among them. The most permeable compounds in the Barratt equation were terbinafine, tazarotene, lidocaine and calcipotriol. In this case, a combination of lipophilicity, MV and MP parameters resulted in a high logKp value.

When comparing permeability coefficients obtained in the two models, both predict the poorest skin permeability for flurandrenolide and clindamycin and the highest permeability for tazarotene. The rest of compounds were similarly ranked, with the exception adapalene whose estimated logKp values varied significantly depending on the model.

3.2. Permeability of compounds in the Skin-PAMPA assay

The effective permeability coefficient (P_e) obtained from the Skin-PAMPA assay provides information about the ability of a compound to diffuse across a synthetic membrane in buffer at pH 5.5, to mimic both the composition and the pH of the *stratum corneum*.

The whole set of compounds were evaluated in the Skin-PAMPA assay and the logPe coefficients were determined (Table 2 and Figure 1). The permeability of the molecules can be explained by the ratio of the charged and uncharged species at the studied pH from the donor compartment. In this case, basic compounds showed a tendency toward slightly lower rank of permeability constants (from -6 to -8) compared with the values obtained for neutral or acidic compounds. Compounds showing medium permeability are acidic compounds with low lipophilicity, as well as neutral and basic compounds with high value of lipophilicity (physicochemical properties detailed in Table 1). Bexarotene, clobetasol and betamethasone appeared to be the most permeable compounds, with logPe values of -5.4, -5.6 and -6 cm/s, respectively. Clindamycin turned out to be the less permeable compound, with a logPe value lower than -8 cm/s.

For each compound, result from Skin-PAMPA in buffer was related to its logKp obtained with the *in silico* models. A positive trend between the experimental Skin-PAMPA and the predicted Potts & Guy permeability values was found, but the correlation coefficient was low (Pearson's r = 0.43). Tazarotene and tacrolimus showed a poor relationship within the two models (Figure 2A), resulting in a much lower observed permeability than predicted by the *in silico* model. Although the reasons for this discrepancy would need further refinement, we hypothesize that both molecules are neutral and display high lipophilicity (logKp>5) that can translate into a high retention into the membrane. When the analysis was repeated removing these two compounds, the correlation improved significantly. In contrast, no relationship between Skin-PAMPA and Barrat data was observed (Figure 2B).

Based on Skin-PAMPA results, representative compounds showing high, medium and low logPe values were selected to be further analyzed in the Franz cell assay.

3.3. Skin permeation of topical drugs in the Franz diffusion cell assay

We used Franz cell assay to evaluate the *in vitro* skin permeability of 9 compounds using pig skin. Compounds were tested in Franz cell assay both in PG solution and in marketed formulations. Formulation forms were mainly creams, except for tacrolimus ointment, metronidazole gel and tazarotene gel.

The study of compounds formulated in PG aimed to gather useful data for new chemical entities in a discovery setting, where final formulations are not yet available. PG was chosen because it is widely used in skin formulations as a co-solvent for poorly soluble materials and can solubilize all actives of this work (Lane et al., 2012). For that purpose molecules were tested at the same concentration than their commercial formulation, except for fluorouracil that was tested at a concentration of 2 mg/g, based on its maximum solubility in PG.

In the Franz cell assay with and PG formulations (Figure 3), tazarotene and ketoconazole exhibited the lowest permeation through skin (below 5% of the applied dose). The permeation of the other compounds did not exceed 10% of the applied dose, except for lidocaine and metronidazole. These two compounds showed the highest skin permeation and more than 30% of the applied amount was

detected in the receptor fluid. Thus, we considered a value of >30% of permeation for lidocaine and metronidazole.

As the formulation plays a critical role in the skin delivery of topically applied molecules, we evaluated the skin permeation of drugs in marketed formulations. Results of the permeated values for the different compounds in their commercial formulations ranged from 0.8 to 11% of the applied dose (Figure 3). In all cases main percentage of the compound was in the wash solution samples (>80%, data not shown) and variability was similar to Franz cell studies in PG. Amongst the commercial formulations tested, betamethasone, fluorouracil, ketoconazole, tazarotene and clobetasol showed the lowest permeation results (0.8-2.2% of the applied dose). In contrast, a higher range of permeation was obtained for lidocaine, flurandrenolide, metronidazole and tacrolimus (6.4-10% of the applied dose).

From the comparative of the results in Franz cell assay, higher or similar permeability of compounds in PG was observed in comparison to formulations. Six over the nine actives assayed presented a significant higher penetration ($p<0.05$) when they were vehiculized in PG than in the commercial formulations. This was expected because PG acts as a penetration enhancer that alters the *stratum corneum* and favors the permeation of the drugs (Schneider et al, 1996; Trommer and Neubert, 2006; Trottet et al, 2004; Carrer et al., in press). Differences in the kinetics of the skin absorption could exist between formulations and PG. Therefore, it would be also interesting to evaluate kinetics at a steady state flux to be compared with the percutaneous absorption results at non-steady state flux.

When the compound permeation values from Franz cell were compared with Skin-PAMPA results (Table 2), similar rank in permeability was found for the studied compounds, except for tacrolimus, metronidazole and ketoconazole. In our hands, tacrolimus showed a good skin permeation in Franz cell assay which was predicted by *in silico* models, but was categorized as a low permeability compound according to the Skin-PAMPA assay. Metronidazole's high skin permeability and ketoconazole's low skin permeability was not estimated by neither the *in silico* models nor Skin-PAMPA assay. Additionally, the low skin permeation of tazarotene was in alignment with the Skin-PAMPA result, in contrast to the *in silico* models (with a high logPe value).

Following the same approach with the in silico and Skin-PAMPA results, we found low correlation between the amount of compound in the skin strata in Franz cell assay with the permeability values in Skin-PAMPA and *in silico* models. The lack of correlation can be explained because of the differences in the readouts (Franz permeation data at one time-point and permeability kinetics in Skin-PAMPA and in silico models), as well as the dose of compound considered in each model (Franz cell experiment was run in a finite dose, whereas Skin-PAMPA and in silico models are set in infinite doses).

In summary, most results from Franz cells studies were aligned with the predicted values in the Skin-PAMPA and/or *in silico* models. Discrepancies were observed with metronidazole that displayed a much higher permeation in Franz cells than predicted permeabilityfrom *in silico* and skin PAMPA models and ketoconazole, which showed a much lower permeation in Franz than the predicted from Potts & Guy, Barrat and Skin-PAMPA models.

3.4. *Overall analysis*

The models used in the present study allow to classify compounds according to their permeability with different degrees of complexity. We have shown that, in general, results from the different methods provide similar rankings, with some exceptions that can be explained by the differences in the methodologies and the diversity in physicochemical properties of the compounds. Franz cell assay could be taken as a reference assay because it uses whole thickness skin. Nevertheless, the real comparison should be done with *in vivo* pharmacokinetic studies in minipigs and validated later on with clinical results.

The value of permeability of a compound alone provides limited information and it needs to be put in context with several factors such as the potency of the drug, its concentration in a formulation, the composition of the formulation as well as properties of the skin aimed to receive the treatment.

We have evaluated marketed formulations in Franz cell model with concentrations in a range from 0.5 mg/g (corticosteroids such as betamethasone) to 50 mg/g (fluorouracil). These drugs showed a similar skin permeation (expressed as % of applied dose), but the total amount of permeated compound was 0.13 µg for betamethasone and 8 µg for fluorouracil. This is in agreement with the

marked differences in *in vitro* potency of these drugs in keratinocytes, IC_{50} of 2.3 nM for bethamethasone and 1400 nM for fluorouracil (Schoepe et al., 2010; Lamberti et al., 2014). To confirm that the permeated compound is exerting a pharmacological activity, studies of target engagement using whole thickness human skin biopsies have been developed (Smith et al., 2016). These studies are feasible when the effect of the drug on the target has a measurable direct readout and can be viewed as a pharmacokinetic/pharmacodynamic assay, providing an integrated view of penetration and target coverage following the application of a formulation to the skin.

The skin barrier function of the skin depends on the properties of the *stratum corneum* which vary depending on multiple factors such as the hydration status of the skin, the anatomic location or the presence of a disease state. Face and flexural areas on one hand, and palms and soles on the other, represent the two opposite bands of the spectrum with the thinnest and the thickest epidermis, respectively (Kakasheva-Mazenkovska et al., 2011). Some skin diseases, for example warts, display reduced penetration in the affected area due to the presence of hyperkeratotic lesions (Sterling et al., 2014); whereas others, such as atopic dermatitis, show higher permeability to drugs due to disruption of the barrier function (Halling-Overgaard et al., 2017). This adds another degree of complexity in the estimation of the skin permeation, because Franz cell studies are generally run in healthy human or pig skin.

Formulation plays a critical role in the delivery of the active into and through the skin. We have been using commercial formulations to see how efficient were those formulations to deliver the actives. In addition, experiments with actives dissolved in PG enabled us to compare permeability in a common solvent. These results, though, need to be taken cautiously since saturation degree of each active in the vehicle has an impact on the final outcome (Lane, 2013).

In the early research process, tools are needed to support selection and characterization of compounds in terms of skin permeability. The complexity of the assays should be considered in the prioritization of these in a drug discovery screening cascade. *In silico* approaches such as Potts & Guy and Barrat models are helpful tools to identify those molecules with challenging physicochemical properties for the topical route. It is important to consider their limitations when predicting permeability of hydrophilic molecules, as demonstrated in the case of metronidazole. Skin-PAMPA stands out as

high throughput assay to evaluate molecules before testing them in the laborious assay such as Franz cell. Finally, the evaluation of advanced molecules from discovery projects in the Franz cell assay in PG is proposed for its characterization in terms of skin penetration before further optimization of an advanced formulation.

Overall, generated data reflects the complexity in the estimation of skin permeability of molecules by topical route. Since skin is an extremely efficient barrier, to overcome the *stratum corneum* becomes a challenge for topical drugs.

4. Conclusions

The prediction of the skin permeation of 20 representative topical marketed drugs using *in silico* models and Skin-PAMPA assay (for 20 compounds), as well as Franz cell assay (a subset of 9 drugs) allowed to grade compounds according to their permeability value in each assay. Comparison of the predicted values showed good overall agreement for all compounds, except for metronidazole and ketoconazole. This reflects the complexity in the estimation of the skin permeability of topically applied drugs, because of the effectiveness of the skin as a barrier. Nevertheless, the employed models are useful tools to support selection and characterization of research compounds in terms of skin permeability in drug discovery.

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Table 1: Physicochemical properties of studied compounds

Compound	MW	MV	MP (ºC)	logKow	Type of compound
Adapalene	412	336.5	316	4.94	Acid
Azelaic acid	188	162.9	110	-0.16	Acid
Betamethasone dipropionate	505	411.6	187	3.96	Neutral
Bexarotene	348	307.3	225	5.50	Acid
Calcipotriol	413	372.5	114	3.84	Neutral
Clindamycin	425	350.5	108	-1.00	Basic
Clobetasol propionate	467	367.4	199	4.18	Neutral
Dapsone	248	180.4	173	1.27	Neutral
Diclofenac	296	212.3	276	2.75	Acid
Diphenhydramine	255	225.0	171	0.52	Basic
Dithranol	226	169.8	179	3.04	Neutral
Fluorouracil	130	84.4	285	-0.66	Neutral
Flurandrenolide	437	355.7	216	1.56	Neutral
Ketoconazole	531	405.8	151	3.65	Neutral
Lidocaine	234	214.4	69	0.61	Basic
Metronidazole	171	129.3	161	-0.46	Neutral
Salicylic acid	138	100.5	161	-0.67	Acid
Tacrolimus	804	692.5	129	5.59	Neutral
Tazarotene	351	290.2	104	5.22	Neutral
Terbinafine	291	269.3	42	2.36	Basic

Table 2: *In silico* log Kp values from Potts &Guy and Barrat models, *in vitro* logPe values from the Skin-PAMPA and skin permeability from Franz cell assays.

Compound	Potts&Guy	Barrat	Skin-PAMPA	Franz cells assays permeation (%)	
	log Kp(cm/s)	log Kp (cm/s)	logPe (cm/s)	PG	Formulation
Adapalene (Ada)	-5.3	-13.8	-6.4		
Azelaic acid (Aze)	-7.6	-8.3	-7.3		
Betamethasone (Bet)	-6.6	-10.2	-6.0	6.4	1.3
Bexarotene (Bex)	-4.5	-9.5	-5.4		
Calcipotriol (Cal)	-6.1	-7.1	-5.6		
Clindamycin (Cli)	-9.6	-10.7	-9.1		
Clobetasol propionate (Clo)	-6.2	-10.1	-5.6	6.9	2.2
Dapsone (Dap)	-6.9	-9.7	-6.5		
Diclofenac (Dic)	-6.2	-12.9	-4.4		
Diphenhydramine (Dip)	-7.5	-10.7	-8.1		
Dithranol (Dit)	-5.5	-8.4	-6.0		
Fluorouracil (Flo)	-7.6	-14.8	-6.7	5.9	0.8
Flurandrenolide (Flu)	-7.8	-12.8	-6.2	6.8	7.2
Ketoconazole (Ket)	-6.9	-9.0	-6.2	4.3	2.2
Lidocaine (Lid)	-7.3	-6.5	-6.1	>30.0	6.0
Metronidazole (Met)	-7.7	-10.2	-8.2	>30.0	9.8
Salicylic acid (Sal)	-7.6	-10.1	-6.5		
Tacrolimus (Tac)	-7.2	-9.3	-9.7	9.1	10.0
Tazarotene (Taz)	-4.7	-4.9	-8.6	2.7	1.8
Terbinafine (Ter)	-6.4	-4.6	-6.9		

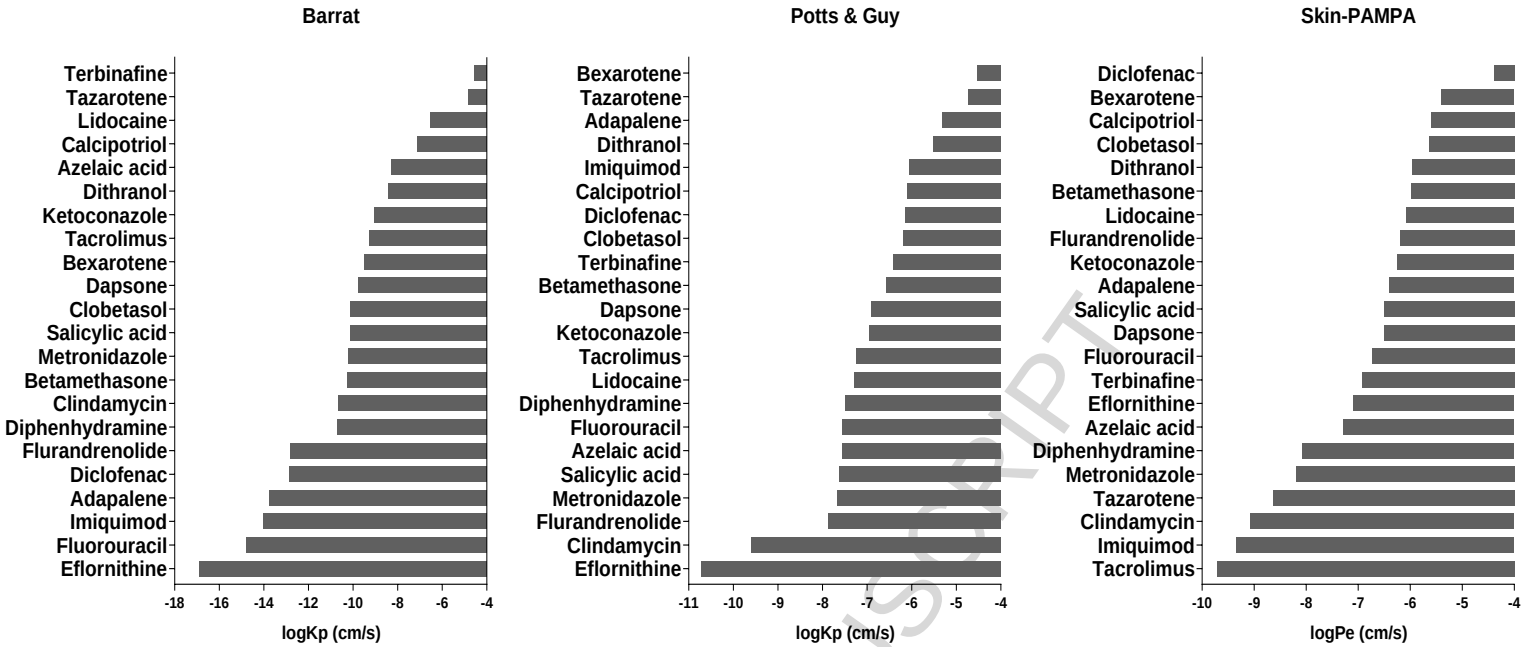

Figure 1. Permeability values of compounds from Potts & Guy model, Barrat models and Skin-PAMPA assay. Compounds are ordered from the one with the highest to the one with the lowest permeability value.

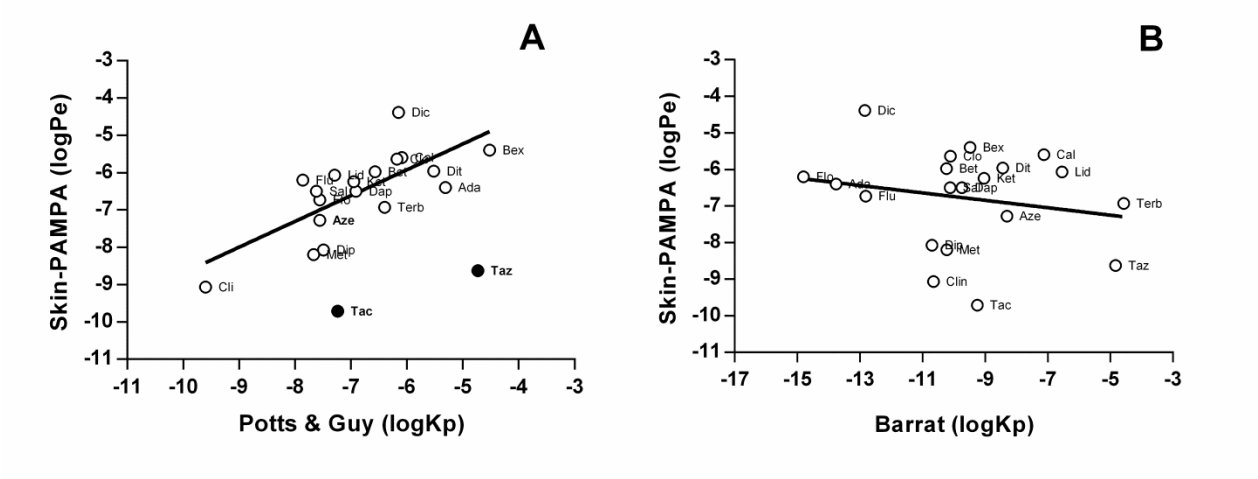

Figure 2. Comparison between the permeability coefficients from the Skin-PAMPA assay with the *in silico* models, Potts & Guy (A) and Barrat (B). (A) Tacrolimus and tazarotene are indicated in black. When these compounds are excluded, a significant positive correlation between permeability from the two models is found (Pearson's r = 0.722; p<0.001; N=18). Line represents linear regression of data (y = 0.6914x – 1.775; R^2 = 0.52; N=18). **(B)** Poor correlation between permeability from the two models is found (Pearson's r = -0.2021; N=20). Abbreviations used per each compound are indicated in Table 2.

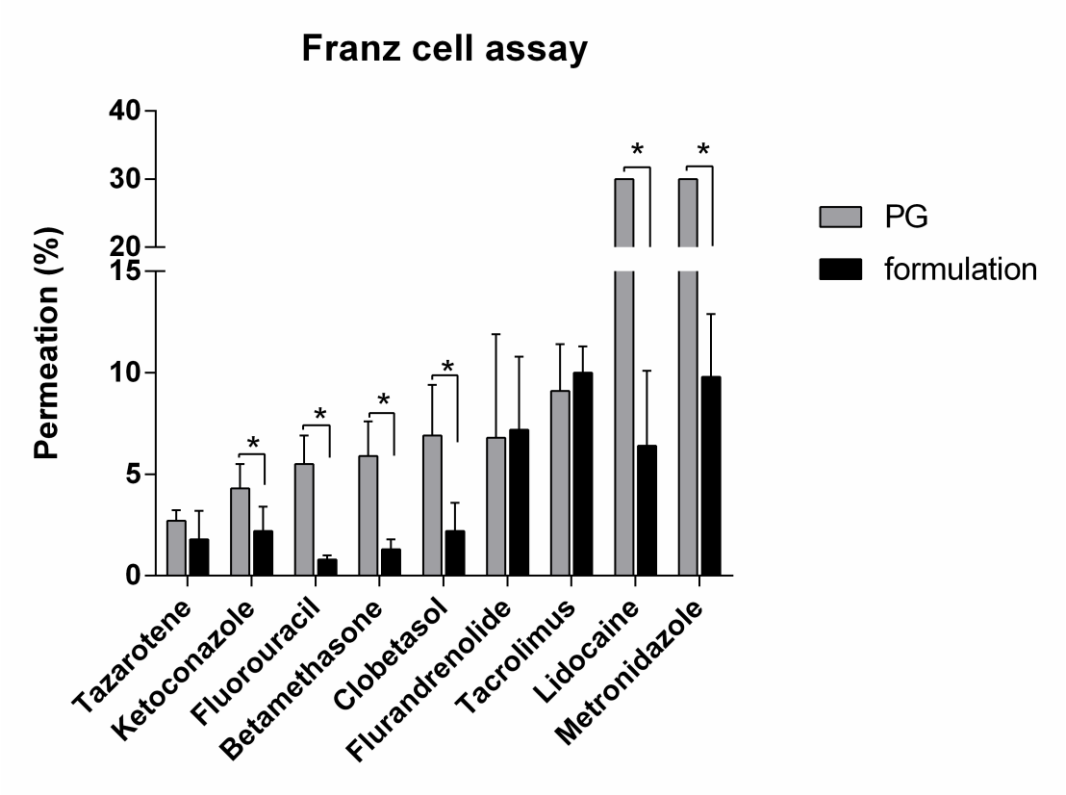

Figure 3. Skin permeation of compounds in PG and formulation in the Franz cell assay. Bars represent mean ± SD from 6 replicates. Permeation value for lidocaine and metronidazole was considered a value of 30%. * indicates statistical significance of $p \leq 0.05$.