Department of Pharmaceutical Sciences[1], School of Pharmacy, University of Catania, Catania, Department Farmaco-Biologico[2] University of Messina, Messina, Dompé S.p.A.[3], L'Aquila, Italy

Vehicle effects on *in vitro* skin permeation of thiocolchicoside

F. Bonina[1], C. Puglia[1], D. Trombetta[2], M. C. Dragani[3], M. M. Gentile[3] and G. Clavenna[3]

Thiocolchicoside, a semi-synthetic derivative of colchicoside, is used in topical formulations for its anti-inflammatory and muscle-relaxant properties. The objective of this study was to evaluate the effect of a (propylene glycol diperlagonate) DPPG and (propylene glycol) PG mixture present in an innovative foam formulation (Miotens®) on the flux of thiocolchicoside through excised human skin. Furthermore, the *in vitro* permeation behaviour of this new formulation (Miotens® foam) was compared to another commercial product (Muscoril® ointment) and to a control gel formulation (thiogel), both enhancer free. The best permeation profile was obtained from the foam formulation (Miotens®) which was able to increase the thiocolchicoside flux about three fold compared to control formulation (thiogel) and about two fold compared to the commercial formulation Muscoril® ointment.

1. Introduction

The therapeutic efficacy of drugs following topical administration is strongly affected by vehicle composition. The importance of the vehicle in determining topical bioavailability is now well recognized [1, 2] and two main approaches are generally followed to improve drug transdermal permeation.

One approach is to favour a high concentration gradient across the diffusion membrane by adding solvents leading to an increase in the thermodynamic activity of the drug in the vehicle and thereby improving the drug diffusion rate [3, 4]. The other approach is to include suitable agents that affect the epidermal barrier functions thus improving drug penetration through the skin [5, 6]. For this purpose, penetration enhancers are currently used in topical formulations; they can act by disrupting lipid or protein structures in the stratum corneum, or by improving drug partitioning into the skin [7]. Low systemic and cutaneous toxicity are prerequisites of the materials used to enhance the percutaneous penetration of drugs.

Thiocolchicoside is a semi-synthetic derivative of colchicoside which has a relaxant effect on skeletal muscle, as well as anti-inflammatory and analgesic activity [8], therefore it is used in topical formulations.

In the present study we investigated the promoting effect of (propylene glycol diperlagonate) DPPG and (propylene glycol) PG enhancers on *in vitro* percutaneous absorption of thiocolchicoside from a new foam formulation (Miotens®), in comparison to another commercial product (Muscoril® ointment) and to a control gel formulation (thiogel), both containing thiocolchicoside but enhancer free.

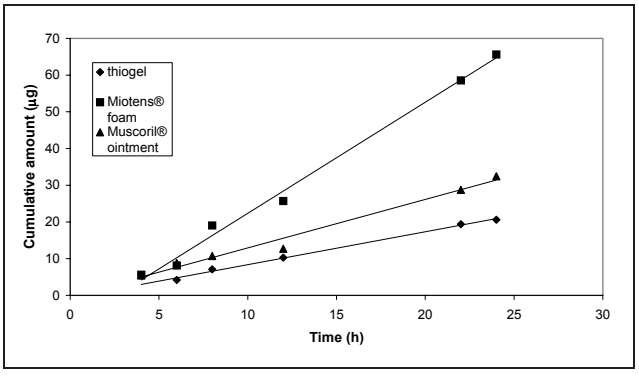

Fig. 1: Typical skin permeation curves of thiocolchicoside from thiogel, Miotens® foam and Muscoril® ointment formulations

The low toxicity of DPPG has already prompted investigation of the ability of this material to promote the percutaneous penetration of drugs [9].

Furthermore, *in vitro* permeation studies were carried out using excised human skin since it consents a better prediction of *in vivo* thiocolchicoside transdermal administration in humans [10] compared to other natural and synthetic membranes.

2. Investigations, results and discussion

In the Fig. the typical skin permeation curves of thiocolchicoside from Miotens®, Muscoril® ointment and thiogel formulations are shown.

The flux values at the steady-state of thiocolchicoside from Miotens®, Muscoril® ointment and thiogel formulations, calculated from the linear segments at the steady-state (see Table 1), were found to be $3.81 \pm 1.18\ \mu g/cm^2 \cdot h$, $1.89 \pm 0.55\ \mu g/cm^2 \cdot h$ and $1.19 \pm 0.38\ \mu g/cm^2 \cdot h$, respectively.

The best permeation profile for thiocolchicoside was obtained from Miotens® formulation: this vehicle was able to increase the thiocolchicoside flux about three fold (F.R.: fluxes ratio = 3.20) compared to control formulation (thiogel) ($p < 0.01$) and about two fold (F.R. = 1.58) compared to the commercial formulation Muscoril® ointment.

The enhanced flux of thiocolchicoside obtained from Miotens® formulation is probably due to the presence of a PG-DPPG mixture. DPPG is a material with a very low polarity and this property enables it to penetrate into the stratum corneum and interact with the lipid bilayers, thus increasing their fluidity or forming, as proposed by

Table 1: Thiocolchicoside steady-state fluxes through excised human skin, lag time, permeability coefficient (P_m) and fluxes ratio (F.R.) from thiogel (control), Muscoril® ointment and Miotens® foam formulations.

Formulation code	Flux ± S.D.* ($\mu g/cm^2 \cdot h$)	Lag time (h)	P_m ($\cdot 10^{-4}$) ($cm \cdot h^{-1}$)	F.R.
Thiogel	1.19 ± 0.38	0.66	4.6	1.00
Muscoril® ointment	1.89 ± 0.55^{a}	0.48	7.5	1.58
Miotens® foam	$3.81 \pm 1.18^{b,c}$	1.14	15.2	3.20

* Each experiment was run in duplicate for 24 h using six different donors (n = 6).
[a] $p > 0.05$ thiogel versus Muscoril® formulation.
[b, c] $p < 0.01$ thiogel versus Miotens® formulation; Muscoril® versus Miotens® formulation.

Table 2: Composition (% w/w) of Miotens®, Muscoril® ointment and thiogel formulations

Constituents	Formulation Code		
	Miotens® foam	Muscoril® ointment	thiogel
Benzyl alcohol	1	–	–
Carbomer	–	–	1
Cetyl ester wax	–	2	–
Dimethicone	–	0.3	–
Ethanol	20	–	30
Ethylparaben	–	0.035	–
Fat alcohols and hydrocarbons	–	7	–
Hydrogenated lanolin	–	12.5	–
Methylparaben	–	0.14	–
Paraffin	–	3	–
Perfume	0.20	1	–
Polysorbate 80	4	5	–
Propylene glycol	4	–	–
Propylene glycol diperlagonate (DPPG)	1	–	–
Propylparaben	–	0.025	–
Sodium alginate	–	0.5	–
Sodium hydrogenphosphate monohydrate	0.83	–	–
Sodium lauryl sulfate	–	2	–
Sodium phosphate dodecahydrate	0.23	–	–
Stearic acid	–	8	–
Thiocolchicoside	0.25	0.25	0.25
Triethanolamine	–	–	1
Water	68.5	58.2	67.7

Walker and Hadgraft [11] for oleic acid, fluid-like channels.

This mechanism is consistent with previous findings [9] showing that a saturated solution of DPPG in PG enhanced both the fluxes and the stratum corneum affinity of caffeine and testosterone. These effects were ascribed to the ability of DPPG to interact with the lipid bilayers and to that of PG to promote DPPG penetration into the stratum corneum creating interaction sites in such a tissue.

In conclusion, while for Muscoril® ointment formulation an appreciable increase of thiocolchicoside flux through excised human skin compared to control gel formulation (thiogel) ($p > 0.05$) was not observed, Miotens® foam formulation appeared the most efficacious in promoting the transdermal administration of this drug in humans.

However, clinical *in vivo* studies are needed to validate the result obtained in the present *in vitro* experimentation.

3. Experimental

3.1. Materials

Thiocolchicoside was supplied by Aldrich. [^{3}H]Water (spec.act. 5 mCi/ml) was obtained from Amersham (U.K.). Carbopol 934 (Carbomer) was supplied by Biochim (Italy), Miotens® foam was obtained from Dompé S.pA. (L'Aquila -Italy), Muscoril® ointment formulation (Inverni della Beffa, Milan, Italy) was commercially available. Their compositions are reported in Table 2. All other materials were of analytical grade. In the present study Miotens® foam corresponds to the liquid formulation of Miotens® foam before pressurization with gas propellent.

3.2. Preparation of hydro-alcoholic gel formulation

The composition of the thiocolchicoside gel formulation (thiogel) used in this study is reported in Table 2. Hydro-alcoholic gel was prepared by dispersing 1% w/w Carbopol 934 (Carbomer) in water and then by adding an alcoholic solution in which thiocolchicoside (0.25% w/w) was solubilized with constant stirring. The dispersion was then neutralized and made viscous by the addition of triethanolamine. The gel was stored at room temperature for 24 h under air-tight conditions prior to use.

3.3. Skin membrane preparation

Samples of adult human skin (mean age 36 ± 8 years) were obtained from breast reduction operations. Subcutaneous fat was carefully trimmed and the skin was immersed in distilled water at 60 ± 1 °C for 2 min [12], after which stratum corneum and epidermis (SCE) were removed from the dermis using a dull scalpel blade. Epidermal membranes were dried in a desiccator at approx. 25% relative humidity (RH). The dried samples were wrapped in aluminum foil and stored at 4 ± 1 °C until use. Preliminary experiments were carried out in order to assess SCE samples for barrier integrity by measuring the in vitro permeability of [^{3}H]water through the membranes using the Franz cells described below. The value of the permeability coefficient (P_m) for tritiated water was found to be $1.6 \pm 0.2 \times 10^{-3}$ cm/h which agreed well with those for tritiated water reported by others authors using human SCE samples [13, 14].

3.4. In vitro skin permeation experiments

Samples of dried SCE were rehydrated by immersion in distilled water at room temperature for 1 h before being mounted in Franz-type diffusion cells supplied by LGA (Berkeley, CA). The exposed skin surface area was 0.75 cm^2 and the receiver compartment volume was of 4.5 ml. The receptor compartment contained a water-ethanol solution (50 : 50), to allow the establishment of the "sink condition" and to sustain permeant solubilization [15]. It was stirred and thermostated at 35 ± 1 °C during all the experiments.

Approximately. 300 mg of each formulation containing 0.25% thiocolchicoside (Miotens® foam, Muscoril® ointment and thiogel) was placed on the skin surface in the donor compartment and the latter was covered with Parafilm®. The experiment was run in duplicate for 24 h using six different donors ($n = 6$). At fixed intervals, samples (200 µl) of receiving solution were withdrawn and replaced with fresh solution. The samples were analyzed for thiocolchicoside content by HPLC as described below. Thiocolchicoside flux through the skin was calculated by plotting the cumulative amount of drug penetrating the skin against time and determining the slope of the linear portion of the curve and the χ-intercept values (lag time) by linear regression analysis. Drug fluxes (µg/cm^2 h^{-1}), at steady state, were calculated by dividing the slope of the linear portion of the curve by the area of the skin surface through which diffusion took place.

The effectiveness of vehicle formulation (F.R.) was determined by comparing thiocolchicoside flux obtained from Miotens® foam and Muscoril® ointment formulations to that obtained with control formulation (thiogel).

Statistical analysis of the data was performed using Student's t-test.

3.5. High-performance liquid chromatography

The HPLC apparatus consisted of a Varian 5000 system (Varian Inc. CA. USA) equipped with a 20 µl loop and a Varian Polychrom 9060 detector (Varian Inc. CA. USA). Chromatography was performed on a Merck

Supersphere-60 RP Select B-LiChroCart column (particle size, 4 μm; 12.5 cm × 4.6 mm i.d; E. Merck, Darmstadt, Germany). The mobile phase for thiocolchicoside was phosphate buffer-water 25 mM (pH = 7.4)/methanol (70 : 30). Detection was effected at 254 nm and the flow rate was set at 1.2 ml/min. The retention time was of 10.5 min.

References

1 Zatz, J. L.; Sarpotdar, P. P.; In Kidonieus, A. F.; Berner, B. (Eds): Transdermal Drug Delivery, Vol. 2, p. 86, CRC press, Boca Raton, 1987
2 Mura, P.; Nassini, C.; Proietti, D.; Manderioli, A.; Corti, P.: Pharm. Acta Helv. **71**, 147 (1996)
3 Schwarb, F. P.; Imanidis, G.; Smith, E. W.; Haigh, J. M.; Surber, C.: Pharm.Res. **16**, 909 (1999)
4 Bach, M.; Lippold, B. C.: Eur. J. Pharm. Biopharm. **46**, 1 (1998)
5 Kanikkannan, N.; Kandimalla, K.; Lamba, S. S.; Singh, M.: Curr. Med. Chem. **7**, 593 (2000)
6 Guy, R. H.; Pharm.Res. **13**, 1765 (1996)
7 Barry, B. W.; Int. J. Cosmet. Sci. **10**, 281 (1988).
8 Janbroers, J. M.; Acta Therapeut. **13**, 221 (1987).
9 Bonina F. P.; Carelli, V.; Di Colo, G.; Montenegro, L.; Nannipieri, E.: Int. J. Pharm. **100**, 41 (1993).
10 Scott, R. C.; Walker, M.; Dugard, P. H.: Int. J. Cosmet. Sci. **8**, 189 (1986).
11 Walker, M.; Hadgraft, J.: Int. J. Pharm. **71**, R1 (1991).
12 Puglia, C.; Bonina, F.; Trapani, G.; Franco, M.; Ricci, M.: Int. J. Pharm. **228**, 79 (2001).
13 Bronaugh, R. L.; Stewart, R. F.: J. Pharm. Sci. **75**, 1094 (1986).
14 Scott, R. C.; Walker, M.; Dugard, P. H.: J. Soc. Cosm. Chem. **37**, 35 (1986).
15 Touitou, E.; Fabin, B.: Int. J. Pharm. **43**, 17 (1988)

Received February 6, 2002
Accepted April 23, 2002

Prof. Francesco Paolo Bonina
Department of Pharmaceutical Sciences
School of Pharmacy
University of Catania
Viale A. Doria n°6
95125, Catania
Italy
boninaf@mbox.unict.it.