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Effect of iontophoresis on in vitro transdermal absorption of almotriptan

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ABSTRACT

The aim of the present work was to characterize the *in vitro* transdermal absorption of almotriptan through pig ear skin. The passive diffusion of almotriptan malate and its iontophoretic transport were investigated using current densities of 0.25 and 0.50 mA/cm^2. *In vitro* iontophoresis experiments were conducted on diffusion cells with an agar bridge without background electrolytes in the donor compartment. Although both current densities applied produced a statistically significant increment with respect to passive permeation of almotriptan ($p < 0.01$), that of 0.50 mA/cm^2 proved to be the best experimental condition for increasing the transport of almotriptan across the skin. Under these experimental conditions, the transdermal flux of the drug increased 411-fold with respect to passive diffusion, reaching 264 ± 24 μg/cm^2 h (mean ± SD). Based on these results, and taking into account the pharmacokinetics of almotriptan, therapeutic drug plasma levels for the management of migraine could be achieved via transdermal iontophoresis using a reasonably sized (around 7.2 cm^2) patch.

1. Introduction

Almotriptan malate is a highly selective serotonin 5-hydroxytryptamine 1B/1D (5-HT$_{1B/1D}$) receptor agonist. According to the FDA *Clinical Review: Almotriptan malate, April 2009* (Rob Harris, 2009), almotriptan is effective in the acute treatment of moderate to severe migraine attacks in adult and adolescent patients with a history of migraine with or without aura. Several studies have reported that almotriptan has the best sustained pain-free rate and the lowest adverse events rate of all the triptans (Sandrini et al., 2007; Pascual et al., 2010), and its efficacy and tolerability have been assessed in a number of randomised, controlled trials in over 4800 adults suffering moderate or severe attacks of migraine (Antonaci et al., 2010; Chen and Ashcroft, 2007). Almotriptan is consistently one of the preferred triptans in multiattribute decision-making analyses (Láinez, 2007).

Almotriptan is administered as oral tablets (basically 12.5 mg) and, although its absorption is good after oral administration, the mean absolute bioavailability is 69.1% (Tfelt-Hansen et al., 2000). After oral dosage, maximal plasma concentrations are achieved between 1.5 and 4 h later, therapeutic levels are in the range of 52–56 ng/mL, its plasma clearance is 36.5 ± 5.8 L/h, the apparent distribution volume is 3.5 L/kg and the terminal elimination half life is between 2.5 and 5 h (McEnroe and Fleishaker, 2005; Tfelt-Hansen et al., 2000). The predominant route of metabolism is via monoamine oxidase-A (McEnroe and Fleishaker, 2005). In humans, after a single subcutaneous dose its bioavailability is complete and time to maximum plasma concentration is about 5–15 min (Jansat et al., 2002). In other animal species oral bioavailability varies in the range of 18.7–79.6% depending on the degree of absorption and first-pass effect metabolism (Aubets et al., 2006).

As mentioned previously, the bioavailability of almotriptan is somewhat limited after oral dosing. Parenteral dosing is an alternative, but involves obvious inconveniences. Bearing in mind the pharmacokinetic properties of this agonist and its superior efficacy and tolerability profile in comparison to other tryptans (Von, 2002), an alternative route of almotriptan delivery, such as transdermal administration, could represent a new and valid pharmaceutical form.

For several decades, there has been interest in using the skin as a port of entry into the body for the systemic delivery of therapeutic agents. However, the stratum corneum, the upper layer of the skin, is a barrier to the entrance of many therapeutic entities (Naik et al., 2000). The passive delivery rate of a compound is often dependent on two major physicochemical properties: its partition coefficient and its solubility. Diffusion through the skin is controlled by the stratum corneum, some of whose properties can be manipulated by application of a penetration enhancer (Kanikkannan et al., 2000), and by using different methodologies such as iontophoresis, a non-invasive technique based on the application of a low-level electrical

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current (with a current density <0.50 mA/cm^2) (Kalia et al., 2004; Mudry et al., 2007; Sieg et al., 2004).

The two main mechanisms by which iontophoresis enhances molecular transport across the skin are electromigration and electroosmosis. Electromigration, also known as electrorepulsion, is the direct interaction of the electrical field and the ions present in the formulation with the skin. In this way, the transport of cationic drugs from the anode and negatively charged drugs from the cathode is enhanced (Guy et al., 2000, 2001; Kalia et al., 2004). The efficiency of drug transport, namely the fraction of the total charge transported by a given drug (its transport number, t_d), can be determined experimentally by measuring its flux (J_d) and applying the relation:

$$J_d = \frac{I \cdot t_d}{F \cdot z_d} \quad (1)$$

where I is the total current passed, F is Faraday's constant, and z_d is the valence of the drug (Phipps and Gyory, 1992).

Electroosmosis is possible because the skin is a negatively charged membrane at physiological pH. When an electrical potential is applied across a membrane containing a fixed charge, there is a bulk volume flow of solution in the direction of the counter ions movement (Pikal, 2001). This means that, for the negatively charged skin, the electroosmotic flow is in the anode-to-cathode direction. This flow of solvent carries through the skin any dissolved solute and, therefore, enhances the transdermal delivery of neutral, polar molecules. The contribution of electroosmosis is greater in the transport of high molecular ions (Guy et al., 2001) due to their competition with the smaller and more mobile ions comprising the background electrolytes. In fact, different parameters, including background electrolytes and current density, have a great impact on iontophoresis efficacy (Femenía-Font et al., 2005a; Phipps and Gyory, 1992; Pikal, 2001; Schuetz et al., 2006).

Almotriptan is a lipophilic drug (log P = 1.6) and its molecular weight is 335.46 g/mol (469.55 as malate). Almotriptan malate is a weak acid with a pK_a value of 8.77 at 22 °C, so the percentage of the ionised form is dependent on the pH. Almotriptan malate is positively charged at pH 7.4, thus being a good candidate for iontophoretic delivery (Burnette and Ongpipattanakul, 1987; Phipps and Gyory, 1992).

The present work was carried out to investigate whether or not almotriptan can be administered transdermally. Thus, we conducted *in vitro* experiments to characterize its passive diffusion through skin and to evaluate the possibility of using iontophoresis in order to enhance almotriptan transdermal passage.

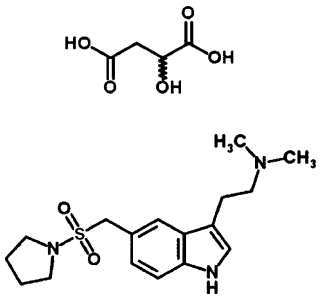
2. Materials and methods

2.1. Materials

The almotriptan malate certified standard (98.9% w/w) (Eur. ATC Code N02C C05) was generously gifted by Laboratorios Almirall S.A. (Barcelona, Spain). Its physicochemical properties are shown in Table 1.

Acetonitrile, ammonium *di*-hydrogen phosphate (96–102%, w/w) and NaCl were obtained from Análisis Vínicos, S.L. (Tomelloso, Spain). Orto-phosphoric acid (85–88%, v/v) was purchased from Panreac Química S.A. (Barcelona, Spain). HEPES (N-[2-Hydroxiethyl] piperazine-*N*′-[2-ethanesulfonic acid]), NaOH, HCl analytical grade and silver chloride (99%), silver, and platinum wire 1 mm (both 99.9%) used to make the Ag/AgCl electrodes were obtained from Sigma–Aldrich S.A. (Madrid, Spain). The agarose ultrapure electrophoresis grade and silicone microtube with 35 mm internal diameter necessary to make the salt bridges employed in the iontophoretic studies were purchased from Sigma–Aldrich Co (St. Louis, USA) and Levantina Laboratorios S.L. (Valencia, Spain),

Table 1
Physicochemical properties of almotriptan malate.

Generic name	Almotriptan malate
IUPAC name	1-[[[3-[2-(Dimethylamino)ethyl]-1H-indol-5 yl]methyl] sulfonyl]pyrrolidine(±)-hydroxybutanedioate (1:1)
Structural formula	
Empirical formula	$C_{17}H_{25}N_3O_2S - C_4H_6O_5$
Molecular weight	469.56 g/mol
Dosage forms	Oral
Oral bioavailability	69.1%
Solubility	Soluble in water
Octanol/water partition coefficient	log P = 1.87

respectively. The electrical current applied to the skin was provided by a Kepco BHK-MG-0-2000 V power supply (Kepco, Inc., Flushing, NY). All reagents were of analytical or HPLC grade. Ultrapure water was obtained with a Barnstead NANOpure system (Barnstead International, Boston, MA, USA).

Pig ears were generously gifted by the Faculty of Medicine, University of Valencia (Valencia, Spain) following the death of the animal. Skin from the outer face was excised from the ear using a surgical blade. Afterwards it was dermatomed to a thickness of 600 μm using an Aesculap-Wagner dermatome C. GA 176 (B. Braun surgical S.A., Barcelona, Spain). Dermatomed skin samples were packed and stored at −80 °C until use.

2.2. In vitro diffusion experiments

Transdermal permeation of almotriptan was evaluated over a 32 h period at 37 °C (36.96 ± 0.14, n = 39) °C. Experiments were performed employing vertical Franz-type diffusion cells (DISA, Milan, Italy) with a diffusion area of 0.567 cm^2.

The dermatomed skin was defrosted and then placed between the two compartments of the cell so that the stratum corneum faced the donor compartment. Previous researchers have reported similar permselective properties for porcine and human skin, which makes the former an appropriate model for iontophoresis studies (Femenía-Font et al., 2006; Marro et al., 2001; Sekkat et al., 2004).

Almotriptan malate is soluble in water. Thus, for passive diffusion experiments, 1 mL of 14.5 mM almotriptan malate solution prepared in an isotonic buffer [HEPES (20 mM)-NaCl (10 mM), pH 7.4] was placed in the donor compartment. The receptor compartment (4 mL volume) was filled with HEPES/NaCl (20/150 mM) saline buffer (pH 7.4), thermostated at 37 °C and stirred to prevent boundary layer effects.

Iontophoresis studies were carried out with a constant current applied to the skin for the first 8 h. Passive permeation was then permitted for up to 32 h. The densities applied were 0.25 and 0.50 mA/cm^2, resulting in intensities of 0.142 and 0.284 mA, respectively. The cathode (−) was placed in the receptor compartment and the anode (+) was localized in a compartment separated from the diffusion cells by means of a salt bridge (Raynauld and Laviolette, 1987). The bridges were prepared by suspending 3% agar in a 0.1 M

NaCl solution and, once the dispersion was warmed, it was introduced in a silicone tube and allowed to dry at room temperature (Shao and Feldman, 2007). The donor compartment was filled with 14.5 mM almotriptan malate solution. The anodal solution consisted of a NaCl solution 84.61 μM for 0.50 mA/cm^2 and 42.30 μM for 0.25 mA/cm^2. The receptor and cathodal compartments were the same as in the passive experiments.

In both passive and iontophoretic diffusion experiments samples of 200 μL were taken from the receptor chamber at the different conditions assayed, at predetermined time intervals. The volume of sample removed was replaced with the same volume of buffer (pH 7.4). The almotriptan malate contained in each sample was analyzed in order to calculate the accumulative amount of drug in the receptor compartment.

At the end of the *in vitro* transdermal permeation experiments, the amount of almotriptan retained in the skin was extracted by shaking the skin during 12 h in 2 mL of the buffer (pH 7.4) (Femenía-Font et al., 2005b).

2.3. Analytical method

The amount of almotriptan in the samples was quantified by High Pressure Liquid Chromatography (HPLC) using a previously described and validated method with some modifications (Kumar et al., 2008). A Waters 600 Controller was used for the analysis and was equipped with a quaternary pump that included a diode-array detector (Waters 996 Photodiode Array Detector, Barcelona, Spain). Separation was carried out using an ammonium *di*-hydrogen phosphate water solution (0.05 M, pH 4.8)-acetonitrile (72:28, v/v) mixture at a flow rate of 1 mL/min as the mobile phase, and an analytical reverse-phase Kromasil® C_{18} column (4.6 mm × 250 mm) (Análisis Vínicos, Tomelloso, Spain). Analysis was performed at room temperature. The wavelength of detection was 283.9 nm. Aliquots of 50 μL were injected.

2.4. Data analysis

Permeation of almotriptan was assessed for 32 h under different experimental conditions and plots were constructed of the accumulated amount of almotriptan (μg/cm^2) against time (h).

Transdermal flux (J) of almotriptan was estimated from the slope of the linear region (steady-state portion) of the plot of the accumulated amount of drug *versus* time.

A one-way ANOVA was followed by a multiple comparison Scheffé post hoc test to compare the transdermal flux of almotriptan during the first 8 h ($J_{8\,h}$) and between 8 h and 32 h ($J_{8-32\,h}$), and to compare cumulative amounts (Q) and amount of almotriptan retained in the skin at the end of the different situations assayed. When statistical differences were detected by means of the ANOVA test ($p < 0.05$), the permeation enhancing activities, expressed as enhancement ratio of flux (ER_{flux}), were calculated as the quotient of the flux value obtained with the iontophoresis that found with control (passive diffusion).

The lag time (t_o) was obtained from extrapolation of the linear region (steady-state portion) of the permeation profile to the intercept on the time axis. As a useful approximation, pseudo-steady-state permeation for most drugs is achieved after approximately 2.7 times the lag time (Barry, 1983).

3. Results and discussion

The transdermal permeation of almotriptan malate has been investigated under different conditions. Accumulated amounts of almotriptan in the receptor compartment as a function of time (for the passive diffusion and 0.25 and 0.50 mA/cm^2 iontophoresis experiments, respectively) are plotted in Fig. 1. Table 2 shows

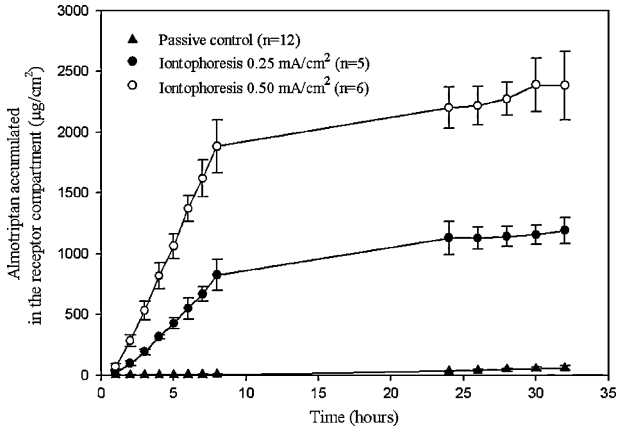


Fig. 1. Accumulated amount of almotriptan (μg/cm^2) *versus* time (h) in the receptor compartment. Passive permeation (▲), iontophoresis 0.25 mA/cm^2 (●) and iontophoresis 0.50 mA/cm^2 (○). Average values ± SD.

the cumulative amount of drug after 32 h of diffusion experiments; as can be observed, the amount of almotriptan in the receptor compartment at the end of the experiments was much lower for passive diffusion conditions than for iontophoresis. Transdermal fluxes (J) were estimated from the slope of the linear region (steady-state portion) and its values across pig ear skin for all conditions assayed are shown in Table 2. The one-way ANOVA test followed by a multiple comparison Scheffé test, revealed significant differences between the transdermal flux obtained during the first 8 h ($J_{8\,h}$) in the conditions studied ($p < 0.01$). The post-iontophoresis fluxes ($J_{8-32\,h}$) were also shown to be statistically different to those of passive diffusion ($p < 0.05$).

As can be seen, iontophoresis application increased the penetration of almotriptan through the skin. The shape of the permeation curve was linear with time for the first 8 h of the experiment. When the current was switched off, the transdermal flux dropped dramatically; even so, passive diffusion after iontophoresis was much higher than when iontophoresis was not applied. Previous research has suggested that the epidermal alterations induced by an electric field result in pore formation and in changes in epidermal membrane permeability (Inada et al., 1994). *In vivo*, these alterations in skin permeability are reverted after a short period of current application, but this is not the case *in vitro*. The reduction of almotriptan flux observed after iontophoresis cessation confirms that electrorepulsion and electroosmosis are the main mechanisms by which transdermal passage is enhanced during iontophoresis. In fact, the $J_{8-32\,h}$ represents less than 13% of the $J_{8\,h}$.

The application of a current density of 0.25 mA/cm^2 produced a statistically significant increment (180-fold) with respect to the passive diffusion control. When a higher current density (0.50 mA/cm^2) was applied, we observed a significant increase flux, not only with respect to passive control (411-fold), but also to the 0.25 mA/cm^2 iontophoretic condition (2.28-fold). The permeation enhancing activities, expressed as enhancement ratio of flux (ER_{flux}), were calculated as the ratio between the flux values obtained with 0.25 or 0.50 mA/cm^2 and that of the passive diffusion control. These results are also shown in Table 2. Our findings support the hypothesis that flux is proportional to the current density applied (Burnette and Ongpipattanakul, 1987; Femenía-Font et al., 2005a; Guy et al., 2001; Schuetz et al., 2006).

The lag time results are also presented in Table 2. There was a delay between applying almotriptan to the outer surface of the tissue and its appearance in a receptor solution, but when permeation was produced by iontophoresis the lag time was approximately only 1 h. Statistical differences were observed between iontophore-

Table 2
Passive and iontophoretic permeation parameters of almotriptan across pig ear skin: cumulative amount in receptor compartment at the end of experiments (Q); transdermal fluxes (calculated after first 8 h ($J_{8\,h}$) and 8–32 h period ($J_{8-32\,h}$)) and lag time (t_o). The enhancement ratio values (ER_{flux}) calculated with respect to control (passive diffusion). Transport number of almotriptan calculated for each iontophoretic condition.

Experimental condition assayed	Cumulative amount in receptor (Q; μg/cm²) (Mean ± SD)	Almotriptan transdermal flux [J; μg/(cm² h)] (Mean ± SD)		Lag time (t_o) (h) (Mean ± SD)	ER_{flux} (Mean ± SD)	Almotriptan transport number (%) (Mean ± SD)
		$J_{8\,h}$	$J_{8-32\,h}$			
Passive control	57.7 ± 17.7	0.64 ± 0.11	2.10 ± 0.64	13.9 ± 2.8	–	–
Iontophoresis 0.25 mA/cm²	1191 ± 106[a**]	115 ± 17[a**]	15.3 ± 3.0[a*]	1.53 ± 0.11[a*]	180 ± 41	2.18 ± 0.33
Iontophoresis 0.50 mA/cm²	2384 ± 282[ab**]	264 ± 24[ab**]	21.2 ± 5.9[a*]	1.01 ± 0.04[a*]	411 ± 80**	2.49 ± 0.23

* Denotes statistical differences with respect to its control ([a]) or 0.25 mA/cm² ([b]) (*$p < 0.05$; **$p < 0.01$).

sis lag times (~1 h) and passive lag times (~14 h) ($p < 0.05$, Scheffé test).

The electromigration of a drug molecule during application of iontophoresis is further complicated in the presence of buffer ions in the solvent, since these small ions are more mobile in the presence of electricity and compete with the drug to carry the charge to the skin surface (Burnette and Ongpipattanakul, 1987). To avoid this competitiveness we used the agar-bridge.

The transport numbers for the delivery of almotriptan across the pig ear skin were calculated from the iontophoretic rate of drug delivery, using Eq. (1), and following the method used in previous reports (Burnette and Ongpipattanakul, 1987; Mudry et al., 2006). These values are shown in Table 2. The transport number values calculated here, with a low increase of 0.0218 (0.25 mA/cm²) to 0.0249 (0.50 mA/cm²), demonstrate the negligible competitiveness between ions and demonstrate that there was no release of Cl^- from the salt bridges, which confirms their effectiveness in *in vitro* iontophoretic studies.

The voltage required to maintain the current across the skin at a density of 0.25 mA/cm² and 0.5 mA/cm² increased by ~6.31% and ~13.8% during the first 2 h of iontophoresis, reaching ~8.91 V and ~15.1 V, respectively. Afterwards declined slightly and remained constant.

After each transdermal diffusion experiment, the amounts of almotriptan retained in the skin were extracted and analyzed. The amount of drug retained in the skin at the end of the different permeation experiments is shown in Fig. 2. The amount of compound retained after passive diffusion was similar to that retained after employing 0.25 mA/cm²: 122 ± 7 and 127 ± 73 μg/cm², respectively. However, the amount of almotriptan retained in the skin after 32 h of transdermal diffusion and iontophoresis at a high current density (0.50 mA/cm²) was significantly higher than in the control (192 ± 62 μg/cm²). A possible explanation for this phenomenon is the formation of intercellular water pools during iontophoresis (Fatouros et al., 2006), which would cause the drug to be retained in the skin. Overall, we have not been able to establish a clear relationship between the transdermal flux through the skin and the amount of almotriptan retained in it.

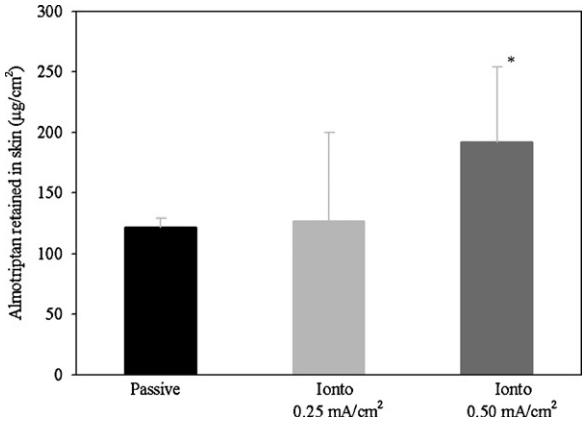


Fig. 2. The amounts of almotriptan retained in skin at the end of the diffusion experiments in the different conditions assayed. * Statistically significant difference with respect to passive diffusion (control) ($p < 0.05$, Scheffé test).

Our previous reports of a vascular 5-HT_{1D} receptor agonist have shown that the transdermal route is also efficient for the administration of sumatriptan succinate. Although the conditions of that study's experiments were slightly different, it is possible to make some comparisons. Passive permeation of sumatriptan was ~0.9 μg/cm² h. Iontophoresis of 0.25 mA/cm², applied to a solution that contained the drug in a solution of 25 mM NaCl, was the best enhancer among the strategies tested, providing a sumatriptan transdermal flux of 526 ± 34 μg/cm² h (Femenía-Font et al., 2005a).

The differences observed in transdermal flux when applying iontophoresis to the two triptans can be explained by their structures and physico-chemical properties. Almotriptan has a slightly higher molecular weight (335.46 g/mol as base and 469.56 g/mol as malate) than the other triptan compared (295.49 g/mol of sumatriptan base and 413.48 g/mol as succinate) and is more lipophilic ($\log P = 1.6$) than sumatriptan ($\log P = 0.9$). Moreover, the structure of almotriptan is less packed than that of sumatriptan, due to the presence of the pyrrolidine group. Taking these three aspects into account it is to be expected that the contribution of electroosmosis to almotriptan transport is higher than in the case of sumatriptan (Guy et al., 2001; Pikal, 2001; Phipps and Gyory, 1992).

The present results emphasize the differences between passive and electrically-assisted transport across the skin and corroborate the idea that different transport mechanisms and pathways are involved in passive and electrically assisted transport. Whereas passive solute permeation occurs mainly via the intercellular lipid matrix of the stratum corneum (Ravikumar et al., 2008), it is likely that ionic shunts (Elias et al., 1981), essentially an aqueous pathway, are predominantly involved in iontophoresis. Moreover, iontophoresis acts mainly on drug molecules rather than on the skin. Physico-chemical properties such as molecular size and lipophilicity are important in determining the efficacy of iontophoresis.

Nevertheless, although almotriptan permeation through the skin is lower than that of sumatriptan, the results obtained in this study are encouraging. Considering the flux obtained when iontophoresis was applied (264 ± 24 μg/cm² h), and using as a reference the pharmacokinetic parameters mentioned in the introduction, a therapeutic plasma level of 52 ng/mL (McEnroe and Fleishaker, 2005) could be reached with a patch of a reasonable surface area (around 7.2 cm²).

The possibility of administering almotriptan transdermally is strengthened by the results obtained with similar studies in triptans. Following the study mentioned previously, the transdermal

delivery of sumatriptan from an iontophoretic patch system *in vivo* was evaluated by Patel et al. The data obtained confirmed that the transdermal delivery of therapeutic amounts of this triptan was feasible using an iontophoretic patch system (Patel et al., 2007). In addition, recent clinical studies of the safety pharmacokinetics and efficacy of transdermal delivery of sumatriptan have been reported with NP101 [Zelrix (NuPathe Inc., Conshohocken, PA)], a single-use, disposable patch that delivers the drug via iontophoresis. Overall, iontophoretic delivery seems to possess several advantages in comparison to more traditional methods (Pierce et al., 2009).

Appart from sumatriptan, the transdermal delivery of zolmitriptan and rizatriptan has also been studied. The iontophoretic delivery of zolmitriptan is currently under investigation by Patel et al. (2009), who have demonstrated by means of *in vitro* and *in vivo* analyses that this drug is appropriate for use in an iontophoretic device. The passive transdermal delivery of an elastic liposomal formulation of rizatriptan has also been evaluated in both *in vitro* and *in vivo* analyses, with results that point to its potential as a future option for migraine therapy (Garg et al., 2008).

The findings of previous studies conducted *in vivo* have demonstrated that the transdermal administration of triptans is possible. Furthermore, it is well known that almotriptan involves a much lower risk of adverse events than sumatriptan, zolmitriptan and rizatriptan, making it a better tolerated and more cost-effective choice for triptan-naïve patients (Pascual et al., 2010; Von, 2002), and fuelling interest in the development of a new route for the therapeutic administration of this drug.

4. Conclusion

In summary, our work demonstrates that almotriptan penetrates the skin and that iontophoresis is a very efficient technique for enhancing its transdermal delivery. The application of a current density of 0.50 mA/cm^2 produces a statistically significant increment not only with respect to a passive control (411-fold) but also to a density of 0.25 mA/cm^2. The results obtained *in vitro* are promising, but further work *in vivo* should be carried out to ensure that therapeutic blood levels of almotriptan can be achieved using iontophoresis.

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