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Combination strategies for enhancing transdermal absorption of sumatriptan through skin

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Abstract

The aim of the present work was to characterize *in vitro* sumatriptan transdermal absorption through human skin and to investigate the effect of chemical enhancers and iontophoresis applied both individually and in combination. A secondary objective was to compare the results obtained with those in porcine skin under the same conditions, in order to characterize the relationship between the two skin models and validate the porcine model for further research use. Transdermal flux of sumatriptan was determined in different situations: (a) after pre-treatment of human skin with ethanol, Azone® (1-dodecyl-azacycloheptan-2-one), polyethylene glycol 600 and *R*-(+)-limonene, (b) under iontophoresis application (0.25 and 0.50 mA/cm^2) and (c) combining chemical pre-treatment and iontophoresis at 0.50 mA/cm^2 current density. All the strategies applied enhance sumatriptan transdermal absorption. A linear relationship between the fluxes in the two skin models in the different conditions assayed can be established. The combination of both strategies, Azone® and iontophoresis, proved to be the most effective of the techniques for enhancing the transdermal absorption of sumatriptan. The flux obtained with porcine skin *in vitro* is approximately double that obtained in human skin.

Keywords: Sumatriptan succinate; Human skin; Percutaneous absorption; Iontophoresis; Chemical enhancers

1. Introduction

Sumatriptan succinate is an agonist of a vascular 5-hydroxytryptamine receptor subtype (a member of the 5-HT$_{1D}$ family), which is present in the human basilar artery and in the vasculature of human dura mater, and which causes vasoconstriction. This action in humans correlates with the relief of migraine headaches. In addition to causing vasoconstriction, experimental data from animal studies have shown that sumatriptan also activates 5-HT$_1$ receptors in peripheral terminals of the trigeminal nerve, thereby innervating cranial blood vessels. Both actions contribute to the antimigraine effect of sumatriptan.

The preparations of sumatriptan succinate currently available are intranasal, oral and subcutaneous, which provide an absolute bioavailability of sumatriptan of approximately 15%, 14% and 96%, respectively (Jhee et al., 2001). The low bioavailabilities of the first two are primarily due to presystemic metabolism and, in a smaller measure, to incomplete absorption.

Over the last two decades, the skin has gained importance as a means for the topical, regional and systemic application of drugs. Nevertheless, human skin is by nature a remarkably efficient barrier, thus, presenting difficulties for the transdermal delivery of therapeutic agents. Moreover, few drugs have the characteristics required to permeate sufficiently across the skin and achieve a therapeutic concentration in the blood.

Different methodologies, consisting of chemical and physical enhancers, have been investigated and developed in order to overcome the barrier properties of the skin and thus enhance drug transdermal absorption. Penetration enhancers have long been used to increase the range of drugs that can be effectively delivered via the skin. To date, a vast array of chemicals have been evaluated as enhancers (Williams and Barry, 2004), yet their inclusion in topical or transdermal formulations are limited due to the lack of knowledge surrounding their underlying mechanisms of action.

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Diffusion through the skin is controlled by its outermost layer, the stratum corneum. Some of its properties can be manipulated by application of a penetration enhancer, which increases the diffusion coefficient of the drug into the stratum corneum (by disrupting the barrier), or by improving partitioning between the formulation and the stratum corneum (perhaps by altering the solvent nature of the skin membrane to improve partitioning into the tissue). On the other hand, enhancers may act by increasing the solubility of the drug in the formulation. Many chemicals have been evaluated as penetration enhancers in human or animal skin, but to date none has been proven to be ideal (Barry, 1983).

On the other hand, iontophoresis is an enhancement technique that improves transdermal drug transport through the application of a low-level electric current ($\leq$0.5 mA/cm^2) (Kalia et al., 2004). The mechanisms by which iontophoresis enhances molecular transport across the skin are: (a) electrorepulsion, in which a charged ion is repelled from an electrode with the same charge, (b) electroosmosis, which is the convective flow of solvent through a charged pore that occurs in response to the preferential passage of counter ions when the electric field is applied, and (c) current-induced skin permeability increment. The conditions in which the iontophoretic field is applied determine the transport of molecules across the skin. For example, iontophoretic transdermal transport becomes more efficient as the background electrolyte concentration is lowered and is directly dependent on the current density applied (Phipps et al., 1989; Delgado-Charro et al., 1995).

Traditionally, the application of chemical enhancers and iontophoresis have been investigated separately, but more recently the combined application of different strategies has been evaluated with the aim of enhancing transdermal drug transport as much as possible (Nair and Panchagnula, 2004; Wang et al., 2005; Pillai and Panchagnula, 2003).

Taking into account the low bioavailability of sumatriptan after oral and intranasal administration, and the inconveniences of parenteral dosing, the development of new preparations of sumatriptan succinate is of considerable relevance. In this context, a transdermal delivery system could represent a valuable new pharmaceutical form that improves treatment of migraine by providing greater and easier relief (lower dosing frequency and nontraumatic administration) and, consequently, improving patient compliance.

Our previous reports show that *in vitro* pre-treatment of porcine skin with chemical enhancers produces a significant increment of the transdermal permeation of sumatriptan with respect to a control (Femenía-Font et al., 2005a). *R*-(+)-limonene has proved to be the greatest enhancer among those evaluated (sumatriptan transdermal flux was 22-fold higher than that observed in the control). Later, our research group reported that iontophoresis is a more efficient technique for enhancing transdermal transport than the use of chemical enhancers (Femenía-Font et al., 2005b).

The primary aim of the work reported here was to characterize the sumatriptan transdermal absorption through human skin and to investigate the possible synergic effect of the application of chemical enhancers and iontophoresis. A secondary objective was to compare the results obtained with those in porcine skin under the same conditions, in order to characterize the relationship between the two skin models and validate the porcine model for further research use.

2. Materials and methods

Initial experiments were focused on the study of the effect of chemical enhancers and iontophoresis, applied separately, on the *in vitro* transdermal permeation of sumatriptan through human skin. Iontophoresis was then combined with each of the chemical enhancers assayed in order to evaluate its effect on the *in vitro* transdermal permeation of sumatriptan. All experiments were performed using human skin and were later reproduced with porcine skin in order to compare the results obtained in the two membrane models.

The sumatriptan succinate [3-[2-(dimethylamino) ethyl]-*N*-methyl indole-5 methane-sulphonamide succinate (1:1)] (Eur. Ph. monograph 1573) was obtained from a commercial source: Imigran® intranasal 20 mg (GSK). The excipients of this dosage form are potassium dihydrogenphosphate, anhydrous disodium phosphate, sulfuric acid, sodium hydroxide and water.

Azone® (1-dodecyl-azacycloheptan-2-one) was obtained from Netqem (Durham, NC, USA). *R*-(+)-limonene and polyethylene glycol 600 (PEG 600) were purchased from Fluka Chemie (Buchs, Switzerland) and HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]) was obtained from Sigma–Aldrich Co. (St. Louis, USA). NaCl, NaOH, HCl and ethanol (absolute) were purchased from Mallinckrodt Baker B.V. (Deventer, Holland). All the compounds were of an analytical grade. Ultrapure water used to prepare the solutions was obtained from a Barnstead NANOpure system (Barnstead International, Boston, MA, USA).

Silver Chloride 99%, and one millimetre silver and platinum wire 99.9%, used for the manufacture of the Ag/AgCl electrodes, were purchased from Sigma–Aldrich Co. (St. Louis, USA).

Human skin was obtained from surgical rejections. After its extraction, the tissue was kept at 4 °C and within 2 h it was stored at −80 °C. One hour before its use, the skin samples were dermatomed to a thickness of 600 μm using an Aesculap-Wagner dermatome C. GA 176 (B. Braun Surgical S.A., Barcelona, Spain).

Pig ears were acquired from a local slaughterhouse immediately following the animal's death (Carnes Estellés, Paterna, Spain). Skin from the outer face was excised from the ear using a surgical blade. Afterwards it was dermatomed to a thickness of 600 μm. The samples of dermatomed skin were packed separately and stored until use at −80 °C.

Transdermal delivery of sumatriptan was analysed at room temperature. The experiments were performed employing standard iontophoresis diffusion cells. The cells used were a modification of horizontal Franz type, flat flange joint cells (SCSIE, Servicios Centrales de Soporte a la Investigación, Universitat de València, Valencia, Spain). These cells have a donor compartment divided into two 2 mL compartments and a 7.75 mL receptor compartment. Skin (600 μm thick) was placed horizon-

tally between donor and receptor compartment with the stratum corneum facing the donor compartments. The diffusion area was 0.9 cm^2.

In the first round of experiments, human skin was pretreated for 12 h with either 600 μL of saline buffered solution pH 7.4 [NaCl 150 mM–HEPES 20 mM] (buffer control), ethanol (vehicle control) or a 5% (w/w) chemical enhancer solution in ethanol (Azone®, *R*-(+)-limonene and polyethylene glycol 600, respectively) to determine whether ethanol or any of the compounds enhanced the percutaneous permeation of sumatriptan with respect to the control.

Sumatriptan succinate is freely soluble in water. Thus, after removing the pre-treatment solution, 1.2 mL of 14.5 mM sumatriptan succinate solution prepared in saline buffer [NaCl (150 mM)–HEPES (20 mM) pH 7.4] were placed in one of the donor compartments. The remaining two compartments (donor and receptor) were filled with the same saline buffer pH 7.4.

In the iontophoresis experiments, we employed a donor anodal solution of 14.5 mM sumatriptan succinate prepared with a saline buffer [NaCl (25 mM)–HEPES (20 mM) (pH 7.4)] of a lower ionic strength than that used in the former studies. The remaining solutions were identical to those described in the chemical enhancer studies.

A constant current was applied to the skin for 8 h using Ag/AgCl electrodes connected to a Kepco BHK-MG 0–2000 V power supply (Kepco, Inc. Flushing, NY, USA). Two different current densities (0.25 and 0.50 mA/cm^2) were applied in order to evaluate the effect of different densities on the iontophoretic transdermal absorption of sumatriptan.

Passive diffusion experiments were performed simultaneously as controls. The experiments were done in the Franz type cells.

Finally, transdermal diffusion experiments were carried out in order to investigate the effect of the combination of chemical enhancers and iontophoresis. In these experiments, skin pre-treatment was performed as previously described with Azone®, *R*-(+)-limonene and polyethylene glycol 600, respectively. Following this, and using the solutions detailed in the iontophoresis experiments, a constant current density was applied (0.50 mA/cm^2).

The sampling protocol was identical in all the experiments performed. One-millilitre samples were withdrawn manually from the receptor chamber hourly for 8 h. The volume of the sample removed was replaced with the same volume of buffer pH 7.4. The amount of drug contained in each sample was calculated to determine the accumulative amount of sumatriptan succinate in the receptor compartment at each time point.

Once the *in vitro* transdermal diffusion experiments had concluded, the amount of sumatriptan retained in the skin was extracted by shaking the skin with 3 mL of isotonic buffer (pH 7.4) for 12 h, after which a 1-mL sample was analysed for sumatriptan content. Previously, the efficiency of the sumatriptan extraction procedure was assayed (Femenía-Font et al., 2005b).

All transdermal absorption experiments were performed maintaining sink conditions in the receptor compartment and maximum concentration conditions in the donor solution.

The amount of sumatriptan in all samples collected was quantified using a HPLC method with UV detection, as previously reported (Femenía-Font et al., 2005c).

3. Results and discussion

3.1. Transdermal absorption of sumatriptan across human skin

Transdermal flux (*J*) was estimated from the slope of the linear region (steady-state portion) of the accumulated amount of sumatriptan versus time plot. The transdermal steady-state flux values across human skin for all conditions assayed are shown in Table 1.

Transdermal flux values obtained for all conditions were statistically compared performing a one-way ANOVA test, followed by a multiple comparison Dunnet T3 test, which showed the existence of significant differences between the conditions studied and the control (buffer) and vehicle control (ethanol) ($p < 0.05$). For this reason, the permeation enhancing activities, expressed as enhancement ratio of flux (ER_{flux}), were calculated as the ratio between the flux value obtained with the strategies applied (chemical compounds, iontophoresis or both) and that observed with the buffer control or vehicle control (ethanol),

Table 1
Transdermal flux of sumatriptan across human skin calculated at the steady-state (nmol/cm^2 h) and sumatriptan retained in the skin (μmol/cm^2) for all conditions assayed

Condition assayed	Sumatriptan transdermal flux (nmol/cm^2 h) (mean ± S.D.)	Sumatriptan retained in skin (μmol/cm^2) (mean ± S.D.)	Number of experiments (*n*)
Buffer (pH 7.4) (control)	0.36 ± 0.14	0.03 ± 0.01	8
Ethanol absolute (vehicle control)	2.34 ± 0.38	0.04 ± 0.01	4
PEG 600	0.83 ± 0.20	0.07 ± 0.01	8
R-(+)-limonene	16.90 ± 4.69	0.05 ± 0.01	6
Azone®	167.3 ± 16.3	0.23 ± 0.01	8
Iontophoresis 0.25 mA/cm^2	281.0 ± 52.0	0.62 ± 0.02	8
Iontophoresis 0.50 mA/cm^2	508.6 ± 45.9	0.95 ± 0.09	8
Iontophoresis 0.50 + PEG 600	497.8 ± 51.0	0.80 ± 0.10	8
Iontophoresis 0.50 + ethanol	591.5 ± 26.1	0.55 ± 0.02	4
Iontophoresis 0.50 + limonene	751.2 ± 67.0	1.04 ± 0.07	8
Iontophoresis 0.50 + Azone®	901.4 ± 80.4	1.36 ± 0.09	8

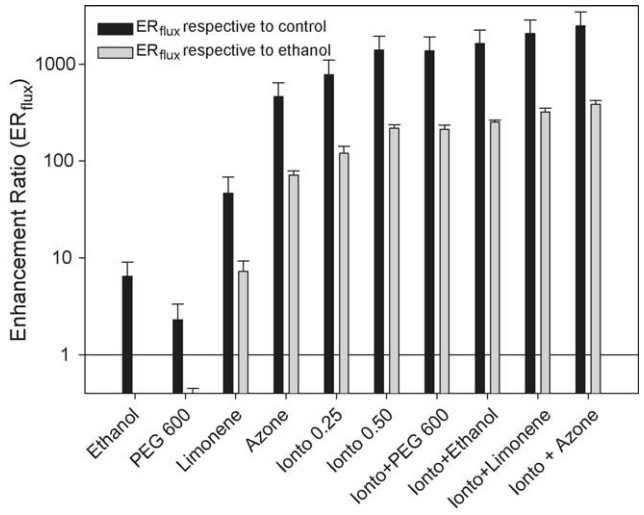

Fig. 1. Enhancement ratio of flux (ER_{flux}) of all conditions studied: chemical enhancers, iontophoresis and a combination of both.

respectively, for all the conditions assayed. The results are shown in Fig. 1.

The amounts of sumatriptan retained in the skin for all conditions assayed are also included in Table 1. The recovery of sumatriptan in the extraction procedures performed after the diffusion studies showed values of 98.6 ± 1.3% (mean ± S.D.). Fig. 2 shows the ratio between the amounts of sumatriptan retained in the skin, extracted after the transdermal diffusion experiments in each condition assayed, and that extracted from the control. It can be observed that the amount of sumatriptan retained in the skin after the experiments varied depending on the strategy applied.

These experiments have confirmed that the vehicle used to prepare the chemical enhancer solutions – ethanol – is capable of increasing the transdermal flux of sumatriptan across human skin. Our results are in concordance with those obtained using porcine skin as a membrane (Femenía-Font et al., 2005a),

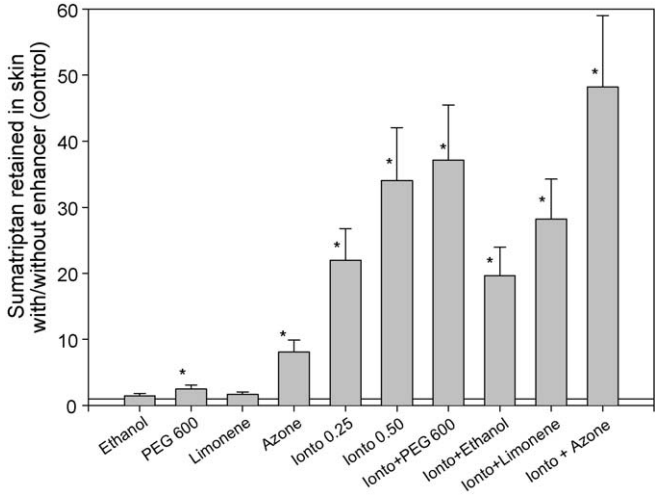

Fig. 2. Ratio between the amounts of sumatriptan retained in skin at the end of the diffusion experiments in all conditions assayed and that in the controls (buffer). *Statistically significant difference ($p < 0.05$, Dunnet T3).

and they also confirm the capacity of ethanol for permeating through the stratum corneum (Berner et al., 1989) and for altering the organization of its intercellular lipids, thereby increasing skin permeability (Megrab et al., 1995) and, consequently, the transdermal transport of the drug. As can be observed in Fig. 2, ethanol does not significantly modify the amount of drug retained in the skin with respect to that retained in the control. Thus, it seems that ethanol acts mainly on the diffusion of the drug and only moderately or poorly on the partition of the drug between the skin and the vehicle.

PEG 600 is the most hydrophilic penetration enhancer compound assayed in this study. It has shown a low ER_{flux} (2.30-fold that of the control), confirming the results previously obtained with porcine skin (Femenía-Font et al., 2005a). As can be seen in Fig. 2, PEG 600 also significantly increases, to the same extent, the amount of sumatriptan retained in the skin with respect the control. In this sense, its enhancing effect can be attributed, at least partially, to the increase of the partitioning of sumatriptan in skin produced by PEG 600. However, the combination of PEG 600 and ethanol resulted in a smaller transdermal flux of the drug than the flux obtained with ethanol alone. It could be attributed to the high viscosity of PEG 600 that would have repercussion on the diffusion of sumatriptan, reducing it with respect to that found when ethanol is used solely.

R-(+)-limonene increases 46.64-fold and 7.23-fold the transdermal flux of sumatriptan across human skin with respect to the control and the vehicle, respectively. However, as occurred with previous studies in porcine skin (Femenía-Font et al., 2005a), the pre-treatment of skin with limonene does not significantly augment the amount of sumatriptan it retains (see Fig. 2). Therefore, the effect of this compound can be attributed to an increase in the diffusion of the drug across the skin and not to any significant influence on the partitioning of sumatriptan in the tissue.

Of all the compounds assayed as penetration enhancers, Azone® revealed itself to be most effective, attaining sumatriptan transdermal flux values across human skin that were 461.83 and 71.62-fold those of the control and the vehicle. This compound was designed as a penetration enhancer for both lipophilic and hydrophilic molecules (Sugibayashi et al., 1992). Its mechanism of action is based on its capability of altering the organization of the lipid structure of the stratum corneum and of increasing the diffusion of the drug into the skin (López et al., 2000). As can be observed in Fig. 2, the amount of drug retained in skin pretreated with Azone® was eight-fold that observed in the control.

The application of iontophoresis produces a much higher increment of transdermal flux than chemical enhancers, resulting in values 775.65 and 1403.92-fold those observed with the control when 0.25 and 0.50 mA/cm^2 are applied, respectively. As in the porcine skin assays (Femenía-Font et al., 2005b), when the current density is doubled the transdermal flux of drug and the amount of drug retained in the skin are also doubled. Both iontophoretic conditions assayed were shown to significantly increase the amount of drug retained in the skin (see Fig. 2).

Due to differences in the mechanisms of action of the chemical enhancers (which increase the transdermal drug penetration by altering the barrier function of the skin) and iontophoresis

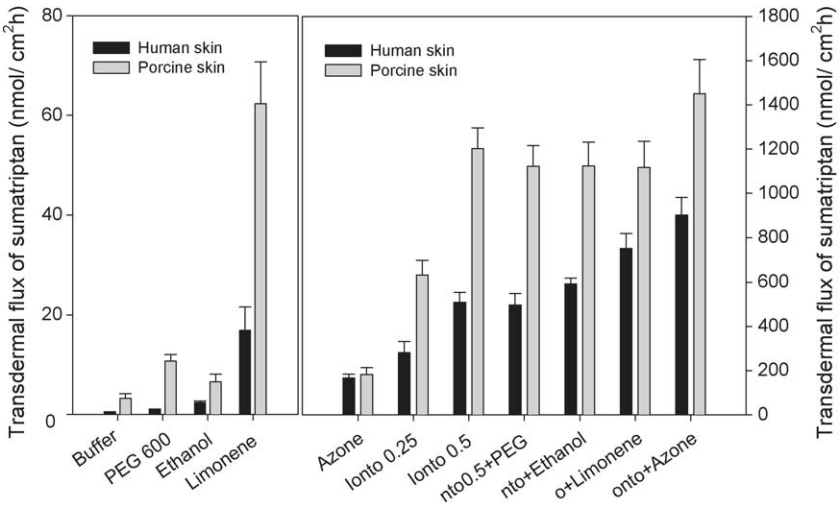

Fig. 3. Comparison of the transdermal flux of sumatriptan observed across human and porcine skin in the experiments performed in different conditions.

(which acts principally on the drug molecule and only moderately on the skin's barrier properties), the possibility that chemical enhancers improve the effect of iontophoresis was explored.

A moderate synergistic effect was observed when pretreatment of the human skin with Azone® or *R*-(+)-limonene was subsequently combined with iontophoresis. As can be seen in Table 1, the combination of both strategies produced a higher increment in the transdermal flux of sumatriptan than the sum of the increments of flux of both strategies. The most noticeable synergistic effect was found when human skin was pretreated with Azone®. On the other hand, and as expected following the studies performed with chemical enhancers as the only enhancement strategy, the combination of ethanol or PEG 600 with iontophoresis did not produce any synergism. Transdermal flux values obtained in combination of ethanol or PEG 600 with iontophoresis were not significantly different from those obtained with iontophoresis ($p > 0.05$, Dunnet T3) (see Table 1 and Fig. 1).

3.2. Human skin versus porcine skin: permeability comparisons

Our group have previously analysed the transdermal flux of sumatriptan after pre-treatment of porcine skin with some of the enhancers assayed in the present work (limonene and PEG 600) and by applying iontophoresis (Femenía-Font et al., 2005b). However, these experiments were performed with sumatriptan succinate powder. In order to compare both skin models and to identify any relationships between them it was considered necessary to repeat all the experiments with porcine skin using Imigran® as a sumatriptan source, as the possible effects of the excipients contained in the commercial sumatriptan source had previously been ignored.

Fig. 3 illustrates that porcine skin is more permeable than human skin. Although the results obtained with porcine skin reproduce the sequence of those obtained with human skin, a more in-depth analysis reveals some differences. In relation to porcine skin, ethanol and PEG 600 do not produce any significant increment of the transdermal flux of sumatriptan with respect to the control. This shows that porcine skin is more permeable than human skin but less sensitive to small variations in transdermal flux. On the other hand, no statistically significant synergistic effect is observed when iontophoresis is combined with chemical enhancers. This could be due to the fact that transdermal transport of sumatriptan across the skin has a maximum limit that is reached with the single strategy of iontophoresis at a current density of 0.50 mA/cm^2.

It should be stressed that, although porcine skin does not reproduce exactly the same results as those obtained with human skin, a linear correlation exists between the transdermal fluxes observed in both membrane models. Even though some of the values deviate from predicted values (see Fig. 4), the correlation is statistically significant ($r > 0.964$). The value of the slope of the correlation is 1.79 ± 0.10. Therefore, the fluxes observed with porcine skin should generally be 1.8-fold higher than those observed with human skin.

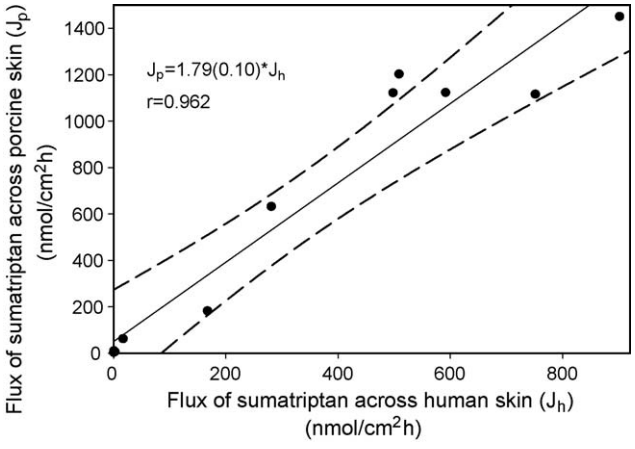

Fig. 4. Lineal correlation between the transdermal flux values obtained with human skin and porcine skin in the experiments performed with Imigran®. Dash lines represent the 95% confidence interval of the regression.

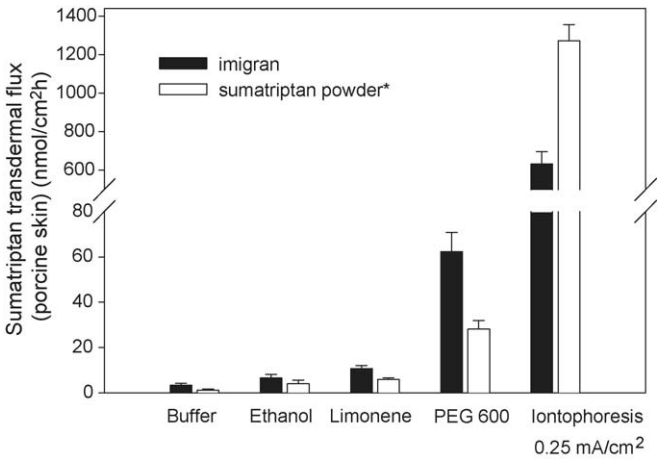

Fig. 5. Comparison of the transdermal flux values of sumatriptan in the experiments performed using Imigran® and those obtained using sumatriptan succinate powder, *from Femenía-Font et al. (2005a,b).

On the other hand, the results of the present study obtained with porcine skin and Imigran® do not reproduce those obtained previously with the same membrane and sumatriptan succinate (powder) (Femenía-Font et al., 2005a,b) (see Fig. 5). It should be pointed out that, in the case of skin pretreated with chemical enhancers, the transdermal flux values attained with Imigran® were double those obtained with sumatriptan succinate powder (Femenía-Font et al., 2005a). However, when iontophoresis was combined with the powder, the transdermal flux values were two-fold those obtained with Imigran® (Femenía-Font et al., 2005b). These findings could be due to the excipients contained in the Imigran® preparation, which is formulated for intranasal administration. It is possible that substances included in the formulation have an additive effect with chemical enhancers. On the other hand, high concentrations of electrolytes compete with sumatriptan and compromise the efficacy of iontophoresis. In fact, we have previously demonstrated that, when the background electrolyte concentration is lowered three times, the iontophoretic transdermal flux of sumatriptan increases 6.8-fold (Femenía-Font et al., 2005b).

4. Conclusions

Iontophoresis is more efficient than chemical substances for increasing the transdermal delivery of sumatriptan through human skin. With respect to the chemical enhancers studied, Azone® exhibits the greatest enhancing activity. Likewise, synergism has been found when Azone® and iontophoresis are combined. Nevertheless, irritation tests must be carried out in order to ensure its safe transdermal application.

Even though the permeability of porcine skin differs from that of human skin, this animal model can be used for the screening and selection of techniques for enhancing the transdermal delivery of drugs. The flux obtained with porcine skin *in vitro* is approximately double that obtained in human skin.

The differences in the transdermal flux values observed with Imigran® and with sumatriptan succinate powder highlight the relevance of the excipients used in the formulation of a drug for its transdermal administration.

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