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Note

Wistar rat skin as surrogate for human skin in nortriptyline hydrochloride patch studies

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ABSTRACT

Six different matrices were prepared containing nortriptyline hydrochloride (NTH) with hydroxypropyl-methyl-cellulose as polymer. A mixture of transdermal enhancers was included as part of the vehicle. Diffusion studies were carried out through Wistar rat full thickness skin using Franz cells. They were compared with previously determined human heat separated epidermis in order to test if this animal can be used as model for *in vivo* assays. A linear correlation was obtained between NTH diffusion coefficients through both skin types ($r^2 = 0.996$).

Smoking is one of the most important risk factors of many prevalent diseases in our society. In this scenario there is a need to improve the current therapies for smoking cessation. Nortriptyline hydrochloride (NTH) has proven to be effective with a recommended oral daily dose of 25 mg the first day before quitting and a maintained dose of 75 mg afterwards (Prohazka et al., 1998). Its main disadvantages are low oral bioavailability (between 30 and 50%) and side effects associated to plasma level fluctuations that may cause lack of compliance and possible failure of the therapy (Stimmel, 1996).

It has been reported that NTH diffuses through human heat separated epidermis (HHSE) from patches (Melero et al., 2008, 2009). The next step for evaluating a pharmaceutical formulation should be to perform *in vivo* studies.

This work presents a comparative study of the diffusion of NTH through HHSE and Wistar rat full thickness skin (WFTS) to test if Wistar rats are a suitable *in vivo* model to address transdermal absorption of NTH and a surrogate to predict dermal drug access in men.

NTH (min. 98%), ethanol (absolute), lactic acid (LA) (min. 85%), propylene glycol (PG) (99%) and hydroxypropyl-methyl-cellulose (HPMC) (2910) were purchased from Sigma Chemical (Madrid, Spain). Oleic acid (OA) (65–88%) and polysorbate 80 (PS80) (Pharmacopoeia Grade) were obtained from Fluka (Buchs SG, Switzerland). The rest of chemicals were of high-performance liquid chromatography (HPLC) analytical grade.

Seven films already studied in HHSE were prepared for the present work. The procedure was as described by Melero et al. (2009). They contain NTH in different concentrations and in the presence of different chemical enhancers (Table 1). The film forming polymer used was hydroxypropyl-methyl-cellulose (HPMC) in all the cases. Briefly, the preparation process consists of dissolution of the different components, gel preparation by addition of HPMC and desiccation of 20 g of gel on a Petri-dish at 50 °C over a heating plate under the extractor hood for 6 h.

The animal skin model was obtained from male Wistar rats belonging to the authorized faculty facility. The study was approved by the Pharmacy Faculty Ethics Commission. After sacrifice of the rat the skin was removed from the back with a scalpel and stored in a freezer at −26 °C for a maximum of 15 days. 25 mm diameter pieces were punched and the hair was cut by means of scissors. After thawing, the samples were submerged in physiologic solution for a maximum of 2 h till they were placed onto the Franz diffusion cells (FDC). FDC were type 4G-O1-00-20 (Perme Gear, Riegelsville, PA, USA), with an available diffusion area of 1.76 cm^2 ($d = 1.5$ cm) and 12 mL of receptor cell volume placed in a heating/stirring module. As receptor phase, bubble-free phosphate buffer solution (1/15 M, pH 5.5) was used. A dialysis membrane was cut and placed over the receptor compartment underneath the skin membrane due to stabilization reasons. 100 μl of the same phosphate buffer

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Table 1
Composition of the formulations.

	A (%)	B (%)	C (%)	D (%)	E (%)	F (%)
NTH	2	2	4	4	4	8
EtOH	30	30	30	30	30	30
PG	30	30	30	30	30	30
OA	1	1	1	1	–	1
PS80	–	1	–	1	1	1
HPMC	2	2	2	2	2	2
H_2O	35	34	33	32	33	28

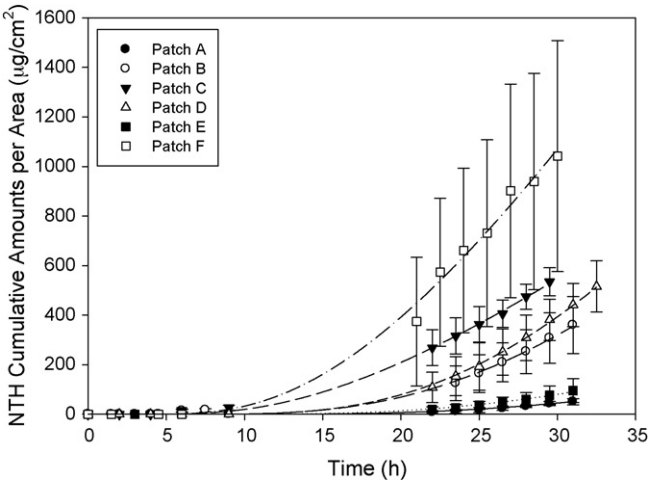

Fig. 1. NTH diffusion through WFTS.

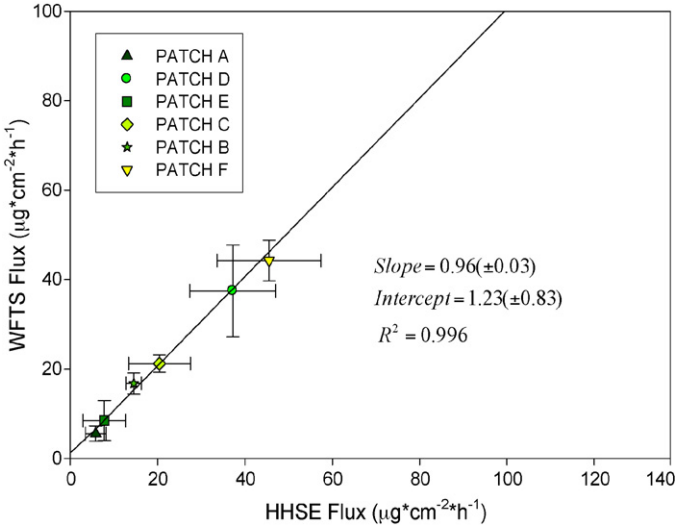

Fig. 2. Correlation between fluxes from HHSE and WFTS.

was placed over the membrane in order to moisture it and assure the adhesion of the film. The cells were equilibrated for 1 h, and then air bubbles were removed off the diffusion cell. Finally, a 13 mm diameter film disc was placed on top of the skin and the donor compartment was covered with Parafilm® and aluminum foil. 400 μl samples were collected at 1.5, 3, 4.5, 6, 7.5, 9, 22.5, 24, 25.5, 27, 28.5, 30 and 31.5 h from the receptor compartment. The same volume of fresh buffer solution was added after each sampling. NTH concentration was measured by HPLC as described in Melero et al. (2008).

Fig. 1 shows the cumulative amounts of NTH versus time obtained using WFTS. The linear regression of the steady state provided the diffusion parameters: flux (J, $\mu g\,cm^{-2}\,h^{-1}$), permeability coefficient (K_p, $cm\,s^{-1}$) and lag time (t_L, h), which are listed in Table 2. Fluxes obtained for the patches through HHSE are also listed in Table 2 for comparison (Melero et al., 2008, 2009).

Results confirm previous findings from many authors concerning the enhancement produced by PS80 and OA in different systems (Gao and Singh, 1998) regardless of the membrane used in the experience of penetration. The comparison E vs. D patch gives an enhancement about 4.5 times due to the presence in the matrix of OA. Concerning PS80, a bigger enhancement effect can be seen in 2%-NTH matrices (A vs. B, about 3 times) than in 4%-NTH matrices (C vs. D, about 1.5 times). This fact can be explained in light of changes due to different NTH concentration. The effect of concentration in the film is studied by the comparison among B, D and F patches; as can be observed, the fluxes increase but not proportionally, that leads to a bias in the K_p estimation. The reason for these results could be due to changes in the network of the gels or different thermodynamic activity.

HHSE-fluxes obtained for the patches were plotted vs. WFTS-fluxes (Fig. 2). They were correlated by means of a statistically significant linear regression (Excel Microsoft Office, 2007). This finding is important and should be studied with different drugs in order to establish the suitability of the rat as model for transdermal absorption from patches. In fact, our data demonstrate that the NTH permeability through both model membranes is similar as the slope is not statistically different from 1. The intercept value, that is significantly different from zero ($\alpha = 0.05$), can be interpreted as a slightly higher permeability in WFTS for NTH than in HHSE. This effect could be due to the presence of more hair follicles in rats that facilitates the overall diffusion process.

Taking these results into account it can be concluded that Wistar rat is a good animal model for future *in vivo* experiments with these patches.

Table 2
Diffusion parameters through WFTS.

	(A) 2% NTH OA	(B) 2% NTH OA + PS80	(C) 4% NTH OA	(D) 4% NTH OA + PS80	(E) 4% NTH PS80	(F) 8% NTH OA + PS80
Flux WFTS ($\mu g\,cm^{-2}\,h^{-1}$)	5.53 ± 1.71	16.75 ± 13.52	21.19 ± 1.93	37.45 ± 1.32	8.45 ± 4.74	44.23 ± 4.57
Flux HHSE ($\mu g\,cm^{-2}\,h^{-1}$)	5.80 ± 2.40	14.52 ± 1.80	20.39 ± 7.09	37.21 ± 9.83	7.76 ± 4.91	45.50 ± 11.9
WFTS K_p ($\times 10^6\,cm\,s^{-1}$)	0.022 ± 0.006	0.067 ± 0.005	0.064 ± 0.006	0.099 ± 0.027	0.026 ± 0.014	0.082 ± 0.008
Lag time (h), mean ± SD	21.70 ± 1.02	7.58 ± 8.04	7.07 ± 1.40	19.70 ± 1.32	20.09 ± 1.75	7.59 ± 9.76

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