The Enhancer Effect of Several Phenyl Alcohols on Percutaneous Penetration of 5-Fluorouracil

A. López,[1,3] M. A. Pellett,[2] F. Llinares,[1] O. Díez-Sales,[1] M. Herráez,[1] and J. Hadgraft[2]

Received November 19, 1996; accepted February 19, 1997

KEY WORDS: percutaneous absorption; enhancers; phenyl alcohols; 5-fluorouracil; monolayer.

INTRODUCTION

The mechanism by which percutaneous penetration enhancers operate is not fully understood but the structured lipids within the intercellular channels play an important role in controlling absorption. Penetration enhancers may act by either interacting with the highly ordered lipid structure or by modifying the partitioning of the drug into the tissue (1,2).

Different phenyl alcohols are included in many pharmaceutical and cosmetic products as bacteriostatic agents and essential oils. Benzyl alcohol which is used as a bacteriostatic agent and also as a solvent has been evaluated as a percutaneous enhancer (3). In the present work three phenyl alcohols, 2-phenylethanol, 3-phenyl-1-propanol and 3-phenyl-2-propen-1-ol were assessed as enhancers for the model polar permeant, 5-fluorouracil (5-FU), which has been used previously in in vitro diffusion experiments (2,4,5). 3-Phenyl-2-propen-1-ol was chosen in order to compare the influence of a double bond in the alkyl chain region of a phenyl alcohol. In order to gain an understanding of the mechanism of action of the phenyl alcohols as enhancers, the epidermal membrane/water partition coefficients of 5-FU with and without treatment of the membrane with the phenyl alcohols were determined. Furthermore, it is thought that the intercellular lipids of the stratum corneum, which are arranged in bilayers, provide the main barrier to xenobiotic penetration through the skin. The interactions of such enhancers with model lipid monolayers can help us understand how such molecules elicit their effect. The intercellular region of the stratum corneum is made up of a heterogeneous mixture of lipids. In order to study the interactions at a molecular level we have chosen to examine a homogeneous system. Although no phospholipids are present in the stratum corneum, we found dipalmitoylphosphatidylcholine (DPPC) very useful, for example, in understanding the mechanism of action of Azone (1). Other authors have also employed DPPC as model structured lipids for the same purpose (6). It is for this reason that mixed monolayers of DPPC and the phenyl alcohols on a Langmuir trough were examined in the present study.

MATERIALS AND METHODS

Materials and Reagents

2-Phenylethanol, 3-phenyl-1-propanol, 3-phenyl-2-propen-1-ol (all Aldrich) and 5-Fluorouracil (Sigma) were used in the experiments.

DPPC (1,2-dipalmitoyl-DL-α-phosphatidylcholine, Sigma) and chloroform (high-performance liquid chromatography (HPLC) grade) were used to prepare the monolayers. The subphase consisted of double distilled water from an all glass apparatus and further purified by a Milli-Q Plus water deionising system (Millipore).

Permeation Experiments

All the permeation experiments were performed on Wistar rat skin (aged 20–25 days), obtained from our laboratory colony. Epidermal membranes were prepared by a heat-separation technique as previously reported (7).

Diffusion studies were performed in a 6-cell battery system. The receiver and donor compartment capacity was 22 ml and the temperature was maintained at 37°C.

In order to analyse the effect of the phenyl alcohols on the percutaneous penetration of 5-FU the following concentrations of the phenyl alcohols were selected: 1, 2.5 and 5 $mg \cdot mL^{-1}$. 5-FU was dissolved in a solution buffered to pH 6.2 (control), or in a solution of the corresponding phenyl alcohol buffered to pH 6.2, at a concentration of 10 $mg \cdot mL^{-1}$ in all cases.

The epidermal membranes were treated with either 22 ml of phosphate-buffered saline (pH 6.2) as a control or the same amount of a buffered solution with the respective phenyl alcohol overnight. Samples of 1 ml were taken from the receptor compartment every 60 minutes over a 32-hour period. The volume withdrawn was always replaced with an equal volume of fresh receptor solution (phosphate-buffered saline pH 7.4).

The 5-FU concentration in samples was determined by HPLC using a Perkin-Elmer liquid chromatograph with UV detector set at 254 nm. An analytical Novapack C-18 column was employed. The mobile phases were composed of mixtures of acetonitrile and phosphate buffer solution (pH 6.2) in variable proportions, depending on the phenyl alcohol assayed, and were delivered at a flow rate of 1 $mL.min^{-1}$ at ambient temperature.

Calibration curves covering the entire range of concentrations assayed for 5-FU were prepared in triplicate. The accuracy of the method was evaluated and the results were considered acceptable (8).

The permeability coeficient (Kp) and the enhancement ratio (ER) for 5-FU was calculated as previously reported (5).

Partitioning Experiments

The effect of phenyl alcohols on 5-FU partitioning into epidermal membranes of Wistar rat, at 37°C, were investigated. Samples of epidermis, approximately 0.1 g, were weighed and hydrated by floating overnight on a solution buffered to pH 6.2

[1] Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Valencia, Spain.

[2] Department of Pharmaceutical Chemistry, School of Pharmacy, Cardiff, UK.

[3] To whom correspondence should be addressed. (e-mail: Alicia.Lopez@uv.es)

0724-8741/97/0500-0681$12.50/0

(control) or in the same solution to which the corresponding phenyl alcohol at the different concentrations had been tested. After this period the membrane was placed for 48 h in a buffered solution which contained the 5-FU (control) or both 5-FU and the corresponding phenyl alcohol. The concentration of 5-FU in the aqueous phase at equilibrium was evaluated by HPLC.

Partitioning of the drug between epidermal membranes and the aqueous solution was estimated in triplicate as previously reported (7).

Monolayer Studies

DPPC solutions were prepared in chloroform, to a concentration of 1.4 mM. Solutions containing both DPPC and the phenyl alcohols assayed were prepared in the same solvent. The concentration of DPPC was kept constant (1.4 mM), whilst the concentration of the corresponding phenyl alcohol was increased (0.3 mM, 0.7 mM, 1.4 mM and 1.7 mM) to give different molecular ratios of the monolayer components (DPPC-phenyl alcohol).

Surface pressure vs molecular area compression isotherms were recorded at 25°C using an automated Langmuir film balance (Nima Technology, Coventry, U.K) equipped with a pressure sensor and filter paper Wilhelmy plate capable of an accuracy of measurement of 0.1 mN/m. To prepare monolayers, 100 μL of chloroform solutions were spread and the solvent was allowed to evaporate for 10 min at an initial area per molecule of between 150 and 170 $Å^2$ before commencing compression at a rate of 10 $Å^2$/molecule per min.

For each combination of phenyl alcohol and DPPC monolayer, three sets of isotherms were obtained at different concentrations of the corresponding phenyl alcohol. The steeply sloping linear section of each isotherm was extrapolated back to zero surface pressure to determine the area per molecule, *Ao*, of each mixture.

RESULTS AND DISCUSSION

The amounts of 5-FU that accumulated in the receptor compartment as a function of time in the experiments without treatment (control) and with treatment of the skin with the phenyl alcohols at the different concentrations, are shown in Figure 1. As can be seen, treatment of the skin with the phenyl alcohols produced an increase in the amount of 5-FU penetrated, except when the membrane was treated with 3-phenyl-1-propanol at 1 $mg \cdot mL^{-1}$. This increase is more apparent after the epidermis was treated with 3-phenyl-2-propen-1-ol.

The permeability coefficients of 5-FU obtained in the different experimental conditions established are shown in Table 1. In order to compare them the U-Mann-Whitney test was used because non homogeneity in variances was found. The statistical analysis is also shown in Table 1. As can be seen, there are significant differences between the permeability coefficients obtained through the membrane without treatment (control) and with treatment with phenyl alcohols except when the membrane was treated with 3-phenyl-1-propanol at 1 $mg \cdot mL^{-1}$.

The penetration enhancing activity of the phenyl alcohols is more clearly demonstrated in terms of the 5-FU enhancement ratio. Fig. 2 shows the 5-FU enhancement ratios calculated for all conditions, and it clearly shows that the highest enhancement ratio (ER = 9.3) corresponds to 3-phenyl-2-propen-1-ol. This

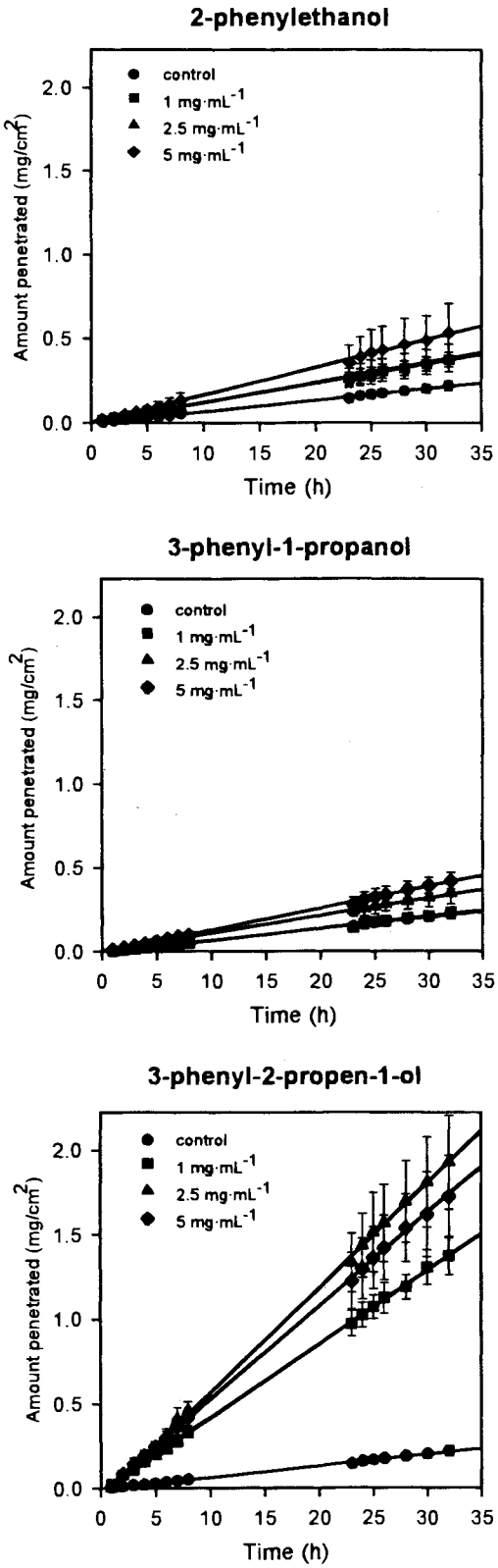


Fig. 1. Plots showing the amount penetrated of 5-FU (Q, mg/cm^2) as a function of time (t, h) under different conditions, without treatment (control) and with treatment of the membrane with the phenyl alcohols at the concentrations selected. Each data point is the mean of four experiments with standard deviation. The symbols for the 1 $mg \cdot mL^{-1}$ 3-phenyl-1-propanol (squares) and the control (circles) plots are in the same position.

t Different Concentrations (1, 2.5 and 5 mg-mL^{-1}) on 5-FU
ning Constant (n = 3 ± Standard Deviation)

›ound			Statistical differences		
Phenyl 1-panol (III)	3-Phenyl 2-propen-1-o1 (IV)		I–II	I–III	I–IV
—	—		—	—	—
4 ± 0.12	4.36 ± 0.35		*a*	NS[b]	*a*
7 ± 0.18	6.21 ± 0.92		*a*	*a*	*a*
2 ± 0.17	5.50 ± 0.78		*a*	*a*	*a*
—	—		—	—	—
—	10.12 ± 0.36		—	—	—
—	10.97 ± 0.82		—	—	NS
± 0.36	11.09 ± 2.56		NS	NS	NS

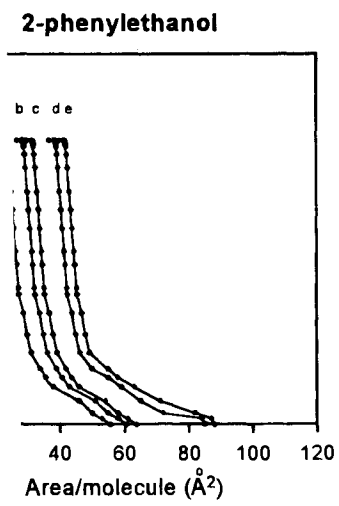
to obtain new experimental data in this field of a series of experiments were carried out to deter-ct of treatment of epidermal membrane with the ls on 5-FU membrane partitioning (Table 1). This been used by other authors in a similar way to compare the 5-FU epidermal partitioning under xperimental conditions a statistical analysis of rformed and is also shown in Table 1. Homoge-ıfirmed by Barlett's test. One- and two-way sed prior to the Tukey test. As can be observed, nificant differences between the partition coeffi-obtained without treatment of the membrane ıembrane was treated with the phenyl alcohols, ts that treatment of the skin with the phenyl ɔt affect drug tissue partitioning.

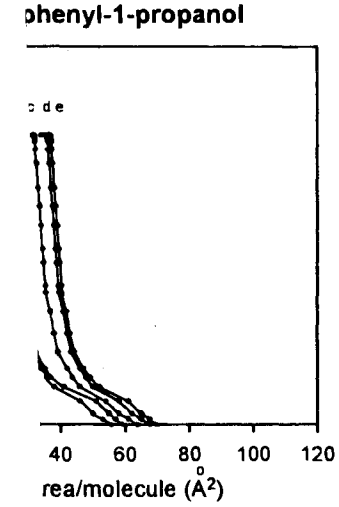
he enhancer effect could be due to a disruption , which makes them more fluid, and facilitates ecules through the hydrocarbon region of the lipid bilayers (11). To test this hypothesis the tions between DPPC, as a model lipid, and ls in mixed monolayers were studied. Despite and large amounts of ceramides, cholesterol ls contained in the stratum corneum (11,12), n of the interactions of enhancers with phos-examined previously and has lead to theories of action of enhancers on skin (1). In fact, ıg phospholipid vesicles after incorporation ules have demonstrated the utility of model ms (6,13). In Figure 3, pressure-area iso-and mixed monolayers of DPPC and the different concentrations (0.3 mM, 0.7 mM, M) are shown. As can be seen, the phenyl expansion in the isotherms which can be raction between the enhancers and DPPC. indicate that the effects of the enhancers d packing by the phenyl alcohols. In fact, l fluidity of the E. coli envelope by 2-ıve been well correlated with lipid fluidity

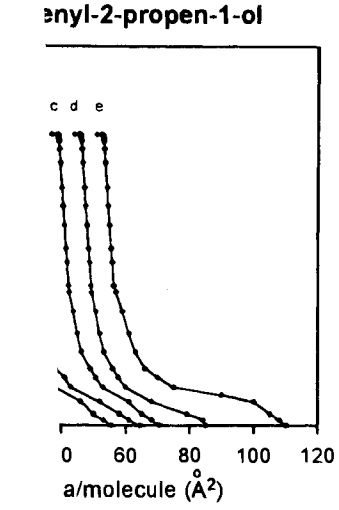
d B. E. Brown. *J. Invest.*

shington. *Int. J. Pharm.*

). **130**:39–61 (1979).
of Wales (1990).







of DPPC (a), and mixed monolayers
l at different concentrations; 0.3 mM
d 1.7 mM (e). All the isotherms were

in DPPC liposomes (15) and could reflect similar interactions occurring in the intercellular lipids of stratum corneum (15).

The relationships between, the average area per molecule (*Ao*) and the concentration of each phenyl alcohol (*Cpa*) in mixed monolayers, are linear and have good correlation coefficients.

3-phenyl-1-propanol $Ao = 15.55(\pm 2.77)Cpa + 30.05(\pm 1.71)$ $(r > 0.954)$ (1)

2-phenylethanol $Ao = 20.62(\pm 1.51)Cpa + 30.24(\pm 0.88)$ $(r > 0.990)$ (2)

3-phenyl-2-propen-1-ol $Ao = 24.63(\pm 1.29)Cpa + 30.74(\pm 0.73)$ $(r > 0.996)$ (3)

Statistical comparison using Student's t-test shows that the slopes of regression lines obtained for all the phenyl alcohols are significantly different ($p < 0.05$) and there is a rank correlation between these slopes and the 5-FU enhancement ratios. The usefulness of monolayer studies in evaluating these type of enhancers was confirmed.

In conclusion, the phenyl alcohols tested in this work act as percutaneous penetration enhancers of hydrophylic drugs such as 5-FU. These compounds are usually included in many pharmaceutical and cosmetic products, so their effect has to be take into account to avoid possible risks of undesirable absorption and to improve the penetration of some compounds through the skin. The mechanism of action of phenyl alcohols as enhancers is not due to partitioning phenomena; these compounds may act by modifying intercellular lipids, thus disrupting their highly ordered structure to increase diffusivity. Furthermore, the double bond present in 3-phenyl-2-propen-1-ol could be responsible for a stronger disorder in the regular packing of the lipids and hence would impart more fluidity.

ACKNOWLEDGMENTS

The present work is part of a research project (PM 92-0149) developed with a grant from the DGICYT, of the Ministry of Education and Science of Spain.

The authors would also like to thank Smithkline Beecham Consumer Healthcare for partly financing this work.

REFERENCES

1. D. Lewis and J. Hadgraft. *Int. J. Pharm.* **65**:211–218 (1990).
2. A. C. Williams and B. W. Barry. *Pharm. Res.* **8**:17–24 (1991).
3. I. Saitoh, K. Ikeda, and Y. Takagishi. *Biol. Pharm. Bull.* **18**:321–325 (1995).
4. O. Díez-Sales, A. C. Watkinson, M. Herráez-Domínguez, C. Javaloyes, and J. Hadgraft. *Int. J. Pharm.* **129**:33–40 (1996).
5. A. López, M. J. Morant, D. Guzmán, J. Borrás-Blasco, O. Díez-Sales, and M. Herráez. *Int. J. Pharm.* **139**:205–213 (1996).
6. A. Rolland, A. Brzokewicz, B. Shroot, and J. Jamoulle. *Int. J. Pharm.* **76**:217–224 (1991).
7. O. Díez-Sales, E. Perez-Sayas, A. Martín-Villodre, and M. Herráez-Domínguez. *Int. J. Pharm.* **100**:1–7 (1993).
8. H. T. Karnes and C. March. *Pharm. Res.* **10**:1420–1426 (1993).

9. M. Goodman and B. W. Barry. *J. Invest. Dermatol.* **91**:323–327 (1988).
10. J. E. Harrison, A. C. Watkinson, D. M. Green, J. Hadgraft, and K. Brain. *Pharm. Res.* **13**:542–546 (1996).
11. G. M. Golden, J. E. McKie, and R. O. Potts. *J. Pharm. Sci.* **76**:25–28 (1987).
12. P. M. Elias, G. K. Menon, S. Grayson, and B. E. Brown. *J. Invest. Dermatol.* **91**:3–10 (1988).
13. J. C. Beastall, J. Hadgraft, and C. Washington. *Int. J. Pharm.* **43**:207–213 (1988).
14. S. Halegoua and M. Inouye. *J. Mol. Bio.* **130**:39–61 (1979).
15. T. M. Murphy. PhD Thesis, University of Wales (1990).