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Enhancement of nortriptyline penetration through human epidermis: influence of chemical enhancers and iontophoresis

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Abstract

Different known percutaneous chemical enhancers and iontophoresis have been tested in-vitro to study their ability to increase transdermal absorption of nortriptyline hydrochloride (20 mg mL^{-1}). The chemicals 1-dodecanol, Span 20, Azone, (*R*)-(+)-limonene or isopropyl myristate were used as an overnight pretreatment at 5% (w/w) in ethanol. Furthermore, isopropyl myristate (20%, w/w) and propylene glycol (15%, w/w) were tested in the same vehicle. Iontophoresis was applied directly to the nortriptyline hydrochloride donor solution for three different concentrations (20, 2 and 0.5 mg mL^{-1}). The chemical enhancers slightly increased the nortriptyline transdermal flux but iontophoresis was more efficient. In this case, nortriptyline transdermal flux was concentration dependent, having a higher flux when the concentration was lowered. Therefore, iontophoresis was the most suitable technique to increase transdermal absorption of nortriptyline and it could be an alternative method to provide therapeutic concentrations of this drug in smoking cessation treatment.

Introduction

Recently, some clinical trials in healthy smokers have proposed the antidepressant nortriptyline, available in capsules and oral solution, as a new alternative in smoking cessation since it is safe and well tolerated in the dose regimen approved for the treatment of depression (Wagena et al 2005).

In general, the delivery of drugs across the skin is gaining wide acceptance among patients (Finnin & Morgan 1999). Advantages of transdermal drug delivery systems include non-invasiveness, prolonged drug levels in the bloodstream, reduced side effects, improved bioavailability, better patient compliance and easy termination of drug administration (Williams & Barry 2004). Nortriptyline has a half-life ranking from 16 to 90 h (McEvoy 2000), and its oral bioavailability varies from between 0.17 and 0.71, depending on the genotype of the cytochrome P450 (CYP) 2D6 (Kvist et al 2001). The main reason to optimize a transdermal system for this drug is based on one of the main problems associated with smoking therapies, i.e. absence of patient compliance. In this context, the development of transdermal systems is justified to facilitate nortriptyline use and reduce adverse effects such as dry mouth and constipation/gastrointestinal upset associated with oral administration. Many years ago, Bailey (1990) studied the percutaneous absorption of different tricyclic antidepressants, including nortriptyline, without employing any vehicle. Although at very low concentrations, the compound was detected in plasma.

In fact, human skin is a remarkably efficient barrier, thus causing difficulty for transdermal delivery of therapeutic agents since few drugs have the characteristics required to permeate sufficiently across the stratum corneum to reach therapeutic blood concentration. To enhance transdermal drug absorption different methodologies have been investigated and developed, including the use of chemical enhancers and physical methods that facilitate the diffusion of drugs through the human skin (Barry 2001).

The addition of penetration enhancers is a widely used approach to increase the range of drugs that can be effectively delivered via this route (Kanikkannan et al 2000). To date, a vast array of chemicals has been evaluated as enhancers (Bauerova et al 2001). However,

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despite the large number of chemicals evaluated as penetration enhancers in human or animal skin, to date none has proven to be ideal (Barry 2001). This makes it necessary to test the effect of different enhancers on the transdermal absorption of each compound intended to be administered by this route.

Different physical methods have been used to increase drug penetration through the skin (Barry 2001). In particular, iontophoresis, a technique based on the application of a low-level electric current ($\leq 0.50\,mA\,cm^{-2}$), has been demonstrated to be more efficient than chemical enhancers (Pikal & Shah 1990). The mechanisms by which iontophoresis enhances molecular transport across the skin are: electrorepulsion, which implies that charged ions are repelled from an electrode with the same charge; electro-osmosis, the convective flow of solvent that occurs in response to the preferential passage of counter ions when the electric field is applied; and current-induced skin permeability increment (Kalia et al 2004). Among these mechanisms electrorepulsion is the most relevant. Electro-osmosis, which is in general of a lower magnitude, becomes more important in the transport of large cations that have low transport numbers due to competition with smaller and more mobile ions comprising the background electrolytes. In fact, different parameters such as background electrolytes and current density have a great impact on iontophoresis efficacy (Phipps et al 1989; Delgado-Charro et al 1995).

We have investigated whether nortriptyline could be administered transdermally. Thus, we have conducted in-vitro standard diffusion experiments to characterize its passive diffusion and to monitor the effect of pretreatment of skin with various chemical enhancers on nortriptyline permeation, to find possible candidates to be included in a transdermal delivery system that could permit it to reach therapeutic blood levels. Nortriptyline has a positive charge which makes it a good candidate to be administered by means of iontophoresis. Accordingly, this technique was applied to the compound using three different drug concentrations (20, 2 or $0.5\,mg\,mL^{-1}$) in the donor solution.

Materials and Methods

Materials

Nortriptyline hydrochloride (98%), (*R*)-(+)-limonene, Span 20 (sorbitan monolaurate), propylene glycol, isopropyl myristate and HEPES (N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]) were obtained from Sigma-Aldrich Co. (Madrid, Spain). Azone (1-dodecyl-azacycloheptan-2-one) was obtained from Netqem (Durham, NC), 1-dodecanol synthesis grade and acetonitrile multisolvent HPLC grade were obtained from Scharlau (Barcelona, Spain). NaCl and ethanol (absolute) were purchased from Mallinckrodt Baker B.V. (Deventer, Holland). Ultrapure water was used to prepare all solutions and was obtained from a Barnstead NANOpure system (Barnstead International, Boston, MA). Silver chloride 99%, and 1-mm diameter silver and platinum wire 99.9%, used for the manufacture of the Ag/AgCl electrodes, were purchased from Sigma-Aldrich Co. (Madrid, Spain).

Permeation experiments were performed on Caucasian abdominal skin samples (from three female donors aged 38–48 years randomly assigned), obtained from cosmetic surgical corrections performed in the Hospital Clínico Universitario (Valencia, Spain). The study was performed in accordance with the provisions of the recent version of the Helsinki Declaration and after approval by the local Investigational Review Board Committee. Informed consent was previously obtained from the patients and their identity was masked to the researchers to guarantee their anonymity. Excess fatty and connective tissues were removed and the samples (full thickness skin) stored in a freezer at −40°C for less than one month.

For the passive diffusion experiments the skin membranes were placed in Franz-type diffusion cells with an effective area available for diffusion of $0.78\,cm^2$ and a receiver compartment capacity of approximately 6 mL (López et al 2000). The experiments employing iontophoresis were carried out in the same kind of diffusion cells, with slight modification. These cells had a diffusion area of $0.9\,cm^2$ and the donor compartment was divided into two 2-mL compartments (Glikfeld et al 1988). All cells were made in the SCSIE (Servicios Centrales de Soporte a la Investigación) of the Universitat de València, Valencia, Spain.

Diffusion experiments

The passive diffusion of nortriptyline was investigated at 37 ± 1°C. The pieces of skin were mounted over diffusion cells with dermal-side in contact with receptor phase, equilibrated for 1 h, and then air bubbles were removed. Then skin membranes were pretreated overnight either with 600 μL saline buffered solution pH 5.5 (buffer control), ethanol or a chemical enhancer solution of Span 20, (*R*)-(+)-limonene, Azone, isopropyl myristate or 1-dodecanol in ethanol (5%, w/w), respectively, to determine whether ethanol or any of the compounds enhanced the nortriptyline percutaneous permeation respective to the control. The skin was also pretreated with isopropyl myristate 20% (w/w) and propylene glycol (15%, w/w) in ethanol.

After pretreatment, the enhancer solution was removed and the skin was rinsed with 1 mL ethanol before the commencement of the diffusion experiments. Nortriptyline hydrochloride solution ($20\,mg\,mL^{-1}$) in HEPES (25 mM) at pH 5.5 (1 mL) was then placed in the donor compartment. The receptor compartment was filled with the same buffer. At fixed times, 200-μL samples from the receptor chamber were taken manually over 34 h, the volume being replaced with buffer pH 5.5. The drug contained in each sample was determined to calculate the accumulative amount of nortriptyline in the receptor compartment. Periodically, the donor solution was replaced to maintain the concentration gradient through the membrane.

To test the integrity of skin samples (Hanafy et al 2001), at the end of the experiments phenol red solution (0.5%, w/w) was added to the donor compartment (200 μL). At the end of the in-vitro transdermal diffusion experiments the amount of nortriptyline retained in the skin was extracted by shaking the skin for 20 h with 5 mL buffer (pH 5.5).

Iontophoresis was applied at room temperature. Three different concentrations of nortriptyline hydrochloride (20, 2 or 0.5 mg mL^{-1}), prepared in the same buffer mentioned above, were analysed. Nortriptyline was placed in the anodal compartment, and a constant current (0.50 mA cm^{-2}) was applied through the skin for 8 h using Ag/AgCl electrodes connected to a power supply (Kepco BHK-MG 0-2000V, Kepco Inc., Flushing, NY). Periodically, the donor solution was replaced (every 30 min) to maintain the concentration gradient through the membrane. The pH was verified at the end of the experiments.

Analytical method

The method used for the nortriptyline hydrochloride determination was based on Ghahramani & Lennard (1996). The amount of nortriptyline in the samples was quantified by high-pressure liquid chromatography (HPLC), using an isocratic pump (Waters 1515), a spectrophotometric detector (Waters 2487) set to 240 nm and Breeze software (Waters, Barcelona, Spain). An analytical column Kromasil 100 C-18, (5-μm particle size, 4.6 × 150 mm) (Scharlab, Barcelona, Spain) was used. A mixture of sodium dihydrogen phosphate anhydrous (1/15 M, pH 3)–acetonitrile (50:50, v/v) was used as mobile phase, at a flow rate of 1 mL min^{-1}. Injection volume was 20 μL.

The analytical method was properly validated. Accuracy of the method was defined as the relative error of known concentration solutions. The values found were within ± 10%, and accordingly it was considered acceptable. Precision of the method was tested as within-day and between-day reproducibility of the assay. Precision of the method was expressed as the residual standard deviation of replicate measurements. In all the concentrations analysed it was lower than 10% (Shah et al 1992).

Data analysis

Plots of the accumulated amount of nortriptyline (Q, mg cm^{-2}) against time (h) were constructed to explore its passive diffusion for the different conditions assayed (34 h) and in the iontophoresis experiments (8 h).

Transdermal fluxes at steady state (J_{ss}), from passive diffusion studies, were estimated by fitting equation 1 (Scheuplein 1967) to the accumulated amounts of nortriptyline vs time.

$$Q = (KH)C\left[\frac{D}{H^2}t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{n=7}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{H^2}\right)\right] \quad (1)$$

Where Q is the cumulative amount of drug permeated per unit area at time t, C is the concentration of the drug in the donor vehicle, K is the stratum corneum/vehicle partition coefficient, D the diffusion coefficient, and H the diffusion path length. The fitting procedures were carried out by means of nonlinear regression using the Win Nonlin 4.1 (Pharsight Corp). An equal weighting scheme was applied. Standard error of the parameters determined was below 30%.

This procedure led to the determination of the drug's partition (P′ = KH) and diffusivity (D′ = D/H^2) parameters. The permeability coefficient (Kp) was then calculated as the product of P′ and D′.

Steady-state flux (J_{ss}) was obtained as:

$$J_{SS} = Kp \times C \quad (2)$$

The lag time of diffusion (L_t) could be determined from the expression:

$$L_t = \frac{H^2}{6 \times D} \quad (3)$$

The data obtained after iontophoresis were explored by means of a linear regression, excluding the first time point only in the experiments with the lowest nortriptyline hydrochloride concentration (0.5 mg mL^{-1}).

The permeability parameters and lag times were statistically evaluated by one-way analysis of variance after homogeneity was confirmed by Bartlett's test. Post-hoc comparisons of the means of individual groups were performed with Scheffe's test, with the level of statistical significance set to $P < 0.05$. When statistical differences were detected, the permeation-enhancing activity, expressed as enhancement ratio of flux (ER_{flux}), was calculated as the quotient of the flux value obtained with the enhancer to that found with control (buffer) or vehicle (ethanol), respectively.

Results and Discussion

Nortriptyline base (molecular weight = 263.4 g mol^{-1}) is a lipophilic compound with a partition coefficient in octanol, log P_{oct}, of 4.7 (data from PubChem Compound database) and a pK_a of 9.7 (Ruiz-Angel et al 2003). Considering its lipophilicity and the frequency of its administration in the treatment of depression, it can be considered a good candidate for transdermal administration (Barry 2001). We used nortriptyline hydrochloride (molecular weight = 297.8 g mol^{-1}) for this study.

Amounts of nortriptyline accumulated in the receptor compartment as a function of time after chemical enhancer pretreatment are plotted in Figure 1 to facilitate the comparison of values. All the enhancers, more or less, increased the penetration of nortriptyline through the skin, with the exception of propylene glycol.

In Table 1, the nortriptyline permeability parameters obtained after skin pretreatment with and without the different enhancers tested are summarized. 1-Dodecanol had the highest enhancing activity for nortriptyline and the flux increment with respect to the vehicle (ethanol) was approximately 5-fold (see Figure 2). The rest of the chemical enhancers increased nortriptyline absorption very moderately, 3-fold with respect to ethanol. Surprisingly, Azone, a good chemical enhancer for many kinds of compounds (López et al 2000), had the lowest enhancing effect among the substances tested.

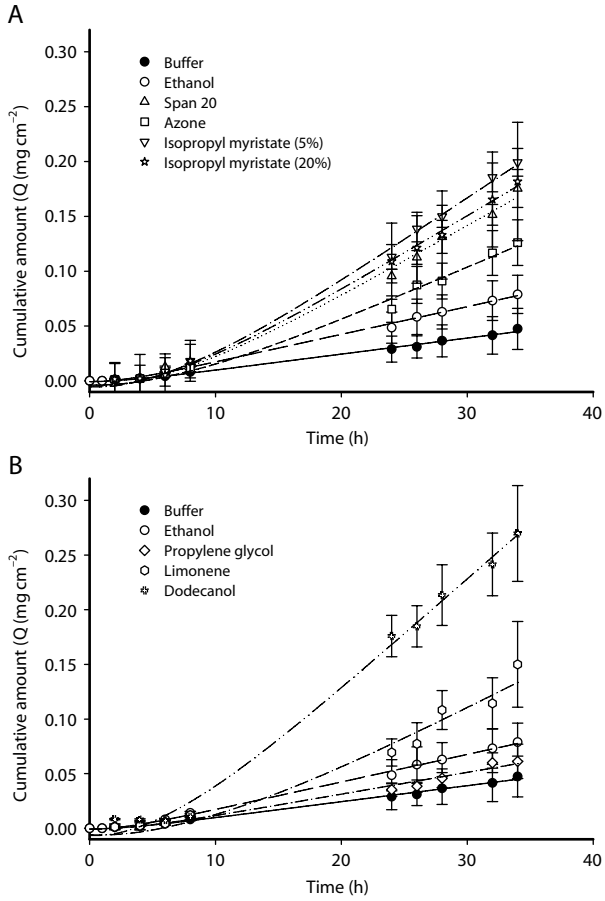

Figure 1 Accumulated amount of nortriptyline hydrochloride (Q, mg cm^{-2}) vs time (h), in the receptor compartment, after treatment of the skin with the enhancers in ethanol (A and B). Error bars show standard deviation.

Guo et al (2006) reported that there was a concentration dependence of the enhancing activity of isopropyl palmitate for different kinds of compounds, with the highest activity at 20%, w/w, concentration. Accordingly, isopropyl myristate was tested at two different concentrations (5 and 20%, w/w). Nevertheless, we did not detect differences in its enhancing activity at the two concentrations assayed.

Since the enhancers used in this study were directly applied onto the skin, previously to the diffusional experiments, their enhancer effects cannot be attributed to variations in nortriptyline solubility in the vehicle. Thus they can act directly on the skin by several mechanisms (Barry 2001; Williams & Barry 2004) that include: disruption of the intercellular lipid domains to reduce the barrier resistance of the bilayer lipids; modification of the solvent nature of the stratum corneum to increase partitioning of the drug or of a co-solvent into the stratum corneum; interaction with intracellular proteins; and change in the desmosomes that maintain cohesion between corneocytes. Nevertheless, for highly lipophilic enhancers the two latter mechanisms might play a relatively small role (Barry 2001).

With the exception of propylene glycol, the enhancers used were compounds of high lipophilicity (Table 1). Therefore, it seemed that the first two mechanisms described could participate in their enhancer activity. For the lipophilic enhancers tested (Table 1), the diffusion parameter (D′) of nortriptyline decreased with respect to the vehicle control (ethanol), and accordingly the lag time calculated from the diffusion parameter increased with regard to the control (ethanol). On the contrary (as could be expected) the apparent partition parameter increased. The highest value of P′ was provided by 1-dodecanol, the most potent enhancer for nortriptyline, but this P′ value was very similar to that obtained with (*R*)-(+)-limonene, with lower enhancing activity.

Three of the lipophilic enhancers (1-dodecanol, Azone and Span 20) had an alkyl chain which was the same (C12); for isopropyl myristate it was C14. This alkyl chain can permit the molecular insertion into lipid bilayers of the stratum corneum (Bouwstra et al 1989; López et al 2000). The local disorders induced by such insertion could allow an increase in drug permeation mainly via the intercellular route (Ribaud et al 1994). However, this effect was not produced by limonene, given its molecular structure. In fact, this compound provided a diffusion parameter with the lowest value ($D' = (14.2 \pm 4.5) \times 10^3$ cm h^{-1}).

Table 1 Partition coefficients in octanol (P_{oct}) of the different chemical enhancers used in skin pretreatment. Partition (P′) and diffusion (D′) parameters, permeability coefficients (K_p), steady-state flux (J) and lag time (Lt) of nortriptyline hydrochloride and amount of the drug retained in the skin.

Skin pretreatment	log P_{oct}[a]	(P′ ± s.d.) × 10^3	(D′ ± s.d.) × 10^3 (cm h^{-1})	(K_p ± s.d.) (nm h^{-1})	J ± s.d. (μg cm^{-2} h^{-1})	Lt ± s.d. (h)	Amount of nortriptyline retained in the skin (mg g^{-1})
Buffer solution	–	1.5 ± 0.4	49.9 ± 13.1	0.75 ± 0.01	1.51 ± 0.02	3.3 ± 1.1	1.87 ± 0.36
Ethanol	–	2.4 ± 0.5	54.9 ± 10.1	1.32 ± 0.02	2.64 ± 0.03	3.2 ± 0.9	1.73 ± 0.39
Propylene glycol	−0.7	2.6 ± 1.4	38.9 ± 18.2	1.01 ± 0.25	2.02 ± 0.51	4.3 ± 1.2	0.52 ± 0.14
1-Dodecanol	5.4	26.6 ± 6.5	24.4 ± 4.8	6.49 ± 0.36	13.01 ± 0.58	6.8 ± 0.7	2.74 ± 0.54
Span 20	3.9	16.2 ± 4.5	20.1 ± 4.1	3.27 ± 0.18	6.54 ± 0.16	8.3 ± 0.2	2.01 ± 0.58
Azone	6.4	13.2 ± 4.2	18.6 ± 4.2	2.45 ± 0.19	4.91 ± 0.21	8.9 ± 0.3	2.02 ± 0.30
Isopropyl myristate (5%)	6.4	19.1 ± 3.6	20.0 ± 2.8	3.82 ± 0.13	7.64 ± 0.33	8.4 ± 0.5	2.59 ± 0.50
Isopropyl myristate (20%)	–	16.3 ± 2.7	21.0 ± 2.5	3.42 ± 0.21	6.82 ± 0.45	7.9 ± 0.8	1.42 ± 0.45
R-(+)-limonene	3.7	21.1 ± 10.4	14.2 ± 4.5	2.98 ± 0.47	5.96 ± 0.93	11.7 ± 1.4	2.55 ± 0.55

[a]Data from PubChem Compound database (see reference). The number of experiments was four or five.

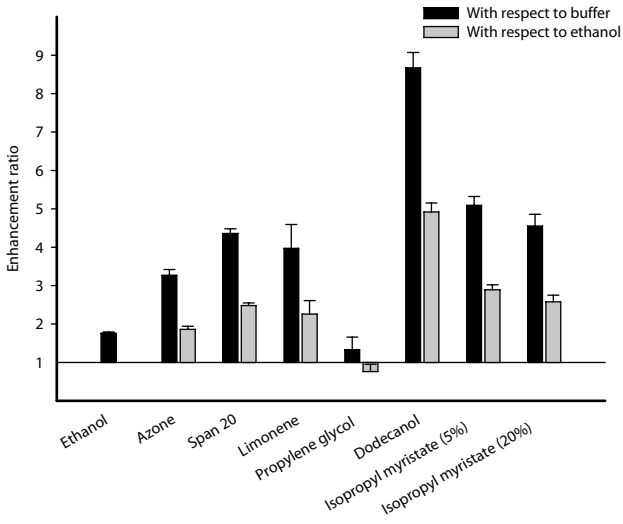

Figure 2 Enhancement ratio of flux of all chemical enhancers used.

On the other hand, since 1-dodecanol, Azone, Span 20, and isopropyl myristate did not have exactly the same effect on nortriptyline permeability parameters, as can be seen in Table 1, to analyse the differences in efficacy of the lipophilic enhancers used, the relationship between nortriptyline permeability coefficients and lipophilicity of enhancers was explored. The maximum enhancement effect was obtained with 1-dodecanol, which had a log P_{oct} value of 5.4, the closest to nortriptyline (log P_{oct} = 4.7). Accordingly, it seemed that 1-dodecanol inserted into lipid bilayers of skin modified its nature and created an environment of lipophilicity very similar to that of nortriptyline, thus increasing the affinity of the skin for this compound. Nevertheless, there were no statistical differences among the amounts of nortriptyline retained in the skin after the experiments when comparing the results (2.74 ± 0.54 mg nortriptyline $(g skin)^{-1}$) with those obtained with no enhancer (1.87 ± 0.36 mg nortriptyline $(g skin)^{-1}$), probably due to the high variability of the results.

This mode of action could explain the absence of an enhancer effect of propylene glycol, with a log $P_{oct} = -0.7$, which could increase the hydration of the lipid bilayers, thus creating surroundings not favouring nortriptyline penetration.

Finally, taking into account nortriptyline clearance in man, $36\,L\,h^{-1}$ (Merlé & Mentré 1999), to reach the minimum plasma level associated with optimal response, approximately $50\,ng\,mL^{-1}$ (Reynolds 1993), an entrance of $1.8\,mg\,h^{-1}$ would be required. In view of the results obtained with the chemical enhancers studied and considering the pharmacokinetic data for nortriptyline mentioned above, with a patch including the most efficient enhancer, 1-dodecanol, a surface of $138\,cm^2$ should be enough to reach therapeutic blood level. This surface may be too large for the design of a transdermal patch.

For this reason, the iontophoresis was employed with the purpose of increasing the penetration of the nortriptyline. Since this drug ionizes to a cation, it was a good candidate to be administered by means of this technique. To compare the results with those using chemical enhancers as a first step, we used a solution of $20\,mg\,mL^{-1}$. Afterwards, since depending on the nature of the compound the flux can be modified by the concentration when using iontophoresis (Kalia et al 2004), we decided to apply the current to lower concentration solutions. The fluxes obtained in these conditions are shown in Table 2. As can be seen in Table 2, iontophoresis increased nortriptyline transdermal flux to a greater extent than the chemical enhancers (Table 1). Therefore, at $0.5\,mg\,mL^{-1}$, the flux of nortriptyline when applying iontophoresis was of a great magnitude, and a fairly small patch (anodal surface less than $7\,cm^2$) could provide the plasma levels required. Moreover, the main problem of the chemical enhancers, the lag time, was dramatically reduced with this technique.

When describing the relationship between the concentration of compound in the donor chamber and the iontophoretic flux, there is some difficulty. For some lipophilic compounds (log P approximately 3) it has been suggested that the transdermal flux observed is not related to the concentration (Marro et al 2001). On the other hand, more lipophilic substances, such as the decapeptides leuprolide and nafarelin, become anchored in the transport path, neutralizing the original charge of the membrane and completely altering the permselective properties of the skin. As a result the electrorepulsion is reduced and electro-osmosis becomes the main mechanism of transport. Moreover, this can be reversed later, that is it, becomes higher from the

Table 2 Steady-state flux (J) and lag time (Lt) determined after applying iontophoresis to three different concentrations of nortriptyline hydrochloride.

Concn in donor compartment ($mg\,mL^{-1}$)	Flux ± s.d. (J, $\mu g\,cm^{-2}\,h^{-1}$)	Lt ± s.d. (h)
20	84 ± 3	0.9 ± 0.1
2	171 ± 6.2	0.8 ± 0.1
0.5	262 ± 39	1.8 ± 0.1

The number of experiments per condition was four or five.

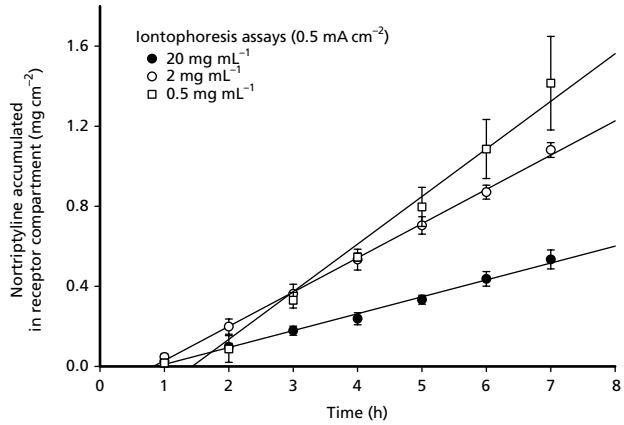

Figure 3 Accumulated amount of nortriptyline hydrochloride $(mg\,cm^{-2})$ vs time (h) in the receptor compartment after iontophoresis application at $0.5\,mA\,cm^{-2}$.

cathodal compartment than from the anodal one (Delgado-Charro & Guy 1995; Rodríguez Bayón & Guy 1996). Nortriptyline, with a log P of approximately 4.7, behaved as the mentioned peptides; it could reduce its own transport through skin because at high concentrations it binds all the terminal groups available in the skin. This fact indicates the importance of selecting the concentration of the donor solution when applying iontophoresis.

Iontophoresis was applied to nortriptyline in the absence of any salt in the solution to avoid competition of small ions with the drug in the transport of current. A further lower concentration would probably allow the optimal relation of concentration/transdermal flux to be reached. Nevertheless, without modifying the design of the experiments it was not possible to reduce further the concentration of the compound in the donor solution, because the chloride in the anode would not be sufficient for the oxidation of silver to be completed.

Conclusion

Among the chemical enhancers tested, 1-dodecanol provided a moderate enhancement of nortriptyline transdermal flux. Iontophoresis was the most suitable technique to increase transdermal absorption of nortriptyline, as it seemed capable of providing therapeutic concentrations. Further studies are required to explore in-vivo the suitability of this technique in terms of irritation, as well as the frequency of administration required.

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