International Journal of Pharmaceutics, 98 (1993) 37–43

IJP03134

In vitro percutaneous absorption of naproxen from gels using a double-layer artificial membrane

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(Received 22 September 1992)
(Accepted 24 November 1992)

Key words: Naproxen; Gel; In vitro percutaneous absorption; Double-layer membrane

Summary

The percutaneous absorption of naproxen from different gels was studied, using a double-layer (hydrophilic/lipophilic) artificial membrane to determine the influence of the viscosity of the preparation and the thermodynamic activity of naproxen. The predominant factor appeared to be the activity of naproxen in the preparation, although viscosity seemed to affect the kinetics of the process.

Introduction

This paper is one of a series in which, taking naproxen as model molecule, we describe attempts to simplify preformulation studies of a topical form of the drug in 5% (w/w) solution (Contreras et al., 1992, 1993a,b). For this purpose the theory of regular solutions (Hildebrand et al., 1970) was applied in the different phases. This paper studies the in vitro percutaneous absorption of naproxen in selected formulations, using a double-layer model membrane. This method was chosen previous to in vivo percutaneous absorption studies, due to the variation in results which the latter usually produce. This type of membrane has been used in numerous topical pharmaceutical preparations (Loth et al., 1979; Proserpio et al., 1982; Szentmiklosi et al., 1984; Velissaratou and Papaioannou, 1989; Valenti et al., 1990) because of its sensitivity, reproducibility and simplicity of manipulation. The results obtained allow qualitative but not quantitative differentiation of the behaviour of the different preparations during the process of percutaneous absorption.

While considering the mechanism of the action of naproxen for the design and selection of formulations, we have taken into account the physical-chemical principles governing the process of percutaneous absorption as established by Higuchi (1960). According to this author and as directly derived from Fick's law, when the stratum corneum is the only barrier to absorption, the flow rate of the substance penetrating the epidermis in the stable solid per surface unit, J (mol

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$cm^{-2}\ s^{-1}$), can be defined as:

$$J = \frac{P_c D_p \Delta C}{h} \quad (1)$$

or by the directly related form:

$$J = \frac{a_v}{\gamma_p} \cdot \frac{D_p}{h} \quad (2)$$

where P_c represents the partition coefficient of the substance between membrane and vehicle and D_p is its diffusion coefficient in the stratum corneum ($cm^2\ s^{-1}$); ΔC denotes the concentration gradient (mol cm^{-3}), h is the thickness of the stratum corneum (cm), a_v represents the thermodynamic activity of the solute in the excipient and γ_p is the activity coefficient of the solute in the stratum corneum.

It can be deduced from these equations that the increase in the values of terms P_c, ΔC and a_v indicates an increase in flow rate, as long as the system as a whole does not endanger the skin. In pharmaceutical preparations the concentration must be established so as to avoid toxic phenomena or adverse effects. The flow rate therefore depends exclusively on the excipient, as the thermodynamic activity (a_v) and the partition coefficient of the solute (P_c) are very susceptible to its composition. Any qualitative or quantitative variation in one or more of the components rapidly affects these parameters (Ashton et al., 1988). The other factors involved in the equations can be considered as constant for the drug under study, regardless of the vehicle (Barry, 1983).

The composition of the formulations tested is shown in Table 1, and a detailed justification can be found in previous papers (Contreras et al., 1992, 1993a,b). We here maintain the same nomenclature for ease of identification of the different samples.

TABLE 1

Formulations assayed (compositions expressed in % w/w and some characteristics)

	Formulations [a]			
	B-1	C-1	D-1	E-1
Naproxen	5.0	5.0	5.0	5.0
Di(2-ethylhexyl) adipate	61.6	–	–	–
Propylene glycol dipelargonate	–	66.8	–	–
Dibutyl adipate	–	–	71.8	67.4
Ethanol	13.4	13.2	8.2	7.6
Ethyl cellulose	20.0	15.0	15.0	20.0
Naproxen concentration				
% w/w	5	5	5	5
mg cm^{-3}	51.42	52.45	55.15	51.42
D_v ($\times 10^{-7}$) $cm^2\ s^{-1}$ [b]	1.74	3.14	5.63	3.28
η (cP) [c]	76265	5412	4446	34373
Naproxen released (% w/w) [d]	12.69	15.75	20.52	15.26

[a] For later identification.
[b] Diffusion coefficient of naproxen in the preparation.
[c] Viscosity of the formulation.
[d] Naproxen released.
[a–d] Contreras et al. (1993b).

Materials and Methods

Materials

Naproxen (D-2-(6-methoxy-2-naphthyl)propionic acid) was provided by Elmu S.A. Its fusion temperature, infrared spectrum and optical rotation conformed to the values included in USP XXII and BP (1988).

The sources of the products were Glyco Ibérica for propylene glycol dipelargonate (Glyco PR-827), Henkel for dibutyl adipate (Cetiol B) and Aldabo-Julia for di(2-ethylhexyl) adipate (Cromadol DOA), all of which are classified by the different companies as technical products for cosmetic use. They were used as received.

Ethanol (Q.P., Panreac); the polymerisation grade of the ethylcellulose used (Ethocel, Dow Chemical S.A.) produced 2% aqueous solutions with 5 cP viscosity.

Methods

Preparation of gels

The naproxen was added and dissolved after mixing the cosolvents in suitable proportions at

approx. 29°C. The samples were gelled by addition of the polymer, which was mixed with the aid of a mechanical stirrer. The samples were then left at 25°C for at least 15 days before undertaking the absorption tests. All the gels were transparent and homogeneous.

In vitro percutaneous absorption

We used the instrument developed by Loth and Holla-Benninger (1978) and marketed by Sartorius (Sartorius ointment chamber, model SM 16754).

The double-layer (hydrophilic/lipophilic) membrane was prepared by soaking a cellophane membrane in water for 1 h and then removing the excess water with filter paper. The lipophilic part was prepared by impregnating a second (probably cellulose acetate, RS filter type) membrane with lauric alcohol, so that its initial weight was not increased by more than 100–110%. Both membranes were joined by pressure and placed in the diffusion chamber (15.55 cm^2 diffusion area).

The receptor liquid used was a 100 ml regulating phosphate solution of pH 7.5 ($Na_2HPO_4 \cdot 2H_2O$, 20.5 g; KH_2PO_4, 2.8 g; per 1000 ml of solution), in which the experimental solubility of naproxen is 0.74% (w/w) at 25°C.

The absorption study was carried out by sampling without replacement and by spectrophotometric determination of the naproxen in the receptor liquid (Perkin-Elmer, model 124; $\lambda = 271$ nm). The volume withdrawn from each sample (normally 4 ml) and the time interval between taking of samples and the total assay time (6 h) depended on the type of determination used and the minimum volume of receptor liquid left in the chamber, which, as indicated by the Sartorius Co., should never be less than 75% of the initial volume.

All the assays were carried out at 35°C, with no apparent alteration of the membrane.

Results and Discussion

Fig. 1 gives a graphic representation of the average accumulative concentrations of naproxen absorbed per area unit ($\mu g\ cm^{-2}$) in three different tests run on the same sample. The different behaviour of each of the processes can be inferred from observation of the plot.

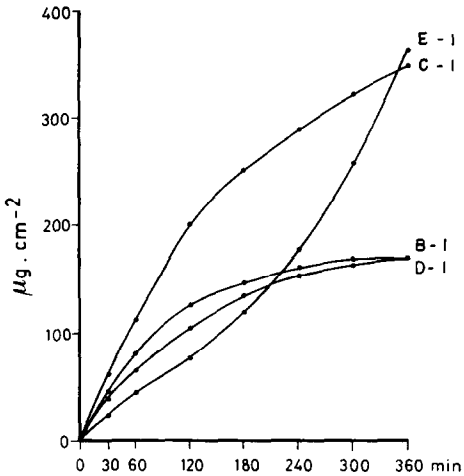

Fig. 1. Accumulated absorption of naproxen per area unit through a double-layer artificial membrane. For identification and composition see Table 1.

In order to determine whether the absorption process depends on the release of solute from the vehicle or by passage of the substance across the membrane, the results obtained were subjected to the kinetic study proposed by Higuchi (1962) to show the presence (in the first case) or absence (in the second case) of linearity in the accumulated quantity of drug absorbed and the square root of the time. Satisfactory linear adjustments were not obtained in any case, by which we may infer that the absorption process is basically governed by the membrane. In other words, the assays were carried out under adequate conditions and, qualitatively at least, the membrane reproduced the behaviour of the stratum corneum.

Three consecutive zones are distinguished in the typical trajectory of an absorption curve governed by a membrane. These are: the time lag, the period of flow rate in the stable state, and a flattening out where the flow rate is lower as a consequence of the decrease in the initial concentration of solute in the excipient.

In formulations C-1, B-1 and D-1 absorption

begins almost immediately (time = 0), with values that constitute a sharply rising trajectory, followed by an almost flat line in the case of B-1 and D-1. The phenomenon occurs rather differently for C-1. Here the trajectory rises more sharply and flattens out gradually until the end of the experiment. No time lag was observed in any of the three formulations, or at least this was less than the time when the first sample was taken (30 min).

The curve obtained in sample E-1 is completely different from the former ones. A gradual increase in absorption is observed, later reaching a practically constant flow rate, which means that the time lag (obtained by extrapolation of the flow rate in the stable state on the time axis) is around 132 min, and the flat section is not reached before the end of the time set for the experiment.

From the analysis of the equations according to Higuchi, which relate the rate of percutaneous absorption in the stable state and the parameters directly dependent on the solute (Eqns 1 and 2), we know that the flow rate of a substance from different excipients is directly related to their thermodynamic activity (a_v) and the membrane/vehicle partition coefficient (P_c) in each of them. The other intervening factors in both equations, such as the diffusion coefficient and the coefficient of thermodynamic activity in the membrane, can be taken as constant and independent of the nature of the excipient. According to the foregoing considerations, if the thickness of the membrane is maintained constant for different assays, the variations in the flow rates (J) are only due to the modifications of the thermodynamic activity of the solute in the vehicle.

Flynn et al. (1974) established the direct relationship existing between flow in the stable state (J) and the value of the permeability coefficient (K_p) of the membrane for the solute (Eqn 3). This coefficient reflects the product of the membrane/vehicle partition coefficient (P_c) and the diffusion coefficient of the solute in the membrane (D_p) (Eqn 4):

$$K_p = \frac{J}{C_v} \tag{3}$$

or

$$K_p = \frac{P_c D_p}{h} \tag{4}$$

If we consider that the latter coefficient (D_p) is taken as constant for a given solute, the variations in the value of the permeability coefficient can only be attributed to the differences in the partition coefficient and, due to their close connection, to modifications of the solubility of the solute in the excipient.

We may therefore conclude that the study of the absorption rate and the permeability coefficient of a substance from different vehicles allows us to examine more closely the relationships between the solute, the excipient and the membrane. With this aim, the flow in the stable state (J) and the value of the permeability coefficient of the membrane for naproxen (K_p) were determined in each of the experimental curves obtained above, in a reasonable space of time. These determinations are possible since the conditions under which the assays were conducted conform to the limits established by Higuchi (1960): constant concentration in the donor phase and sink conditions in the receptor phase.

As regards the absorption rate, the similarity between samples C-1 and E-1 can be seen in Table 2. According to the preceding observations, this value confirms that the naproxen in both excipients presents the same thermodynamic activity, and thus corroborates the supposition based

TABLE 2

Absorption rate of naproxen (J) and permeability coefficient of the membrane (K_p)

Formulation [a]	Interval [b] (min)	J ($\mu g\ cm^{-2}\ min^{-1}$)	K_p ($\times 10^{-5}$) (cm^{-1})
B-1	30–120	0.819 (SD ± 0.7) [c]	1.592
C-1	30–120	1.564 (SD ± 5.0) [c]	2.982
D-1	30–120	0.764 (SD ± 0.7) [c]	1.385
E-1	240–360	1.584 (SD ± 16.3) [c]	2.597

[a] For identification see Table 1.

[b] Time interval in which flow rate is detected in the stable state.

[c] In parentheses: standard deviation in three assays.

on the analyses of the data from the release test and the values of the diffusion coefficient of naproxen in the excipient (Table 1) (Contreras et al., 1993b). However, the trajectories of their kinetic behaviour (Fig. 1) are considerably different. In the case of C-1 there is no time lag, whereas it is significant in the case of E-1 (approx. 132 min). Whether or not flow in the stable state takes place at zero time probably depends on the value of the dynamic viscosity of the preparations (Table 1). In formulation C-1, which has low viscosity, equilibrium between membrane and excipient is almost instantaneous, whereas for E-1 this equilibrium takes some time to be established, due to the high viscosity of the preparation.

Samples D-1 and B-1 present absorption rates in the stable state which are practically half those analyzed above, and slightly higher in the case of B-1. As deduced from the release studies (Contreras et al., 1993b) the thermodynamic activity of naproxen is higher in D-1 than in B-1 and this difference seems to have a direct effect on the flow rate. Despite a markedly higher value for the viscosity of the preparation in D-1 compared with C-1 (Table 1), this does not appear to affect percutaneous absorption, unlike what took place in the release of naproxen from the excipient. Since absorption occurs much more slowly than release, the effect of viscosity is probably eliminated.

The values of the permeability coefficients of the membrane for naproxen (K_p) present variations similar to those of the absorption rate in the stable state (J). As already mentioned, the differences in the permeability coefficient can be attributed to the difference in the partition coefficient of the solute between the membrane and the vehicle, which in turn is directly related to the solubility of the solute in the excipient. The results obtained agree with the observations of Blank (1964) and Marzulli et al. (1965), as the highest permeability coefficient values of the membrane for the solute were obtained for those vehicles in which the naproxen was presumably at a practically saturated concentration, i.e., in those excipients in which the thermodynamic activity of the solute was highest (C-1 and E-1).

The value of the diffusion coefficient of naproxen in the membrane assayed can be deduced from the time lag of preparation E-1 (t_r = 132 min), since the following relationship exists between the two (Flynn et al., 1974):

$$t_r = \frac{h^2}{6D_p}$$

where h represents the thickness of the membrane and D_p the diffusion coefficient of the solute in the membrane.

If we accept the approximate thickness of the membrane to be 100 μ, as suggested by Delonca et al. (1977) for a membrane similar to that used here, the diffusion coefficient of naproxen is around 2.1×10^{-9} cm^2 s^{-1}. This value is similar to that obtained for levonorgestrel and hairless rat skin (Catz and Friend, 1990) and for different solutes and human epidermis (Sheuplein and Blank, 1971), but lower than expected as regards the differences between the characteristics of the membrane used and the stratum corneum, whatever its origin. This may in part be due to the proximity of the values of the solubility parameter of the oily (lauric alcohol) component of the membrane (10 cal$^{1/2}$ cm$^{-3/2}$) (Barton, 1975) and that of naproxen (9.7 cal$^{1/2}$ cm$^{-3/2}$) (Contreras et al., 1992). The similarity in the values of both parameters probably also explains the higher values of the permeability coefficient obtained for the naproxen (around 10^{-5} cm s^{-1}) compared to other substances and human skin (around 10^{-6} cm s^{-1}) (Provost et al., 1989).

According to the studies by Liron and Cohen (1984) and Sloan et al. (1986), the solubility parameter of the corneum stratum can be considered to be between 9.7 and 10 cal$^{1/2}$ cm$^{-3/2}$, which is similar to that of the oily component of the membrane used in the in vitro absorption tests. This would mean that, although this membrane is only a model, the results obtained with it for naproxen are probably close to those which would be obtained if stratum corneum were used. This would have to be confirmed by further research comparing in vitro and in vivo absorption.

Finally, Fig. 2 shows the quantity of naproxen

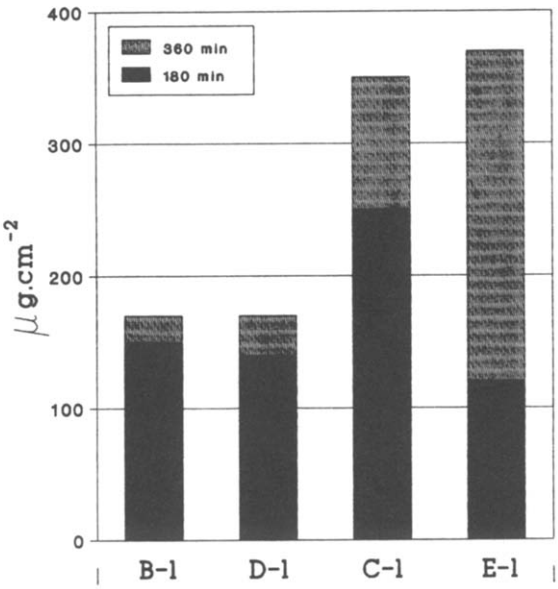

Fig. 2. Differences in the accumulated amount of naproxen absorbed from the different formulations for the same time (180 and 360 min). For identification and composition see Table 1.

absorbed during the same assay time (3 and 6 h) for the different formulations. It can be inferred from these data that, although all the preparations contain the same quantity of solute 5% (w/w), the two preparations containing naproxen seem to present less thermodynamic activity (B-1 and D-1). The total amount of solute absorbed over the entire period considered is half that of samples C-1 and E-1, where the thermodynamic activity is higher. In C-1 and E-1 the same amount is absorbed, but the kinetic behaviour of the absorption process is different. After 180 min the accumulated amount of naproxen absorbed in the 'dermic' zone in the case of C-1 is 120 μg cm^{-2}, whereas E-1 presents practically double this amount (250 μg cm^{-2})

These results show that, for two preparations with the same concentration of solute and the same value of thermodynamic activity, the kinetic behaviour of absorption depends on the type of components in the formulation. Although this difference may be insignificant for fields other than therapeutics, it may have considerable clinical importance.

Of the samples tested here, C-1 would be the most suitable for topical application, given the anti-inflammatory properties of naproxen, as it would take effect rapidly and maintain local concentrations for at least 6 h, which is the time usually thought suitable for this type of preparation to remain on the skin. This would be true as long as an adequate concentration is used and the membrane model conforms to the equivalence conditions with the stratum corneum described above.

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