Topical treatment of infantile haemangiomas: a comparative study on the selection of semi-solid vehicle

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Running Head: Critical features of propranolol loaded semi-solid preparations intended for treating haemangiomas.

Keywords: Haemangiomas, propranolol, topical delivery, semi-solid preparations, skin permeation, vehicle selection

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Abbreviations

PR-Cl	Propranolol HCl
IH	Infantile haemangiomas
$Q_{R,t}$	Cumulative amount released at sampling time (t)
J	Permeation flux at steady state
$Q_{P,t}$	Cumulative permeated amount at sampling time (t)
Q_{ret}	Retained drug amount within the epidermis after 24 h
PBS	Phosphate buffer solution

Abstract

Background/Aim: The topical β-blockers have been recently proposed as a valid alternative to oral drugs for treating cutaneous infantile haemangiomas (IH), but the clinical results in literature were inconsistent due to the empirical choice of topical preparations. The current investigation aimed to rationalize the selection of semi-solid vehicle for locally-applied drug product containing 1% w/w Propranolol HCl (PR-Cl). **Methods**: A hydrophobic ointment of PR-Cl, two lipophilic creams and a hydrophilic one were prepared. *In vitro* release and skin permeation through human epidermis and full-thickness skin studies were performed by Franz's diffusion cells. **Results**: The overall results highlighted that PR-Cl is able to permeate the human epidermis and its penetration pattern is strongly influenced by the composition of semi-solid vehicle. PR-Cl release and permeation from lipophilic vehicles is extremely limited and influenced by their composition. Best results were obtained by using the hydrophilic cream. Furthermore, the retention study evidenced that epidermis acted as a reservoir releasing the PR-Cl accumulated after preparation removal. **Conclusion**: The 1% w/w PR-Cl cream resulted the most suitable candidate for improving the drug permeation through human epidermis. On the contrary, the negligible permeation profile through full-thickness skin pointed out that PR-Cl cannot diffuse significantly to reach the deeper layers of human skin.

1 Introduction

Propranolol (PR) is widely accepted by the paediatrician community as first-line therapy for the management of infantile haemangiomas (IH) [1, 2], which are the most common benign tumours of infancy and affect 2% of infants and around 12% of children under 12 years old. IH are characterized by a rapid and intermittent growth of the tumour mass, followed by a spontaneous regression in most cases. Although IH spontaneously involve in the 90% of cases, pharmacological treatment or surgery is frequently required to prevent disfigurement and functional impairment (*e.g.*, obstruction of airways and vision, cardiac insufficiency, hypothyroidism) [2].

The PR effectiveness was firstly documented in 2008, when Léautè-Labréze et al. observed a rapid regression of IH in a patient who received the beta-blocker for treating a pre-existent cardiovascular disease [3]. In the last decade, other clinical trials have supported the replacement of oral corticosteroids with oral PR as first-line treatment, since the higher effectiveness and the lower risk of side effects [4-6]. Meanwhile, topical preparations containing propranolol hydrochloride (PR-Cl) and timolol were proposed as an alternative treatment for superficial IH, especially for treating new-borns (**Table 1**) [7-11]. Although the published data seem to demonstrate the clinical efficacy of topical PR, no therapeutic protocols and guidelines have been established yet. Indeed, the results of clinical studies published in literature are ambiguous due to the empirical choice of semi-solid bases other than the differences in clinical protocols (*e.g.*, study design, selection of patients, endpoints) [11].

To be efficacious, the semi-solid drug product has to release enough PR-Cl to guarantee a sufficient drug concentration at the interface with human epidermis for sustaining its permeation through the s*tratum corneum* and, consequently, for reaching therapeutic concentrations at the target site. In general, the drug permeation through the human epidermis is a passive diffusion process that depends on both the physicochemical properties of drug (*e.g.*, molecular weight, Log P) and vehicle. In the case of PR, several studies have demonstrated a good permeation profile through human skin, but this process is strongly related to its ionization rate and the type of counter ions [12, 13]. On the other side, the composition of semi-

solid vehicle is critical as well because it strongly influences the drug physicochemical stability and its release and permeation profiles [14-16]. Moreover, the introduction of other excipients, such as preservative, to the composition of the semi-solid product should be also considered carefully since they can alter the inner environment of the semi-solid matrix, modifying its effectiveness in drug delivering. Therefore, the selection of an appropriate vehicle and the rationalization of its composition should be carefully studied as it might influence the clinical efficacy of the drug product by the modification of drug permeation profile; the previously reported case of PR-Cl is an example.

This study was focused on investigating *in vitro* how the permeation profile of PR-Cl could be influenced by the choice of the semi-solid vehicle. In particular, four vehicles were chosen among well-known semi-solid bases that were widely used by compounding pharmacists and were similar to preparations already tested *in vivo* in the existing literature [1, 9, 10]. The formulations were compared in terms of drug release and permeation through human epidermis and full-thickness skin. These model membranes were selected for sustaining with *in vitro* data the clinical evidences of topical-propranolol in treating IH with superficial and deep elements, respectively. Furthermore, the addition of the preservative methyl-paraben and its influence on the permeation pattern were also investigated.

The outcomes of the study may be of particular interest to compounding pharmacists in preparing extemporaneous preparations in hospital and community pharmacies, especially when, as in the case of the use of PR-Cl for treating IH, no industrial medicinal products containing are available in the market.

2 Materials and methods

2.1 Materials

Propranolol HCl (PR-Cl) and sodium methyl-paraben were purchased from Farmalabor srl (Italy). White petrolatum, lanolin and lanolin alcohols were purchased form A.C.E.F. Spa (Italy). Mineral oil, cetostearyl alcohol and cetomacrogol 1000 were purchased from Carlo Erba srl (Italy). Cuprophan® membrane were

purchased by Akzo Nobel Faser, (Germany). HPLC-grade acetonitrile was purchased from VWR International PBI srl. (Italy). All other reagents and solvents were purchased from Sigma-Aldrich Srl (Italy) and used without further purification.

2.2 Preparation of aqueous solutions containing propranolol HCl

Pr-Cl solutions (1% w/w) were prepared in different solvents: HPLC-grade water, phosphate buffer solution (PBS) at pH 9.0 (4008300, Eur.Ph. 8.8) and PBS at pH 5.8 (4002100, Eur.Ph. 8.8). The solutions were obtained dissolving a weighed drug amount in three solvent media with (S_1, S_3, S_5) or without the addition of the methyl-paraben (S_2, S_4, S_6; **Table 2**).

2.3 Preparation of semi-solid formulations containing propranolol HCl

The PR-Cl was incorporated at the concentration of 1% w/w into a hydrophobic ointment, two lipophilic creams and a hydrophilic cream (**Table 2**). The hydrophobic ointment (F_0) was made adding the PR-Cl to white petrolatum by geometric trituration. The lipophilic (F_1, F_2) and hydrophilic creams (F_3) were made by a melting-emulsion process. Briefly, the lipophilic components were melt at 45-50°C under constant magnetic stirring. At the same time, the PR-Cl and sodium methyl-paraben were dissolved in purified water for dispersing homogeneously the drug in the semi-solid matrix, warmed up to 40°C and then mixed to the melt lipophilic phase. The magnetic stirring was kept constant during the entire preparation process. At the end, the system was cold to room temperature and the final preparation was stored at 25°C until use. In order to evaluate the effect of preservative on *in vitro* skin permeation profiles, a batch of hydrophilic cream without sodium methyl-paraben was also prepared (F_4).

2.4 *In vitro* release study

The *in vitro* release studies were performed using Franz diffusion cells (permeation area: 0.636 cm^2; volume of receptor chamber about 3mL) and Cuprophan® as synthetic release membrane. Prior to experiments, the Cuprophan® membrane was hydrated in 0.9% w/v NaCl solution for 1 h. At the beginning of experiment, amount of PR-Cl loaded semi-solid formulations (120 mg) were applied to the surface of Cuprophan® membrane in 1-mm thick layer by means of excavated polyethylene disk. Then, the sample was mounted on

the Franz diffusion cells, whose receptor compartments were filled with degassed 0.9% w/v NaCl solution containing 100 µg/mL NaN_3 as preservative. Special care was given to avoid air bubbles between the membrane and the solution in the receptor compartment. The upper and lower parts of the Franz cell were sealed with Parafilm® and fastened together by means of a clamp. The system was kept at 37°C with a circulating water bath, so that the membrane surface temperature was at 32 ± 1°C throughout the experiment. At predetermined times (1, 3, 5, 7, 24 h), 200 µL samples were withdrawn from the receiver compartment and analysed by HPLC. The withdrawn aliquot was replaced with the same volume of fresh receiver medium. Sink conditions were maintained throughout the experiments. The results were expressed as the average of parallel experiments performed in triplicate. The cumulative amount released ($Q_{R,t}$) from the semi-solid formulation per unit area was calculated from the drug concentration in the receiving medium and plotted as a function of time. In agreement with Higuchi equation [17], the rate of drug release is calculated as the slope of the cumulative released amount plotted versus the square root of time between 1 h and 7 h.

2.5 *In vitro* skin permeation study

The *in vitro* permeation and retention studies were performed using human epidermis as membrane. The human epidermis originated from the abdominal skin of a donor who underwent cosmetic surgery. The full-thickness skin was sealed in evacuated plastic bags and stored within 6 h after removal. Epidermis samples were prepared following an internal standard procedure [18]. Briefly, prior to experiments, the skin was thawed at room temperature, and the excess of fat was carefully removed. The skin sections were cut into squares of about 4.0 cm^2 and, after immersion in water at 60°C for 1 min, the epidermis was gently separated from the remaining tissue with forceps.

At the beginning of permeation experiment, tested formulations were applied on the *stratum corneum* of each epidermis sample. In particular, 0.5 mL of PR-Cl solutions was loaded directly in the donor compartment of each cell, whereas about 120 mg of semi-solid formulation containing PR-Cl was applied using the same polyethylene disk of the release study. Then, the *in vitro* permeation study was carried out following the same experimental protocol described above. The receptor phase samples collected during the experiment

were analysed by HPLC. The results obtained were expressed as the average of parallel experiments performed in triplicate. Furthermore, the best formulation in terms of release and permeation profiles were re-tested using full-thickness skin as membrane.

The cumulative amount permeated through the skin per unit area (Q_P) was calculated from the drug concentration in the receiving medium and plotted as a function of time. The steady state flux (J) was determined as the slope of the linear portion of the plot.

2.6 *In vitro* retention studies

At the end of the permeation experiments, the epidermis sheet was removed from Franz diffusion cell and each side was gently treated with 5 mL of methanol to wash out the unabsorbed drug. Subsequently, the sample was dried, thinly sliced and placed in 5 mL of fresh methanol. The suspension was soaked in a sonicator for 30 min and then maintained for 24 h at 2-8°C. Finally, the supernatant was 0.22 µm filtered and analysed by HPLC. The results were expressed as the average of parallel experiments performed in triplicate. The drug-retained amount (Q_{ret}) was expressed as micrograms of PR-Cl per unit area of epidermis.

In order to evaluate the ability of epidermis to act as reservoir compartment during the drug biodistribution, the PR-Cl amount released from epidermis after removal of the formulation used in the permeation experiments was further investigated. At the end of permeation experiments, the epidermis was removed from Franz diffusion cell and the semi-solid formulation was accurately discarded from the upper side. To wash out the unpenetrated drug, the epidermis was gently treated with 10 mL of 0.9% w/v NaCl solution, to avoid any possible damages to the epidermis structure. Then, the epidermis sheets were re-mounted on the Franz diffusion cells filled with fresh receptor phases following the same procedures of in vitro permeation study. At predetermined times (1, 3, 5, 7, 24 h), 200 µL samples were withdrawn and analysed by HPLC according to the method described above. The results were expressed as the average of parallel experiments performed in triplicate. The cumulative amount released from the epidermis per unit area was calculated from the drug concentration in the receiving medium and plotted as a function of time.

2.7 HPLC analyses

The amount of PR-Cl was determined by high performance liquid chromatography (HPLC; HP 1100 ChemStations, Agilent Technologies, Santa Clara, US), equipped with ultraviolet detector at 230 nm. Phosphate buffer pH 2.5/acetonitrile (70/30, v/v) was used as mobile phase at a flow rate of 1.5 mL/min and the analysis temperature was fixed at 25 °C. The compound separation was carried out using reverse-phase column (Hypersil Gold, 5 µm, 150 x 4.6 mm, Thermo Fisher Scientific Inc., Waltham, USA) and the injection volume was set at 20 µL. The retention time of propranolol HCl was 3.0 min and three calibration curves were constructed in the overall range of 0.02-160 µg/mL.

2.8 Solubility parameter

The solubility parameter (δ) is defined as the square root of the cohesive energy density as described by **Eq.1**:

$$\delta = \sqrt{\frac{\Delta EV}{V_m}} \quad (1)$$

where ΔEV represents the energy of vaporization and V_m is the molar volume of the material. As reported in **Table 2**, the δ value of each lipophilic ingredients of semi-solid formulation were derived from literature [15, 19, 20]. Since no δ values for Lanolin and its alcohols were reported in literature, their solubility parameters were estimated equal to the δ value of the cholesterol, which was the most abundant component in the lanolin compositions. As the solubility parameter was an additive property, the δ values for tested formulations (**Table 3**) were derived according to the following equation (**Eq.2**)

$$\delta = \sum \delta_i \varphi_i \quad (2)$$

where δ_i is the solubility parameter of the excipient and φ_i is its volume fraction [21].

2.9 Data analysis

The performances of the samples and the correlation between release data and permeation/retention ones were compared by t-student tests (Excel 2013® Microsoft, Redmond, US). The level of significance was taken as p-value < 0.05.

3 Results

3.1 *In vitro* release study

As shown in **Table 3**, the hydrophilic cream (F_3) was the best formulation in terms of $Q_{R,24}$ and release rate. Indeed, the formulation F_3 released around 20% w/w of drug loaded within 1 h and 74.70 ± 14.90 % at 24 h. On the other side, the hydrophobic ointment (F_0) showed a negligible released rate and less than 1% of loaded drug was released during all the experiment. The release performances of the two lipophilic creams could be considered between these two extremes. In particular, the release rate of both creams F_1, F_2 was significantly higher in comparison of F_0 (p-value < 0.03) and more than twenty-time lower than cream F_3 (p-value < 0.01; **Table 3**). However, the release rate of the formulation F_2 was three times higher than F_1 within 7 h (p-value < 0.05), whereas the $Q_{R,24}$ resulted twice higher (p-value < 0.01).

3.2 *In vitro* skin permeability study

The PR-Cl permeated amounts measured by using the semi-solid preparations were lower in comparison to that of the reference solution, S_1 (**Figure 1**). After a time lag of about 2 h, the permeation profile of solution S_1 resulted linear (R^2 = 0.992) with a steady state flux of 15.40 ± 3.17 μg/cm^2/h. A similar trend was also observed for the hydrophilic cream (F_3), although the lower permeation flux (*J:* 2.70 ± 0.33 μg/cm^2/h) and a slightly higher lag time (*i.e.*, 2 h 44 min) than those of S_1. On the other side, the steady state fluxes of creams F_1 and F_2 cannot be calculated within 24-hours since the lag time resulted higher than 8 h. The formulation F_2 resulted in having a higher $Q_{P,24}$ in comparison to formulation F_1 (**Table 3**). The $Q_{P,24}$ was always lower the limits of quantification of the analytical method in the case of hydrophobic ointment. Anyhow, the cumulative permeated amounts at the end of experiments did not exceed the 5% w/w of the initial drug loading both for solutions and for all the semi-solid formulations.

The results of retention study confirmed the permeability trend and they highlighted a linear correlation between $Q_{P,24}$ and Q_{ret} (R^2: 0.92). When PR-Cl was used as water solution (S_1), the Q_{ret} was 171.39 ± 7.95 μg/cm^2 (Table 4), which was statistically different from the Q_{ret} values of all the semi-solid preparations (p-value < 0.05). Among them, the Q_{ret} decreased in this order (**Table 3**): hydrophilic cream F_3 > lipophilic cream

F_2 > lipophilic cream F_1 > hydrophobic ointment F_0. The retained amount ranged from 5.00% (F_3) to 0.80 % (F_0). The **Figure 2** showed that the epidermis was able to release the retained PR-Cl after F_3 application: the resulting flux was*:* 1.25 ± 0.18 µg/cm^2/h. Nevertheless, the released amount after 24 h is 41.10 ± 11.92 % of the Q_{ret}, suggesting that the human epidermis acted as reservoir compartment of PR-Cl and released the drug for more than 24 h.

Finally, the hydrophilic cream, which was used also in the permeation studies performed with full-thickness skin, showed only a negligible PR-Cl permeated amount after 24 h (< 0.01 µg/cm^2), suggesting that tested semi-solid vehicles might not be suitable in a no-damaged skin for allowing drug to reach the deep skin levels.

3.3 The influence of the preservative on skin permeation profile

As shown in **Table 4**, the highest fluxes of PR-Cl were observed in the presence of methyl-paraben: *J* of PR-Cl loaded in the hydrophilic cream was three-times higher in presence of sodium methyl-paraben (F_3 vs F_4; p-value < 0.01). These findings were also confirmed by studies carried out with water solutions of PR-Cl (S_1, S_2), where the effect appeared even more significant. However, comparing results of S_1-S_2 solutions to those obtained by the pH-buffered ones (S_3-S_6), it is noteworthy that the enhancement effect attributed to sodium methyl-paraben was due to the pH increase of the vehicle. Indeed, the *J* value of S_1 was similar to S_3 and S_4, where pH was buffered at value near to the pK_a of propranolol (9.5 ± 1.2) [22]. On the contrary, adjusting the vehicle at the value of 5.8, the impact of preservative was negligible (*i.e.*, S_5 vs S_6) and *J* value was comparable to S_2. The pH of 5.8 was used because it was similar to the value of skin surface and of propranolol was almost all ionized. Finally, the paraben also improved the Q_{ret}, but this effect was less significant in the case of the hydrophilic cream than water solution. Indeed, the Q_{ret} of S_1 was slightly higher than the value of S_2 (p-value < 0.05), whereas the results obtained by F_3 and F_4 were not statistically different (p-value = 0.20).

4 Discussion

The present study is focused on the evaluation of the permeation and retention profile of PR-Cl delivered by four semi-solid vehicles. Since contradictory data about the effectiveness of topical-propranolol treatment of IH were reported in literature, the impact of vehicle composition on the *in vitro* skin permeation through

human skin was investigated in depth. Therefore, the tested formulations were chosen among those well-known by compounding pharmacist [23] that were similar to those already tested in clinical trials [1, 9, 10]. All the selected preparations were feasible in community or hospital pharmacies and contained cheap excipients that have a good compatibility with the drug substance. In particular, the four types of considered semi-solid preparations were a hydrophobic ointment (F_0), two lipophilic creams (F_1, F_2) and a hydrophilic cream (F_3). The two lipophilic creams differ for the content of emulsifiers or dispersing agents and have a very limited water amount. The four preparations also differentiated each other in relation to the way to incorporate the PR-Cl: directly as solid salt (F_0) or as water solution (F_1-F_3). All of them showed an acceptable uniformity of drug content and resulted stable at room temperature for at least 6 months after the preparation (data not shown).

The PR-Cl concentration at the therapeutic site after topical application has been directly related to its ability to penetrate the *stratum corneum* and to permeate through human epidermis. However, the drug should firstly diffuse through the semi-solid matrix and be released for allowing the skin penetration.

In general, the drug released from a semi-solid matrix can be described by models derived by the Higuchi equation [17, 24]. In particular, if the drug was dissolved in the matrix, the cumulative released amount (m) can be calculate by the following equation:

$$m = 2 \times C_0 \sqrt{\frac{D_m t}{\pi}} \quad (3)$$

where, D_m is the drug diffusion coefficient, C_0 is the drug solubility in the releasing matrix and t is the time.

On the other side, when the drug is dispersed as solid in the semi-solid matrix, **Eq. 3** cannot be applied and m can be described by the **Eq. 4**:

$$m = \sqrt{2 \times D_m \times C_s \times \left(Q - \frac{C_s}{2}\right) \times t} \quad (4)$$

where, C_s is the saturated drug solubility and Q is the total drug amount in the semi-solid matrix. If Q resulted >> C_s, as it is expected in the case of formulation F_0, **Eq.4** is transformed in **Eq.5**.

$$m = \sqrt{2 \times D_m \times C_s \times Q \times t} \quad (5)$$

According to **Eq.3**, the drug release from the hydrophobic ointment F_0 was not only governed by the drug loading (Q), but also by C_s and D_m. In particular, considering that the drug loading of F_0 was equal to the other semi-solid formulations, the negligible release rate reported in **Table 3** for F_0 suggested that C_s and D_m were the bottleneck parameters of drug release process due to the low drug solubility and diffusion through the white petrolatum. On the contrary, the dissimilarities observed in release rates for the other semi-solid formulations might be related only to changes in D_m (**Eq.3**). Indeed, since PR-Cl was dissolved before incorporation in the semi-solid matrix, C_0 could be considered similar among formulations F_1-F_3 and, therefore, the release profiles could be influenced only by the changes in the drug diffusion through the semi-solid matrix due to the dissimilar composition of vehicles. To support such hypothesis, solubility parameters (δ) were calculated for the lipophilic part of F_0-F_2 (**Table 2**), as proposed by Vaughan [19]. The derived δ values increased in function of the percentage of amphiphilic excipients in the semi-solid formulation, ranging: F_0 (7.33 cal/cm^3) > F_1 (7.47 cal/cm^3) > F_2 (8.44 cal/cm^3). Considering that δ value of propranolol was 12.02 cal/cm^3, the higher the δ value of semi-solid matrix, the higher the drug solubility and, therefore, D_m. Indeed, the release profiles of F_0-F_2 resulted strongly influenced to the solubility parameters (R^2: 0.99). In particular, the release rate of F_0 was the lowest since the PR-Cl cannot be solubilized significantly in white petrolatum (lowest δ value), whereas the highest affinity of propranolol for the matrix of F_2 resulted in the highest drug release.

In case of creams, the solubility parameters cannot be applied since creams were more complex systems and the higher water amount. In particular, the results suggested that the water percentage could also influenced the drug release from the cream formulations (*i.e.*, F_1-F_4). Indeed, comparing the release rate of the three creams, it is worthy observing that the higher the water percentage, the higher the release through the semi-solid matrix. Indeed, the release rate from hydrophilic cream (F_3) was twenty-five-times higher than hydrophobic cream F_2, which contained four-times less water (**Table 2**, **Table 3**).

These results were also substantiated by the human epidermis permeation/retention profiles. The hydrophobic ointment confirmed to be the worst semi-solid matrix in terms of permeated amount, whereas the hydrophilic cream guaranteed the best performance. In the case of lipophilic creams, the results showed that formulation F_2 guaranteed a higher permeation amount after 24 h than F_1. However, both formulations were not able to permit a linear permeation profiles within 24 h.

The permeation of a molecule through the human epidermis may be modelled as a passive diffusion process described by the modified first Fick's law (**Eq.6**).

$$J = K_{SC/W} \cdot D_{SCE} \cdot A \cdot \frac{C_d - C_r}{h} \quad (6)$$

where *J* is the permeation rate through the human epidermis, $K_{sc/w}$ is the partition coefficient between donor phase and stratum corneum, D_{SCE} is the diffusion coefficient, A is the application area, C_d and C_r are the drug concentrations at the two layers of the human epidermis and h is the thickness of the membrane.

By comparison of the release and permeation results, direct correlations between release rate and $Q_{P,24}$ (R^2: 0.93) and Q_{ret} were observed (R^2: 0.79), suggesting that permeation and retention processes were mainly influenced by vehicle effectiveness in drug releasing at the skin surface. In particular, as shown by **Eq.4**, the higher release rate, the higher C_d and, therefore, *J*. However, the formulation performances in drug release cannot describe completely the results obtained of permeation and retention studies. Indeed, although the release rate of the cream F_3 was about twenty-times higher than cream F_2, the ratios between the $Q_{P,24}$ and Q_{ret} values of the two formulations were equal to 3.5 and 1.7, respectively. On the other side, unlike permeation data, the retention results suggested that hydrophobic ointment was able to release enough PR-Cl to reach detectable retained drug amount in the human epidermis after 24 h. Considering drug affinity for white petrolatum previously discussed, the observed Q_{ret} of F_0 might be related to the dissolution of drug crystals, which were homogenously dispersed in the hydrophobic ointment, at the interface between skin and semi-solid formulation. On the contrary, the retention process of PR-Cl delivered by other formulations was strictly related to the permeation process sustained by the concentration gradient between membrane surface and receptor compartment. As a matter of fact, the semi-solid vehicle itself is able to influences in

many ways the permeation of molecules through the human epidermis [14, 15]. In addition to a direct effect on the drug concentration gradient, characteristics of semi-solid bases impact indirectly on $K_{SC/W}$ and D_{SCE}, inducing modifications in the inner structure of the *stratum corneum* [12, 25]. For example, the higher *in vivo* performance of lipophilic creams in comparison to the observed *in vitro* pattern could be due to the enhancement of drug permeation related to the occlusive properties of the semi-solid matrix [16, 25]. Indeed, lipophilic creams, which are more occlusive than hydrophilic ones, can alter the skin permeability of molecules by increasing the water content in human epidermis. Higher occlusive properties may explain why clinical studies showed comparable clinical efficacy for PR-Cl loaded hydrophilic, lipophilic creams or ointments [1, 9].

The results obtained by the epidermis drug release study highlighted that the human epidermis was able to be a reservoir compartment of PR-Cl [26], allowing to prolong the drug diffusion to the lower layers of human skin after the removal of topical preparation. **Figure 2** shows that human epidermis was able to release about 40% of the retained drug amount within the first 24 hours after removal. Indeed, the amount of drug retained into the human epidermis could diffuse to the superficial IH maintaining a clinical effect. Therefore, beyond permeation profiles, the vehicle effectiveness in sustaining the drug partition in the human epidermis resulted interesting since the penetrated drug amount could then diffuse slowly in deeper layer. In particular, lipophilic creams should also take advantage of the occlusive effect and to be able to sustain drug penetration in the human epidermis. Furthermore, the possibility to have a lowest PR-Cl permeated amounts seems interesting for the design of topical preparation intended to treat new-born patients. The limited drug release and permeation profiles of lipophilic creams can be advantageous in such patients because of their skin barrier resulted more permeable to xenobiotics in comparison to adults or children elder than three-year old [27-28].

Finally, the negligible permeation through the full-thickness skin may suggest that the topical-propranolol preparations were not able to increase the concentration gradient between the membrane layers enough to allow the permeation PR-Cl through the dermal layer. Therefore, the use of permeation enhancers should be

considered for improving the PR-Cl flux if the goal is to reach therapeutic sites located in deeper layers of the human skin.

If such results can explain the clinical evidences reported in literature on the low efficacy of topical propranolol in IH with deeper elements [1, 7], the findings suggested also that the use of topical preparation might be related to low PR-Cl systemic concentration, with a low incidence of systemic side effects due to the low permeation profile.

The addition of a preservative to semi-solid preparation that containing water was required for inhibiting the microbiological growth. Aiming to investigate the technological criticisms related to the topical delivery of propranolol by semi-solid formulations, sodium methyl-paraben was used as model preservative since its physicochemical properties and it's widely used in cosmetics and pharmaceutical products. About parabens, it is also noteworthy that antimicrobial activity is pH-dependent: for methyl-paraben, the optimum activity was when pH ranged between 4 and 8 [29]. Moreover, even if the use of parabens in pharmaceutical preparation is common, they have been recently associated with hormonal dysfunctions due to their penetration through the human epidermis [30, 31]. In particular, Dardreet al. published in 2004 a study revealing that methyl-paraben was found in human breast tumors [32] and opening the scientific debate about parabens' role in such tumors. Therefore, as final extemporaneous creams might or not have parabens, a batch of hydrophilic cream was prepared without preservative (F_4) for investigating how such excipient altered the PR-Cl permeation profiles. Interestingly, the results showed a decrease of permeation and retention profiles for the formulations without the preservative. Further studies on aqueous solutions of PR-Cl with or without the preservative at different pH (*i.e.*, 5.8, 9.0) suggested that the improvement related to the presence of the preservative was due to the pH increase in the hydrophilic environment of the formulation (**Table 4**). From the comparison of the pH results obtained by solutions and creams, it is possible to observe that in the former the gap was bigger than latter ones. This incongruity may be justified considering that creams are more complex systems than solutions and that their inner pH is influenced also by the excipients added to the formulation, which can minimize the pH changes induced by the addition of acid or bases, like sodium methyl paraben. As shown in **Table 4**, sodium methyl paraben induced in solution

a shift of pH to value near the pK_a of propranolol (9.5 ± 1.2) [20]. In line with Henderson–Hasselbalch equation, the inner pH of the aqueous phase of the semi-solid formulation modifies the ionization equilibrium of PR-Cl and the propranolol free base, which penetrate easily through the human epidermis [12]. In particular, when pH was near to the pK_a of propranolol, the concentration of free base increases, inducing an increase of the permeation flux due to more appropriate physicochemical characteristics for absorption process. In agreement with Chantasart et al., these evidences highlighted that an increase of delivery system pH was related to higher permeability coefficients of propranolol through the human epidermis [13].

Therefore, the addition of excipients, which influence formulation pH, should be critically considered and tested to avoid a high variability of topical bioavailability of a drug. Furthermore, if pH-sensitive excipients should be included in the semi-solid formulation, their impact on the excipient-related properties (*e.g.*, antimicrobial effectiveness) should be also investigated. If their use could not be avoided, the selection of an appropriate buffer system should be taken in account. Although the hydrophilic cream was tested as preparation model for studying the effect of methyl-paraben, due to the high water amount, the outcomes were also applicable to all the formulations considered in this paper where the preservative was added to the formulation. In particular, the pH increase might be particularly critical also in the case of lipophilic creams since propranolol base might diffuse easier than PR-Cl through the hydrophobic semi-solid base.

5 Conclusion

The use of propranolol in the treatment of IH has become popular in the last decades according to the amazing effectiveness and the low incidence of side effect. Starting from the success of oral treatment, the topical delivery of propranolol started to be considered for the treatment of superficial IH, especially for patients more sensitive to the side effects of oral treatment (*e.g.*, new-borns). The present study rationalized the selection of semi-solid vehicle by an *in vitro* screening, highlighting the most critical formulative aspects for dermal delivery of PR-Cl and identifying different vehicle candidates according to the patient age. The results confirmed that PR-Cl was able to permeate and to concentrate in the upper layer of the human skin,

although its behaviour was strongly affected by formulative choice. In particular, the impact of different technological features of semi-solid vehicles, such as the importance of a good drug solubilization in the semi-solid matrix and influence of pH value on the microenvironment of the matrix, was pointed out on the PR-Cl penetration. Moreover, the *in vitro* results on full-thickness skin suggested a negligible permeation patter that could suggest lower systemic side effects induced by topical propranolol rather than oral treatment and confirm the low effectiveness of topical-propranolol in treating IH with deep elements. Besides the direct implications of this evidence, it may be considered a useful example to emphasize that compounding pharmacists should carefully choose the excipients of an extemporaneous preparations, considering both their well-known function-related properties rather than their possible physicochemical interactions with the other formula components, to rationalize the technological properties of the final formulation.

The overall results discussed in this paper may be, therefore, useful for compounding pharmacists and physicians for driving the selection of the semi-solid matrix according to the therapeutic needs and on the base of the patient features. In particular, the results obtained by the *in vitro* permeation studies might be helpful to compounding pharmacists for choosing the best semi-solid drug product for the treatment of IH in patients with a higher risk in incurring in cardiac side effects (*e.g.*, new-borns).

TABLES

Table 1 – Studies evaluating topical propranolol in treating cutaneous IH.

Reference	Study design	N° of patients	Topical preparation	PR-Cl concentration	Daily dose	Response rate
Zaher et al. (2013) [8]	Randomized trial	15	Hydrophilic ointment	1%	Twice a day	67%
Wang et al. (2012) [33]	Prospective cohort	51	Chitosan gel	3%	Thrice a day	92%
Xu et al. (2012) [10]	Retrospective cohort	25	Solid dispersion	1%	Thrice a day	90%
Kunzi-Rapp (2011) [9]	Prospective cohort	45	Hydrophilic ointment	1%	Twice a day	64%
Bonifazi et al. (2010) [1]	Prospective cohort	23	Oil based-cream	1%	Twice a day	73%
Bonifazi et al. (2008) [7]	Prospective cohort	6	Oil based-cream	1%	Twice a day	67%

Table 2 – Solubility parameters (δ) of Pr-Cl and lipophilic ingredients; the composition of semi-solid preparations (F_0-F_4) and water solutions (S_1-S_6) containing propranolol HCl 1% w/w.

Ingredients	**δ (cal/cm³)**	**Formulation composition (%, w/w)**										
		F_0	*F_1*	*F_2*	*F_3*	*F_4*	*S_1*	*S_2*	*S_3*	*S_4*	*S_5*	*S_6*
Propranolol HCl	12.02 [a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
White petrolatum	7.33 [b]	99.00	79.30	41.40	15.00	15.00	-	-	-	-	-	-
Lanolin alcohol	9.55 [c]	-	5.10	-	-	-	-	-	-	-	-	-
Cetostearyl alcohol	9.49 [b]	-	0.40	-	7.25	7.25	-	-	-	-	-	-
Mineral oil	7.09 [b]	-	-	-	6.00	6.00	-	-	-	-	-	-
Lanolin	9.55 [c]	-	-	41.40	-	-	-	-	-	-	-	-
Cetomacrogol 1000	9.40 [b]	-	-	-	1.80	1.80	-	-	-	-	-	-
Sodium methyl-paraben	-	-	0.05	0.05	0.05	-	0.05	-	0.05	-	0.05	-
Phosphate buffer at pH 9.0	-	-	-	-	-	-	-	-	98.95	99.00	-	-
Phosphate buffer at pH 5.8	-	-	-	-	-	-	-	-	-	-	98.95	98.95
Purified water	-	-	14.15	16.15	68.90	68.95	98.95	99.00	-	-	-	-

[a] Maitani et al. [20]. [b] Minghetti et al. [15]. [c] Vaughan [19].

Table 3 – In vitro release ($Q_{R,24}$ and release rates) and skin permeability ($Q_{P,2}$ and Q_{ret}) parameters of the tested semi-solid formulations (F_0-F_3; mean ± St. Dev.; n =3).

Form.	***$Q_{R,24}$ (µg/cm²)***	***Release rate (µg/cm²h$^{1/2}$)***	***$Q_{P,24}$ (µg/cm²)***	***$Q_{ret,24}$ (µg/cm²)***
F_0	6.11 ± 2.73	0.57 ± 0.59	-	13.19 ± 6.61
F_1	31.11 ± 5.60	4.16 ± 1.88	0.50 ± 0.27	34.27 ± 4.27
F_2	69.42 ± 9.81	12.71 ± 3.35	14.99 ± 8.52	54.57 ± 19.72
F_3	1306.66 ± 211.32	307.55 ± 84.86	52.22 ± 7.34	94.96 ± 4.11

Table 4 – Effect of sodium methyl-paraben and vehicle pH on skin permeability parameters of 1% w/w PR-Cl delivered by solutions (S_1-S_6) and semisolid preparations (F_3; F_4).

Form.	*Sodium methyl-paraben*	*Vehicle*	*pH*	*J (µg/cm²h)*	*$Q_{P,24}$ (µg/cm²)*	*$Q_{ret,\ 24}$ (µg/cm²)*
S_1	+	Water	9.1	15.40 ± 3.17	329.85 ± 67.70	171.39 ± 7.95
S_2	-	Water	7.7	1.53 ± 0.88	30.64 ± 19.35	147.11 ± 9.68
S_3	+	PBS pH 9	9.0	29.00 ± 2.92	623.57 ± 67.26	320.65 ± 103.61
S_4	-	PBS pH 9	9.0	23.94 ± 7.69	494.79 ± 119.10	178.49 ± 17.50
S_5	+	PBS pH 5.8	5.8	0.66 ± 0.14	12.97 ± 2.93	341.48 ± 92.69
S_6	-	PBS pH 5.8	5.8	0.88 ± 0.81	19.50 ± 12.32	249.01 ± 117.42
F_3	+	Hydrophilic cream	6.2	2.70 ± 0.33	52.23 ± 7.34	94.96 ± 4.11
F_4	-	Hydrophilic cream	6.2	0.87 ± 0.29	19.82 ± 15.02	79.74 ± 16.82

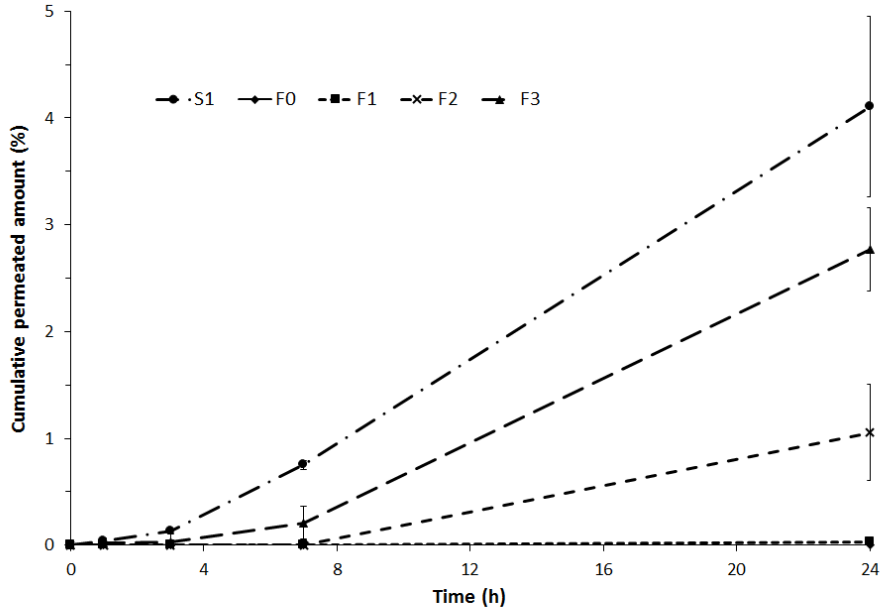
Note: PBS= ….

FIGURE LEGENDS

Figure 1 – *In vitro* permeation profiles through human epidermis of the reference solution (S_1) and the semisolid preparations (F_0-F_3) containing 1% w/w of PR-Cl (n = 3, mean value ± St. Dev.).

Figure 2 – Propranolol permeated amounts through human epidermis during a 24-hour permeation study delivered by 1% PR-Cl hydrophilic cream (continuous line) and those released from the same membrane after the preparation removal (dashed line) (n = 3, mean value ± St. Dev.).

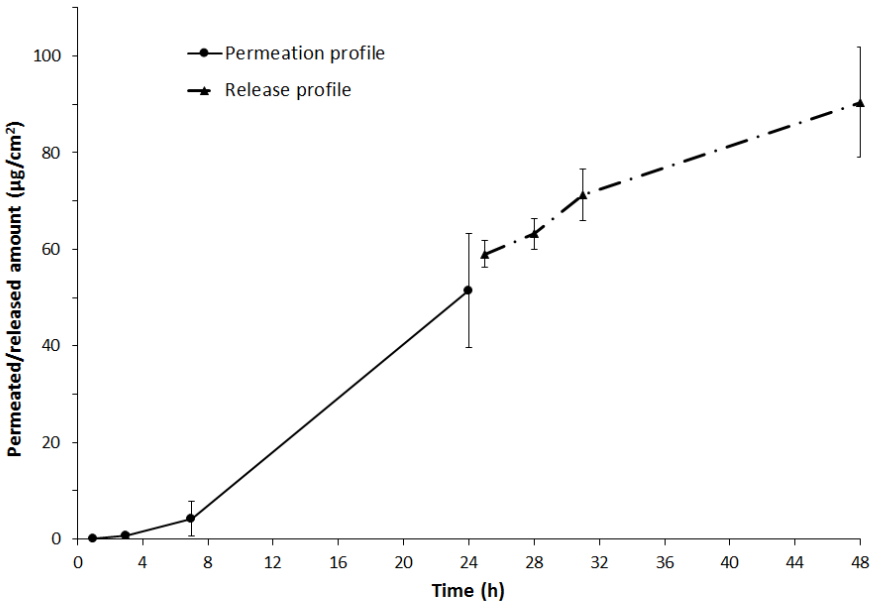
1 Figure 1


S1
F0
F1
F2
F3
Cumulative permeated amount (%)
Time (h)
0
1
2
3
4
5
0
4
8
12
16
20
24

2

3

1 Figure 2



2

3

4

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