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Short Communication

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Transdermal Delivery of Probenecid: the Effects of Vehicles and Enhancers on Permeation Through Pig Skin

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SUMMARY. Vehicles and enhancers effect on *in vitro* probenecid permeation through dermatomed abdominal pig skin was investigated. The permeability of different probenecid percentages dispersed in vehicles as vaseline, carbopol/ethanol/water and carbopol/propylene glycol was tested. The 1.3% L-menthol addition, as permeation enhancer, over probenecid/vaseline formulations showed the highest values for both, flux and permeation coefficient. Permeation experiments of the probenecid formulations in carbopol/propylene glycol showed that the carbopol/probenecid concentration relation is the most important issue to be considered. Comparatively to lipophilic vehicle (vaseline), carbopol dispersions seen to be more convenient as vehicle for topical administration of probenecid. The results obtained from this study may be helpful in the development of a probenecid transdermal drug delivery system.

INTRODUCTION

Gout is a disease characterized by the crystallyne deposit of uric acid salts into the joints and sinews and it is generally associated with high uric acid levels in blood, which give rise to acute joint inflammation and may also lead to formation of kidney stones [1,2]. Probenecid administration can prevent the deposit of urates in tissues. This uricosuric benzoic acid derivative is a tubular renal blocking agent [3,4] which inhibits urate tubular reabsorption, thus increasing the urinary excretion of uric acid and decreasing serum urate levels [5].

In transdermal preparations, drug penetration is limited by the properties of the corneous layer (stratum corneum) [6,7]. The chemical barrier functionality of the skin makes it necessary to optimize the system by selecting suitable vehicles and permeation enhancing agents [8-11]. These enhancers have to possess certain properties such as chemical inertia, stability, effectiveness at low concentrations, atoxicity and effect reversibility. Even though hundreds of substances have been identified as permeation enhancers to date, the understanding of the structure-activity relationships is still limited. In general, enhancers can be divided into two large groups: small polar solvents and amphiphilic compounds containing a polar head and a hydrophobic chain [12]. Other families of compounds such as terpenes have also been suggested as promising non-toxic, non-irritating transdermal penetration enhancers [13]. These compounds may increase drug diffusion by disjointing lipid structure of stratum corneum. Among these, L-menthol has demonstrated a remarkable improvement in the penetration of a great variety of compounds [14-18].

Pig skin has been widely used for permeation measurements since it is more comparable to human skin than any other animal skin [19]. Pig and human skin exhibit similar lipidic surface, barrier thickness, morphological aspects, histological characteristics and permeability properties, which makes pig skin suitable for in vitro estimation of human skin permeability [20,21].

Oral route administration of probenecid has frequent adverse effects such as gastrointestinal

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intolerance (causing nausea, sore gums and vomiting), headache, dizziness and fever; therefore, the design of a transdermal drug delivery system (TDDS) is highly recommendable.

In this study the effect of vehicles and enhancers on *in vitro* probenecid permeation through dermatomed abdominal pig skin was investigated. Additionally, possible detrimental effects on the pig skin were evaluated by histological examination after formulation administration.

MATERIALS AND METHODS

Materials

4-(dipropylsulfamoyl) benzoic acid (probenecid), $C_{13}H_{19}NO_4S$ (MW: 285.36) (Sigma); vaseline (Britania); carboxypolymethylene 940 (carbopol) (Britania); phosphate saline buffer pH = 7.4 (PBS); ethanol (Merck); propylene glycol (Merck); L-menthol (Sigma) and dermatomed abdominal pig skin.

Abdominal depilated skin of cadaver pig (aged less than three months) was carefully excised, cleaned and kept at 0 °C. Subcutaneous fat and other extraneousadhered tissue were completely removed. The skin was cut into size necessary for the Franz cell employed and stored at -10 °C. Skin samples thickness was measured by a coating thickness measuring instrument Fischer Dualscope MP0R. A histological study of pig skin including the tissue samples in paraffin stopper and employing hematoxylin-eosin coloration was performed.

All assays were carried out in a period of 12 h after death. Five-µm thick crosscuts (Reichert-Jung microtome) were observed and photographed on an Olympus BX-40 microscopy.

Solubility determination and stability studies

Due to the low aqueous solubility of probenecid (27.1 mg/L), its solubility was assayed in ethanol, ethanol/water and propylene glycol [22]. An excessive amount of solid probenecid (enough to get a saturated solution) was added into a screw-capped test tube containing 5 mL of different solvents. Test tubes were periodically rotated for 24 h and maintained at constant temperature. This experiment was repeated at different temperatures between 17 °C and 35 °C. After centrifugation, probenecid in the clear supernatant was analysed by UV-VIS spectroscopy (wavelength 245 nm) in a Shimadzu UV-160A spectrophotometer.

Stability studies were performed at 25 °C under light exposure. Probenecid was dissolved in three different solvent systems: ethanol/water (15/85), ethanol and propylene glycol. Probenecid concentration was quantified by UV-VIS spectroscopy as described above.

Probenecid formulations

Formulations using vaseline as vehicle of probenecid were prepared (F1 to F6) by dissolving probenecid in vaseline and stirring until homogeneity. L-menthol effect over the probenecid permeation was studied by adding it in adequate proportions (F2 to F6).

Probenecid formulations in carbopol with different solvents and probenecid percentages were assayed. A standard formulation containing carbopol was obtained by dissolving probenecid in the working solvent (ethanol F7 to F12; propylene glycol F13 to F15) and adding the necessary amount of water (F7 to F12). After gradual addition of carbopol, the formulation was stirred until homogeneity was obtained and then stored for 24 h before the experiment.

In vitro permeation of probenecid through abdominal pig skin

Permeation experiments were performed using a Microette System automatic sampler (Hanson-Research) with 4.5 ml Franz-type diffusion vertical cells and equipped with a thermostatic bath, injection system, vacuum pump, agitation clamp, computerized sip control module and sampler. The frozen abdominal pig skin was thawed at room temperature before mounting on cell between the donor and receptor compartments. The diffusion area, this is, the effective permeation surface, was 1.767 cm^2. The receptor compartment (in contact with the dermis side of the skin) was filled with 4.5 mL of PBS with constant stirring (180 rpm) and all the system was maintained at 32 ± 0.5 °C (human skin temperature) with a circulating water jacket. Next, 0.265 cm^3 of formulation containing different probenecid amounts were introduced into the donor compartment, which was in contact with the stratum corneum side of the pig skin. At predetermined times, 0.1 mL samples were withdrawn from the receptor compartment and analysed for probenecid quantification by UV-VIS spectroscopy (Shimadzu UV-160A) at 245 nm. The receptor compartment volume was maintained by addition of freshly prepared PBS (32 °C ± 0.5 °C). Finally, the amount of permeated accumulated drug in the receptor compartment was recalculated. Experiments were performed in triplicate.

Theory of permeation processes

Most substances are absorbed through skin by passive diffusion according to Fick's law [23]. Drug flux (J_m) through stratum corneum under stationary conditions can be described by Fick's law:

$$J_m = Kp \cdot \Delta C \quad [1]$$

where J_m is the amount of mass diffusing across a plane of area unit per time unit, Kp is the permeability coefficient and ΔC is the concentration increment through membrane. Assuming negligible concentration in the receptor compartment, ΔC is the substance concentration in the donor compartment (g/cm^3).

Determining the cumulative quantities of active principle per membrane area unit (Q_m /A) versus time (t) the permeation profile can be obtained, and then J_m value from the slope of the curve linear portion is evaluated.

$$J_m = Q_m / (t. A) \quad [2]$$

Furthermore, equation [**1**] permits an estimation of the permeability coefficient Kp (cm/s).

RESULTS

Rationale for solvent selection in formulation design

Various solvent systems as ethanol, ethanol/water and propylene glycol were initially investigated for their abilities to dissolve probenecid (Table 1). These systems are known for their pharmaceutical uses, their enhancer capacity and effects on *in vitro* permeation through abdominal pig skin.

Probenecid spectra and concentration remained unchanged after three months, indicating that this drug is stable in solvents employed, at normal storage temperature.

Solvents (%)	Probenecid solubility (mg/mL)
Et (100)	78.23
Et/W(15/85)	15.50
PG (100)	8.981

Table 1. Probenecid solubility in different solvents at 25 °C.

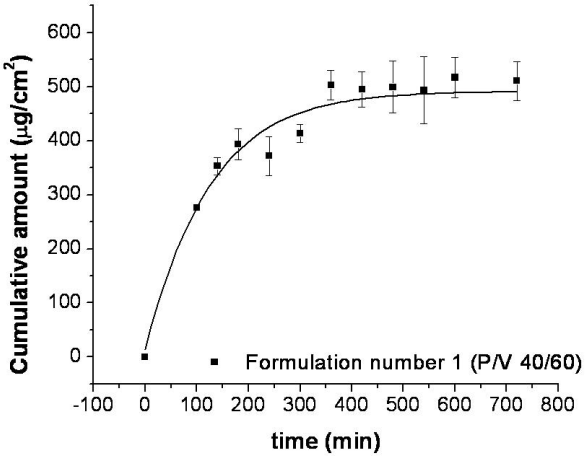

Figure 1. Cumulative quantities of probenecid as a time function.

Permeation experiments

Probenecid formulations were prepared in the vehicle solid vaseline, a hydrophobic excipient, in 40/60 ratio. Permeation profiles of probenecid/vaseline systems through abdominal pig skin were obtained. Figure 1 shows probenecid cumulative amounts as a function of time for this formulation. In order to improve drug permeation an enhancing agent was incorporated in the composition of the formulae. The formulation containing probenecid/vaseline (40/60) was added of L-menthol at concentrations between 0 and 4%, which would further

Formulation number	Composition (%)	J_m x 10^7 (g/cm^2·s)	Kp x 10^7 (cm/s)
1	P/V (40/60)	0.976 ± 0.133	1.93 ± 0.262
2	P/V/L-m (40/59.2/0.8)	4.23 ± 0.765	8.34 ± 1.51
3	P/V/L-m (40/58.7/1.3)	4.83 ± 0.533	9.55 ± 1.05
4	P/V/L-m (40/58.2/1.8)	4.41 ± 0.802	8.70 ± 1.54
5	P/V/L-m (40/57.5/2.5)	1.40 ± 0.161	2.76 ± 0.319
6	P/V/L-m (40/56/4.0)	1.15 ± 0.0212	2.26 ± 0.0340

Table 2. Physicochemical parameters for probenecid permeation through abdominal pig skin using vaseline as vehicle. J_m: flux; Kp: permeation coefficient; P: probenecid; V: vaseline; L-m: L-menthol.

Formulation number	Composition (%)	Carbopol/ Probenecid Ratio	Jm x 10^7 ($g/cm^2 \cdot s$)	Kp x 10^5 (cm/s)
7	P/C/Et/W (0.66/1.5/15/82.8)	2.27	0.716 ± 0.212	0.651 ± 0.193
8	P/C/Et/W (0.66/1.5/20/77.8)	2.27	0.803 ± 0.189	0.684 ± 0.161
9	P/C/Et/W (1/1.5/15/82.5)	1.50	1.99 ± 0.0955	1.20 ± 0.00558
10	P/C/Et/W (1/1.5/20/77.5)	1.50	2.48 ± 0.645	1.44 ± 0.389
11	P/C/Et/W (0.66/3/15/81.3)	4.54	2.87 ± 0.475	2.61 ± 0.432
12	P/C/Et/W (1/3/15/81)	3.00	2.37 ± 0.306	1.43 ± 0.184
13	P/C/PG (0.3/3/96.7)	10.0	2.09 ± 0.589	3.80 ± 1.07
14	P/C/PG (1/3/96)	3.00	3.30 ± 0.890	1.99 ± 0.536
15	P/C/PG (2/3/95)	1.50	1.88 ± 0.120	0.563 ± 0.0359

Table 3. Permeation physicochemical parameters for probenecid/carbopol formulations through abdominal pig skin. J_m: flux; Kp: permeation coefficient; P: probenecid; C: carbopol; Et: ethanol; W: water; PG: propylene glycol.

increase probenecid permeation coefficient through abdominal pig skin [24,25].

The physicochemical parameters for probenecid permeation from the exponential phase for formulations F1 to F6 (with/without enhancer) are summarized in Table 2.

On the basis of these results we judged necessary to design an alternative system that might increase probenecid permeation. With this purpose, and taking into account solubility studies results, formulations with different percentages of probenecid in 1.5% and 3% of carbopol were prepared, employing ethanol/water and propylene glycol as solvents. Probenecid permeation experiments through abdominal pig skin were made for each formulation and permeation profiles were obtained. In Table 3 permeation rates flux and permeability coefficients for formulations F7 to F15, are shown.

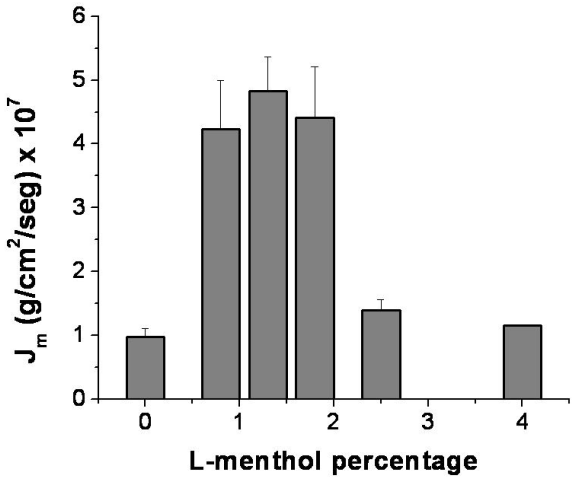

Figure 2. Probenecid permeation flux dependence with L-menthol percentage.

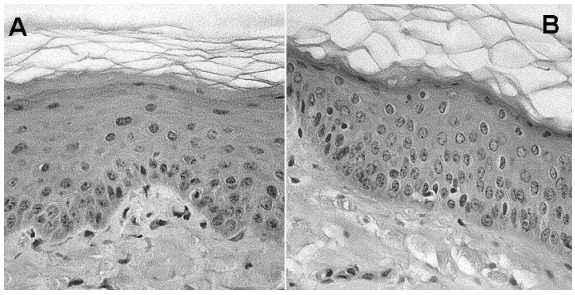

Figure 3. Abdominal pig skin microscopies. **A**: skin dermatomized storage at -16 °C; **B**: after 2.5% of L-menthol enhancer exposition.

DISCUSSION

The permeation rate flux and permeability coefficient values obtained from experiments carried out with L-menthol as enhancer of probenecid permeation (F2–F6) are higher than the ones obtained in its absence (F1). L-menthol showed the best performance as enhancer at concentrations about 1.3 % (F3, Figure 2), where probenecid diffusion was increased over 5-fold as compared to F1.

This effect can be attributed to the direct action of L-menthol on membrane structure, promoting its distension by reversible disruption of the lipidic barrier. Abdominal pig skin samples with different exposure times to 2.5% of L-menthol were observed on the microscope. Skin integrity showed not to be affected by the freezing process since dermatomed pig skin stored at –16 °C conserved its histological characteristics, with well conserved stratified epithelial layer and normal histoarquitecture (Fig. 3A). After exposure of pig skin to L-menthol for several hours (Fig. 3B), certain disorganization of the epithelial weave was observed, evidenced by a greater separation of queratinized cell layers.

Regarding formulations containing carbopol dispersions as vehicle, the analysis of the results for formulations 7-8 (probenecid/carbopol/ethanol/water: 0.66/1.5/15/82.8 and 0.66/1.5/

20/77.8) and 9-10 (probenecid/carbopol/ ethanol/ water: 1/1.5/15/82.5 and 1/1.5/20/77.5) indicated that small changes in ethanol percentage did not lead to significant modifications of the probenecid permeability coefficient. Probenecid solubility increased markedly with ethanol percentage, but amounts above 20% of this solvent proved to liquefy the formulation. Furthermore, although ethanol is commonly used as a transdermal penetration enhancer, skin irritation induced by high doses has been reported [26].

The rise of carbopol/probenecid ratio in gels, increased the flux and permeation coefficients values (formulations F7, F12 and F11). Carbopol dispersion used as vehicle, due to gel matrix strengthening, difficults the probenecid availability when this compound is present in high concentration.

In probenecid formulations the employment of propylene glycol as solvent allows to obtain higher permeation coefficient values than ethanol-water as solvent. In this case, the increase in the concentration ratio carbopol/ probenecid provoked a significant rise in the permeation coefficient (F15, F14 and F13).

CONCLUSIONS

In vitro probenecid permeation experiments through dermatomed abdominal pig skin permitted to conclude that: a) with regard to probenecid formulations containing vaseline as vehicle, the incorporation of L-menthol (1.3%) seems to be the most effective for absorption enhancement; this compound acts provoking certain disorganization of the epitelial weave, promoting the membrane structure distension by reversible disruption of the lipidic barrier, b) in the case of carbopol dispersions, probenecid permeation was highly influenced by the drug concentration in the system, and c) comparatively to lipophilic vehicle (vaseline), carbopol dispersions seen to be more convenient as vehicle for topical administration of probenecid. These researches may be helpful in the development of a probenecid transdermal delivery system.

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REFERENCES

1. Kelley, W.N. and R.L. Wortmann (1997) *"Gout and Hyperuricemia",* in: *"Textbook of Rheumatology",* (W.B. Saunders, W.N. Kelly, E.D. Harris Jr., S. Ruddy, C.B. Sledge, eds.) W.B. Saunders, Philadelphia, pp. 1313-51.
2. Groft, G.D., W.A. Franck & D.A. Raddarz (1990) *Semin. Arthritis. Rheum.* **19**: 329-36.
3. Paulus, H.E., L.H. Schlosstein & R.L. Godfrey (1987) *Arthritis. Rheum.* **17**: 609-14.
4. Reinders, M.K. & K.N. Van Roon (2007) *Clin. Rheumatol.* **26**: 1459-65.
5. Hanson, G.R. (2003) *"Drogas Diuréticas",* in: *"Remington Farmacia",* (A.R. Gennaro, ed.) Editorial Médica Panamericana, Buenos Aires, pp. 1588-600.
6. Barry, B.W. (2004) *Nat. Biotechnol.* **22**: 165-7.
7. Jantharaprapap R. & G. Stagni (2007) *Int. J. Pharm.* **343**: 26-33.
8. Barry, B.W. (2004) *"Administración de fármacos por vía transdérmica",* in: *"Farmacia: la ciencia del diseño de las formas farmacéuticas",* (M.E. Aulton, ed.) Elsevier España S.A., Madrid, pp. 499-533.
9. Thomas, N.S. & R. Panchagnula (2003) *Pharmazie.* **58**: 895-8.
10. Narishetty, S.T. & R. Panchagnula (2004) *Biopharm. Drug. Dispos.* **25**: 9-20.
11. Furuishi, T., O. Sachiko, H. Saito, T. Fukami, T. Suzuki & K. Tomono (2007) *Biol. Pharm. Bull.* **30**: 1350-3.
12. Vavrova, K., J. Zbytovska & A. Hrabalek (2005) *Curr. Med. Chem.* **12**: 2273-91.
13. Olivella, M.S., L. Lhez, N.B. Pappano & N.B. Debattista (2007) *Pharm. Dev. Technol.* **12**: 481-4.
14. Narishetty, S.T. & R. Panchagnula (2004) *J. Control. Release.* **95**: 367-79.
15. Sunil, T.K., S.T. Narishetty & R. Panchagnula (2005) *J. Control. Release.* **102**: 59-70.
16. Amnuaikit, C., I. Ikeuchi, K. Ogawara, K. Higaki & T. Kimura (2005) *Int. J. Pharm.* **289**: 167-78.
17. Boix, A., C. Peraire, R. Obach & J. Domenech (2005) *Pharm. Res.* **22**: 94-102.
18. Nokhodchi, A., K. Sharabiani, M.R. Rashidi & T. Ghafourian (2007) *Int. J. Pharm.* **335**: 97-105.
19. Singh, S., K. Zhao & J. Singh (2003) *Drug. Chem. Toxicol.* **25**: 83-92.
20. Rastogi, S.K. & J. Singh (2003) *AAPS Pharm. Sci. Tech.* **4**: 1-9.
21. Suwanpidokkul, N., P. Thongnopnua & K. Umprayn (2004) *AAPS Pharm. Sci. Technol.* **5**: 82-9.
22. Aulton, M.E. (2004) *"Disolución y solubilidad",* in: *"Farmacia: la ciencia del diseño de las formas farmacéuticas",* (M.E. Aulton, ed.) Elsevier España S.A., Madrid, pp. 15-32.
23. Barry, B. (2004) *"Administración de fármacos por vía transdérmica",* in: *"Farmacia: la ciencia del diseño de las formas farmacéuticas",* (M.E. Aulton, ed.) Elsevier España S.A., Madrid, pp. 499-533.
24. Aqil, M., A. Ahad, Y. Sultana & A. Ali (2007) *Drug Discov. Today* **12**: 1061-7.
25. Kunta, J.R. (1997) *J. Pharm. Sci.* **86**: 1369-73.
26. Thomas, N.S. & R. Panchagnula (2003) *Eur. J. Pharm. Sci.* **18**: 71-9.