Delivery of Timolol through Artificial Membranes and Pig Stratum Corneum

D.F. STAMATIALIS, H.H.M. ROLEVINK, G.H. KOOPS

European Membrane Institute Twente, University of Twente, Chemical Technology Department, P.O. Box 217, NL-7500 AE Enschede, The Netherlands

Received 18 September 2002; revised 8 January 2003; accepted 9 January 2003

ABSTRACT: The *in vitro* passive and iontophoretic (applied current density: 0.5 mA/cm^2) timolol (TM) permeability from a liquid solution through pig stratum corneum (SC) is found to be $0.9 \pm 0.5 \times 10^{-6}$ and $3.9 \pm 0.9 \times 10^{-6}$ cm/s, respectively. The *in vitro* iontophoretic TM delivery through the combination of artificial porous membranes with pig SC is investigated as well. When the meso-porous PES-30 membrane is applied, the SC mainly controls the TM delivery. When the microporous NF-PES-10 membrane is applied, both the membrane and the SC contribute to controlling the delivery of TM. When the microporous LFC 1 membrane is applied, the TM delivery is membrane controlled. In all cases, however, the efficiency of the TM delivery is low and would need to be improved for the development of a commercially viable product. J Pharm Sci 92:1037–1039, 2003
Keywords: pig stratum corneum; artificial membranes; iontophoresis; timolol

INTRODUCTION

Transdermal iontophoresis achieves enhanced transport of a charged drug molecule through the interaction between it and the applied electric field (electrorepulsion). The field imposes a force on the drug molecule, which adds to the pure passive diffusion driven by a concentration gradient.[1]

For most drugs, the rate-determining step for drug transport across the skin is transit across stratum corneum (SC). According to some researchers,[2,3] it would preferable that a rate control resides within the delivery patch to attain drug delivery independent of variability in skin permeability. However, other studies have shown that constant current iontophoresis provides a similar degree of control for transdermal delivery of charged drugs.[1] In a transdermal patch the artificial membrane can play an important role in the control of the drug delivery. If the drug permeability through the membrane is significantly lower (higher) than through the skin, then the drug delivery is membrane (skin) controlled.

In a previous study,[4] we investigated the permeability of timolol (TM, p$K_a = 9.21$); a nonselective beta-adrenergic blocking agent; through artificial membranes. We found that the incorporation of a porous membrane within a transdermal TM patch seems feasible. In the present note, we apply three artificial membranes, a mesoporous (PES-30) and two microporous (NF-PES-10 and LFC 1) membranes in combination with pig SC. We place the membrane in between the TM liquid reservoir and the pig SC as it actually happens for a transdermal patch. We calculate the fractional rate control of the membrane (device) by the equation:

$$M_M = \text{fractional control by membrane} = \frac{Q_{\text{total}}}{Q_M} \quad (1)$$

where Q_{total} and Q_M represent the amount of the TM transported in a given period of time under

Correspondence to: Dimitrios F. Stamatialis (Telephone: +31-53-489-4675; Fax: +31-53-489-4611; E-mail: d.stamatialis@ct.utwente.nl)

Journal of Pharmaceutical Sciences, Vol. 92, 1037–1039 (2003)

steady-state conditions through the combination of membrane and pig SC and the membrane alone (in mg/cm^2), respectively.[5] Thus, $M_M = 1$, implies that the delivery is controlled entirely by the membrane; however, when $M_M < 1$, then the SC is contributing to the control process.

EXPERIMENTAL

We dissolved timolol maleate salt (MW = 432.5, Sigma, The Netherlands) in phosphate buffer saline solution (PBS) 0.153 M at pH 7.4. Under these conditions, TM was positively charged. The concentration of timolol base used was in the range of 23–25 mg/cm^3. The PES-30 (Sartorius, Germany), NF-PES-10 (Nadir, Germany) and LFC 1 (Hydronautics, Oceanside, CA) artificial membranes were purchased. The Institute of Animal Science and Health (ID-Lelystad, The Netherlands) generously provided us with pig skin, and we prepared the SC following the procedure described in Ref. 6.

We performed all transport experiments (passive diffusion or iontophoresis) in a continuous flow-through diffusion cell[7] following the procedure described in Ref. 4. The SC was very fragile, and was supported by a dialysis membrane (MWCO: 5kDa, Diachema, Germany). We applied a current density of 0.5 mA/cm^2, the maximum acceptable for the iontophoretic transdermal delivery. A silver plate electrode and a silver/silver chloride electrode were used as driving electrodes in the anode and cathode, respectively. Silver/silver chloride electrodes were also used as reference electrodes for the measurement of the electrical resistance. We performed all experiments for at least 8 h and for 8–12 samples for each membrane or pig SC. The TM concentration was determined by HPLC.[4]

RESULTS AND DISCUSSION

We found that the passive and iontophoretic TM permeability through supported pig SC was $0.9 \pm 0.5 \times 10^{-6}$ and $3.9 \pm 0.9 \times 10^{-6}$ cm/s, respectively. A typical result is presented in Figure 1. During the first minutes of the current application, the electrical resistance of the SC droped sharply from 15.8 ± 6.5 kΩ cm^2 to 2.9 ± 2.5 kΩ cm^2 and stayed at the lower levels for several hours of iontophoresis.

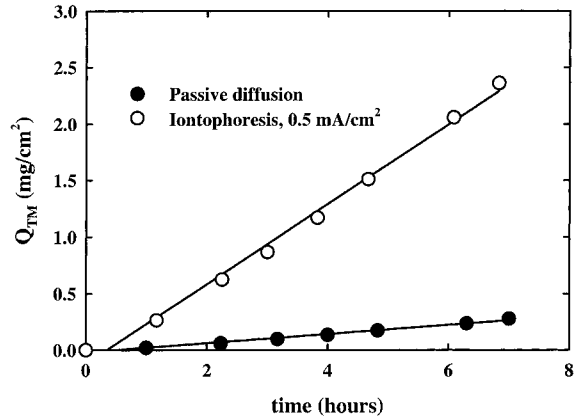

Figure 1. Typical result of the TM transport through supported pig SC under passive diffusion (filled symbols) and iontophoresis (open symbols).

In previous work,[4] we found that the passive and iontophoretic permeability of TM through the dialysis support membrane alone was practically the same ($56.5 \pm 8.0 \times 10^{-6}$ cm/s and $58.5 \pm 6.3 \times 10^{-6}$ cm/s, respectively). The fact that the overall TM permeability through the supported pig SC was much lower than through the support membrane alone indicated that the SC strongly inhibited the transport of TM. Therefore, the contribution of the dialysis membrane to the overall permeability through the SC and dialysis can be ignored.

In Table 1, we present the overall TM permeability through the rate controlling membrane and the pig SC together with the TM permeability through the membranes alone (found in Ref. 4). The application of the artificial membranes in front of the SC caused an increase of the overall electrical resistance during iontophoresis. This phenomenon became more significant in the order PES-30 ~ NF-PES-10 < LFC 1. Therefore, if the LFC 1 membrane is used in a TM iontophoretic

Table 1. Iontophoretic TM Permeability Through Rate-Controlling Artificial Membranes Alone and Their Combination with Supported Pig SC

System	$K_p \times 10^6$ (cm/s) Iontophoresis	Elect. Resistance (kΩ cm^2)
PES-30[a]	41.0 ± 8.6	0.2 ± 0.1
NF-PES-10[a]	6.7 ± 0.8	0.4 ± 0.1
LFC 1[a]	0.9 ± 0.1	1.2 ± 0.1
PES-30 + pig SC	3.2 ± 0.9	4.4 ± 3.3
NF-PES-10 + pig SC	2.6 ± 0.9	4.7 ± 2.4
LFC 1 + pig SC	0.9 ± 0.1	8.5 ± 4.7

[a]Results from Ref. 4.

patch, the depletion of the electrical power source (in some commercial products, it is a "walkman-type" device with batteries) will be faster than for the other two membranes. For all cases, the mass balance for TM fits well (with a deviation of $\pm 10\%$) allowing us to conclude that the adsorption of TM to the pig SC is not significant.

From the results of Table 1, we calculate the Q_M, Q_{total} and M_M using eq. 1 (with $t = 24$ h and TM solution of 25 mg/cm^3). When we applied the mesoporous PES-30 membrane, the pig SC controlled the iontophoretic TM delivery ($M_M = 0.1$). When we applied the microporous NF-PES-10 membrane, both the membrane and SC contributed to controlling the iontophoretic delivery of TM ($M_M = 0.4$). In this case, the membrane worked as a safe guard and controlled the TM delivery if for any reason the skin was compromised. When we applied the microporous LFC 1 membrane, the membrane controlled the iontophoretic TM delivery ($M_M = 1.0$). Fatouros and Bouwstra (Leiden University, The Netherlands) studied the permeability of TM through human skin under the same conditions of this work. They reported that the K_P for iontophoresis (current density: 0.5 mA/cm^2) was $3.1 \pm 0.4 \times 10^{-6}$ cm/s (unpublished results, personal communication), in close agreement with our findings for the pig SC. Therefore, the pig SC can be considered as a reasonable model for the human skin TM iontophoretic studies and the previous conclusions concerning the overall TM transport through the membrane and pig SC would also be valid for the human skin.

In all cases we present in this note the efficiency was low (1.5–6%). More research is required to understand the physicochemical parameters (TM concentration, background electrolyte) that condition the transport efficiency.

ACKNOWLEDGMENTS

The European Community is gratefully acknowledged for the financial support of this work (ACTRADEL project within the 5th RTD Framework program). The authors thank Dr. D. Fatouros and Dr. J. Bouwstra (Gorlaeous Laboratories, Leiden University, The Netherlands) for their assistance with the preparation of the pig SC.

REFERENCES

1. Banga AK. 1998. Percutaneous absorption and its enhancement. Chapter 1. In: Rubinstein MH, Wilson CG, Todd JP, editors. Electrically assisted transdermal and topical drug delivery. London: Taylor & Francis Ltd.
2. U.S. Pat. 5167616 (1992); Haak RP, Yieh J, Gyory RJ (Applicant Alza Corp., US) Iontophoretic delivery method.
3. U.S. Pat. 5322502 (1992); Theeuwes F, Gyory RJ, Haak RP (Applicant Alza Corp., US). Membrane for electrotransport transdermal drug delivery.
4. Stamatialis DF, Rolevink HHM, Koops GH. 2002. Controlled transport of timolol maleate through artificial membranes under passive and iontophoretic conditions. J Controlled Release 81:335–345.
5. Guy RH, Hadgraft J. 1992. Rate control in transdermal drug delivery? Int J Pharm 82: R1–R6.
6. Li GL, Danhof M, Bouwstra JA. 2001. Iontophoretic delivery of apomorphine in vitro: Physicochemic considerations. Pharm Res 18:1509–1513.
7. van der Geest R, Danhof M, Bodde HE. 1998. Validation and testing of a new iontophoretic continuous flow through transport cell. J Controlled Release 51:85–91.