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Iontophoretic transport of verapamil and melatonin I. Cellophane membrane as a barrier

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Summary

In this work the relative importance of the transport contributions involved in iontophoresis, namely diffusive, iontophoretic and electro-osmotic fluxes, were investigated using a barrier constituted by a cellophane membrane. An ionizable drug, verapamil, and an unionizable one, melatonin, were used. The transport behavior of drugs, alone or in combination, was studied in order to know the influence of ionized drug transport on the movement of liquid inside the membrane. The application of an electric current increases the flux of verapamil from its solution at pH 5 through cellophane membranes. At these conditions the total flux of verapamil is a result of two contributions: passive diffusion and iontophoretic flux. The contribution of electro-osmosis appears to be negligible.

Introduction

The transdermal route for controlled administration of drug is already a reality. However, the permeability characteristics of a drug limit its transport, since passive flux through the skin is significant only for lipophilic substances. Iontophoresis is a technique that improves the transport of ionizable drugs through porous barriers. Thus, higher permeation rates of ionizable molecules through the skin, compared to passive flux, can be attained.

Transdermal devices in which drug delivery rate is adjusted to therapeutic needs by modifying current intensity and duration, have already been advertized (Panoderm®, Elan Corp., Monksland, Ireland; Lectro Patch®, General Medical Company, Los Angeles, U.S.A.).

Iontophoretic transport occurs through the pores of the skin (Burnette and Ongpipattanakul, 1988). Three contributions to the total amount of drug transported through the membrane have been identified: passive diffusion, according to Fick's law, iontophoretic transport, due to the electric potential difference across the barrier, and electro-osmotic flux (Tojo, 1989; Srinivasan and Higuchi, 1990; Pikal and Shah, 1990). The presence of the three mechanisms has recently been discussed (Sims et al., 1991).

The aim of this work was to ascertain the relative importance of the three described mechanisms in iontophoretic transport using an ioniz-

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able drug, verapamil, and an unionizable one, melatonin. In order to know the influence of ionized drug transport on the movement of liquid inside the membrane the transport behavior of drugs, alone or in combination, was studied. To set up the conditions for verapamil transport under the effect of electric field, in this first part, a cellophane membrane, very permeable compared to the skin, was used.

Materials and Methods

The following materials were used: verapamil · HCl (Prodotti Gianni, Milan, Italy), Mol. Wt 491.08, pK_a 8.9, m.p. 143.5°C; melatonin (Janssen Chimica, Beerse, Belgium), Mol. Wt 232.27, m.p. 116–118°C; Ag/AgCl electrodes (In Vivo Metric, Healdsburg, CA, U.S.A.); HPLC acetonitrile (Labscan, Dublin, Ireland); 2-amino epthane 99% (Aldrich Chimica, Milan, Italy); acetic acid, HPLC reagent (J.T. Baker B.V., Deventer, The Netherlands); sodium acetate RPE-ACS (Carlo Erba, Milan, Italy); cellophane membranes (Viscora, Bagnolet, F), thickness 0.0065 cm, pore size 0.0025 μm.

Permeation through cellophane membranes

For the determination of drug permeation through cellophane membranes, a Resomat type absorption simulator was employed. The donor compartment contained 4 ml of drug solution in McIlvaine buffer pH 5 and the receptor compartment contained 220 ml of the same buffer. The area available for permeation was 10 cm^2.

Verapamil concentration in the donor compartment was set to 30, 60 and 90 mg/ml. Melatonin concentration in the donor compartment was set at 1 mg/ml. The apparatus was maintained at 37°C and two stirrers eliminated the boundary layer effects in each compartment. At predetermined time intervals, samples were withdrawn from the receptor compartment and assayed for verapamil content. The samples were filtered through 0.45 μm membranes and injected in a 3×3 CR C18 column of HPLC apparatus (Perkin Elmer, Norwalk, CT, USA) according to USP XXII verapamil monograph.

For the iontophoretic transport experiments two platinum electrodes, positive in the donor and negative in the receptor compartment, respectively, were inserted in the Resomat apparatus. The electrodes were connected to a current generator (Neuromed CBR 2, IREM, Parma, Italy) and constant direct current was applied for 30 min.

In the case of the experiment performed using melatonin and verapamil in the donor compartment, the concentration of melatonin in the receptor compartment was determined at the same HPLC conditions used for verapamil.

Determination of voltage drop across the membrane

Two reference electrodes, constructed by placing Ag/AgCl probes into two capillary tubes, filled with agar-agar KCl saturated gel, were used for measuring the voltage drop across the membrane. These reference electrodes were located very close to the membrane, both in donor and receptor compartments. The potential difference, due to the application of the electric current by means of the working electrodes, was measured with an oscilloscope (Hitachi Denshi, Tokyo, Japan) connected to the reference electrodes. The voltage differences obtained at different current densities were plotted against the corresponding current densities. From the slope of the straight line the electric resistance of the membrane was calculated.

Results and Discussion

Iontophoretic transport experiments were performed in buffer solutions at pH 5, at this pH value verapamil being mainly present in ionized form. Permeation profiles of verapamil at current densities of 0, 0.5, 1 and 2 mA cm^{-2}, from a donor solution containing 30, 60 and 90 mg/ml of drug, respectively, were obtained. The first portion of the permeation profiles was linear with time in all cases; its slope allowed the calculation of flux (J), assuming pseudo steady-state conditions. The results are illustrated in Fig. 1 and Table 2: the flux of verapamil increases with

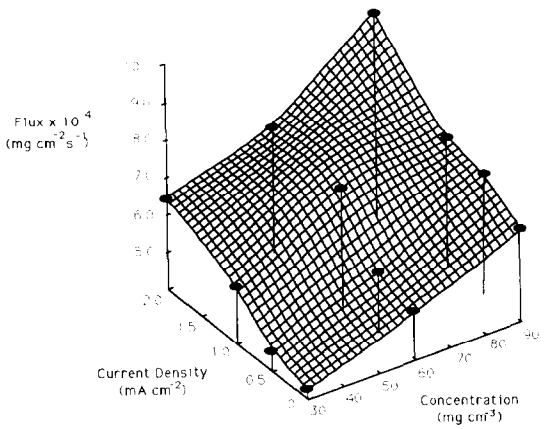

Fig. 1. Flux of verapamil as a function of donor concentration and current density.

current density and donor solution concentration. Permeability coefficients (P) were then calculated using the relationship:

$$P = \frac{J}{C_d} \tag{1}$$

where C_d is the verapamil donor concentration.

Permeability coefficient values, for different verapamil donor concentration and current density, are reported in Table 1. The permeability coefficient values decrease with increasing verapamil donor concentration, probably due to the lower activity of the drug in more concentrated solution. For all concentrations examined, the permeability coefficient increases significantly with increasing current density.

According to a recent iontophoresis study (Sims et al., 1991) the total flux (J_{tot}) of an ionized molecule, at a given current density, is equal to the sum of three possible contributions: passive diffusion (J_0), iontophoretic flux ($J_{\Delta V}$) and electro-osmotic flux (J_{osm}) *:

$$J_{tot} = J_0 + J_{\Delta V} \pm J_{osm} \tag{2}$$

First, the diffusive contribution to the total flux is determined in experiments without current application, assuming that iontophoresis does not change the passive flux of the drug when the current is applied.

* In Eqn 2 the sign ± preceding J_{osm} indicates that the electro-osmotic flux can assume either the same direction as the total flux or the opposite direction, depending on the membrane and electrode polarity.

TABLE 1

Transport parameters of verapamil through cellophane membranes (mean values ± SE)

Current density (mA cm^{-2})	Verapamil concentration (mg cm^{-3})	Permeability coefficient (cm s^{-1})	Enhancement factor	Peclet number
0	30	$1.43 \times 10^{-5} \pm 0.07 \times 10^{-5}$	–	–
	60	$0.89 \times 10^{-5} \pm 0.06 \times 10^{-5}$	–	–
	90	$0.72 \times 10^{-5} \pm 0.01 \times 10^{-5}$	–	–
0.5	30	$1.52 \times 10^{-5} \pm 0.13 \times 10^{-5}$	1.067 ± 0.029	0.036
	60	$0.94 \times 10^{-5} \pm 0.01 \times 10^{-5}$	1.065 ± 0.002	0.040
	90	$0.81 \times 10^{-5} \pm 0.02 \times 10^{-5}$	1.125 ± 0.010	0.052
1.0	30	$1.85 \times 10^{-5} \pm 0.05 \times 10^{-5}$	1.295 ± 0.035	0.184
	60	$1.19 \times 10^{-5} \pm 0.02 \times 10^{-5}$	1.352 ± 0.018	0.284
	90	$0.83 \times 10^{-5} \pm 0.01 \times 10^{-5}$	1.165 ± 0.020	0.036
2.0	30	$2.15 \times 10^{-5} \pm 0.06 \times 10^{-5}$	1.511 ± 0.043	0.197
	60	$1.24 \times 10^{-5} \pm 0.01 \times 10^{-5}$	1.402 ± 0.007	0.027
	90	$1.06 \times 10^{-5} \pm 0.02 \times 10^{-5}$	1.482 ± 0.031	0.157

TABLE 2

Total flux of verapamil through cellophane membranes in iontophoretic experiments and the passive and electric contributions (mean values ± SE)

Current density (mA cm^{-2})	Verapamil concentration (mg cm^{-3})	Total flux [a] J_{tot} (mg cm^{-2} s^{-1})	Diffusive flux [b] J_0 (mg cm^{-2} s^{-1})	Iontophoretic flux [c] $J_{\Delta V}$ (mg cm^{-2} s^{-1})
0.50	30	$4.57 \times 10^{-4} \pm 0.40 \times 10^{-4}$	$4.28 \times 10^{-4} \pm 0.21 \times 10^{-4}$	$0.72 \times 10^{-4} \pm 0.02 \times 10^{-4}$
	60	$5.63 \times 10^{-4} \pm 0.14 \times 10^{-4}$	$5.31 \times 10^{-4} \pm 0.11 \times 10^{-4}$	$0.89 \times 10^{-4} \pm 0.02 \times 10^{-4}$
	90	$7.26 \times 10^{-4} \pm 0.08 \times 10^{-4}$	$6.45 \times 10^{-4} \pm 0.08 \times 10^{-4}$	$1.08 \times 10^{-4} \pm 0.03 \times 10^{-4}$
1.00	30	$5.54 \times 10^{-4} \pm 0.15 \times 10^{-4}$	$4.28 \times 10^{-4} \pm 0.21 \times 10^{-4}$	$1.44 \times 10^{-4} \pm 0.04 \times 10^{-4}$
	60	$7.14 \times 10^{-4} \pm 0.09 \times 10^{-4}$	$5.31 \times 10^{-4} \pm 0.11 \times 10^{-4}$	$1.78 \times 10^{-4} \pm 0.05 \times 10^{-4}$
	90	$7.51 \times 10^{-4} \pm 0.10 \times 10^{-4}$	$6.45 \times 10^{-4} \pm 0.08 \times 10^{-4}$	$2.17 \times 10^{-4} \pm 0.06 \times 10^{-4}$
2.00	30	$6.46 \times 10^{-4} \pm 0.18 \times 10^{-4}$	$4.28 \times 10^{-4} \pm 0.21 \times 10^{-4}$	$2.88 \times 10^{-4} \pm 0.08 \times 10^{-4}$
	60	$7.44 \times 10^{-4} \pm 0.03 \times 10^{-4}$	$5.31 \times 10^{-4} \pm 0.11 \times 10^{-4}$	$3.57 \times 10^{-4} \pm 0.10 \times 10^{-4}$
	90	$9.55 \times 10^{-4} \pm 0.20 \times 10^{-4}$	$6.45 \times 10^{-4} \pm 0.08 \times 10^{-4}$	$4.34 \times 10^{-4} \pm 0.12 \times 10^{-4}$

[a] Values measured in iontophoretic transport experiments.
[b] Values measured in passive transport experiments.
[c] Values calculated according to Eqn 3, under the assumption that the current does not modify the permeability of the membrane.

Then, from the potential difference across the membrane, assuming a uniform electric potential inside the membrane, the iontophoretic contribution is calculated, according to the following equation:

$$J_{\Delta V} = \frac{DC_d}{h} \frac{zF\Delta V}{RT} \tag{3}$$

where D is the diffusion coefficient, C_d the concentration of drug in the donor compartment, z the charge of the permeant, F the Faraday constant, ΔV the potential difference, R the gas constant and T the absolute temperature.

From the ratio between the flux at a given current density and the passive flux, quantitative evaluation of the transport improvement, determined by current application, is achieved. This value is defined as the enhancement factor (E) (Srinivasan and Higuchi, 1990):

$$E = \frac{J_{tot}}{J_0} \tag{4}$$

From the enhancement factor and the electric potential difference across the membrane, a dimensionless number known as the Peclet number (Pe) can also be iteratively calculated from the following equation (Sims et al., 1991):

$$E = \frac{K\left(1 + \frac{Pe}{K}\right)}{1 - e^{K(1+(Pe/K))}} \tag{5}$$

where $K = zF\Delta V/RT$.

The Peclet number represents the ratio between the electro-osmotic flow and passive diffusion rates. It depends on the velocity of the solvent (v), membrane thickness (h) and drug diffusion coefficient (D), according to the following relationship:

$$Pe = \frac{vh}{D} \tag{6}$$

Finally, from the velocity of solvent (v), obtained from the Peclet number, the electro-osmotic flux, due to the solvent flow which drags all the present solutes, can be derived as:

$$J_{osm} = v \cdot C_d \tag{7}$$

Examining the experimental data, in the case of the transport of verapamil, the enhancement

factor value obtained increases with increasing current density (Table 1). However, it is practically independent of the concentration of verapamil in the donor solution. The values show that current application through cellophane membranes did not produce more than a 50% increase in transport relative to the passive diffusive transport. Thus, the cellophane membrane can be readily permeated by verapamil molecules.

The Peclet number values, calculated using Eqn 5, are very close to zero, indicating negligible solvent flow through this type of membrane (Table 1).

The total flux experimentally determined at three increasing current densities and for three different verapamil concentrations is shown in Table 2. The passive and electric contributions to this flux are also reported. Comparing the total flux (J_{tot}) under current application with the sum of diffusive (J_0) and iontophoretic ($J\Delta_V$) contributions, it appears that the two contributions can justify the value of total flux measured. Thus, no significant electro-osmotic flux contribution seems to be involved.

In order to verify the absence of electro-osmotic flux, experiments using melatonin as a non-ionizable molecule were set up. First, the flux of melatonin was measured as a function of current density ranging from 0 to 2 mA cm^{-2}, at both positive and negative polarity. The results obtained confirm that melatonin transport

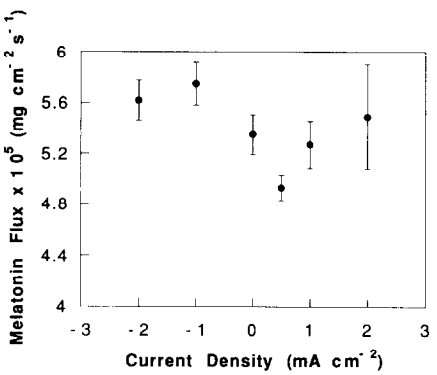

Fig. 2. Melatonin flux as a function of current density (mean value ± SE).

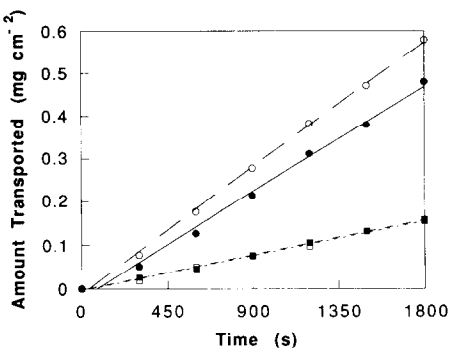

Fig. 3. Amount of melatonin and verapamil transported; verapamil (●) and melatonin (■) passive transport, verapamil (○) and melatonin (□) transport at a current density of 1 mA cm^{-2}.

through cellophane membranes is not significantly affected by current application (Fig. 2).

Next, melatonin and verapamil were used concomitantly in the donor compartment. An eventual electro-osmotic flux, induced by verapamil iontophoretic transport, could improve the transport of melatonin. Experiments in which the donor compartment contained a solution of 30 mg/ml verapamil and a solution of 3 mg/ml melatonin were performed under the same conditions. The permeation profiles obtained, in the presence and absence of current, are shown in Fig. 3. The application of electric current does not modify the flux of melatonin, whereas the flux of verapamil increases significantly. Thus, the absence of an electro-osmotic contribution on verapamil transport through cellophane membranes at pH 5 was confirmed.

Conclusions

From the data obtained it can be concluded that the application of an electric current increases the flux of verapamil from a solution at pH 5 through cellophane membranes. The permeability of cellophane membranes to verapamil appears to be high enough to limit the relevance of the current application for transport.

Under these conditions, the total flux of verapamil is a result of two contributions: passive

diffusion and iontophoretic flux. The contribution of electro-osmosis appears to be negligible with the artificial membranes used. The absence of electro-osmotic flux in the cellophane membranes used can be attributed both to the high permeability of this membrane to verapamil and to the lack of fixed charges in the pores of the membrane itself.

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