Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **31:** 213–218, 1998.

In vitro Release Study of Tretinoin from Tretinoin/Cyclodextrin Derivative Complexes

PATRICIA MONTASSIER[1], DOMINIQUE DUCHÊNE[2] and MARIE-CHRISTINE POELMAN[1]
[1]*Département de Dermopharmacie et Biophysique Cutanée, Faculté de Pharmacie, Université René Descartes (Paris V), 4 Avenue de l'Observatoire, 75006 Paris, France*
[2]*Laboratoire de Physico-chimie, Pharmacotechnie, Biopharmacie, URA CNRS 1218, Faculté de Pharmacie, Université de Paris-Sud (Paris XI), Rue Jean Baptiste Clément, 92290 Châtenay Malabry, France.*

(Received: 30 May 1997; in final form: 25 August 1997)

Abstract. The effects of β-cyclodextrin, hydroxypropyl β-cyclodextrin and dimethyl β-cyclodextrin complexes on the *in vitro* release of tretinoin gels were investigated. The experiments were carried out in a Franz cell using a silicone membrane as a barrier for the diffusion of the vehicle. Two types of vehicle were compared: a hydroalcoholic gel in which both tretinoin and the inclusion complexes are soluble, and an aqueous gel in which only the complexes are soluble but tretinoin is dispersed. As expected, the release rate of free tretinoin in the hydroalcoholic gel is much faster than in the aqueous gel. However,with the aqueous gel, the cyclodextrin complexation enhances the diffusion rate of the active drug through the membrane, especially with the hydroxypropyl cyclodextrin inclusion compound. The release of tretinoin is related not only to the stability constant of the inclusion, but also to the binding properties of the inclusion compounds to the vehicle.

Key words: tretinoin, β-cyclodextrin, hydroxypropyl β-cyclodextrin and dimethyl β-cyclodextrin complexes, diffusion cells.

1. Introduction

For a number of years, topical all trans tretinoin has been widely used in the treatment of acne, psoriasis and, more recently, photo-ageing [1]. Tretinoin exhibits many biological actions and is a valuable drug in skin disorders that involve cell differentiation, proliferation and inflammation [2,3]. Moreover, topical treatment of Kaposi's sarcoma with all trans retinoic acid has been proposed [4]. But its extensive use has been reduced due to side effects, especially local primary irritation [5]. It should also be mentioned that its poor solubility in water and its weak stability in the presence of oxygen and light could lead to technical drawbacks.

All these reasons led us to search for an improvement by inclusion in various cyclodextrins [6]. Numerous studies have reported the effect of cyclodextrin complexes on the release of drugs designed for topical use. Otagiri et al. [7] found an enhanced release of betamethasone from ointments containing β- and γ-cyclodextrin complexes. From these data, the authors suggested an improvement in the topical bioavailability of betamethasone by means of γ-cyclodextrin

complexation. Uekama et al. [8] confirmed, in man, the improvement in the percutaneous absorption of beclomethasone dipropionate by γ-cyclodextrin complexation. More contradictory data were reported by Okamoto et al. [9] on the percutaneous absorption of butylparaben and indomethacin. β-Cyclodextrin and dimethyl β-cyclodextrin decreased percutaneous absorption of these drugs.

The aim of the present work was to survey the possible interest of tretinoin complexation in various mainly hydrophilic cyclodextrins, in topical aqueous or hydroalcoholic gels of hydroxypropyl cellulose. The release of tretinoin and its inclusions from gels was assessed by using Franz diffusion cells with a non-porous membrane of polydimethylsiloxane.

2. Materials and Methods

2.1. PRODUCTS

Tretinoin was provided by Produits Roche (Paris, France), β-cyclodextrin and hydroxypropyl β-cyclodextrin by Roquette Frères (Lestrem, France), and dimethyl β-cyclodextrin by Cyclolab (Budapest, Hungary).

These three types of cyclodextrin were studied because they differ in their solubility, and their properties may have an effect on the rate of release of tretinoin. β-Cyclodextrin is very soluble in water and poorly soluble in organic solvents, hydroxypropyl β-cyclodextrin is also soluble in water and more soluble in polar solvents, such as ethanol, and dimethyl β-cyclodextrin is soluble both in water and in organic solvents. The cyclodextrin complexes were prepared in the same manner as described previously [10].

Hydroxypropyl cellulose (Klucel HF) was purchased from Aqualon (Rueil Malmaison, France). Ethanol 95% was of analytical grade, and distilled water was used. Oleth 20 was provided by Croda (Trappes, France), and potassium phosphate, sodium chloride, ammonium acetate and acetic acid by Prolabo (Paris, France).

2.2. GEL FORMULAE

The gel formulae are shown in Table I. A 0.01% concentration of tretinoin or 0.075% of its inclusion compound (which corresponds to about the same amount of tretinoin) was chosen according to the solubility of the drug in the hydroalcoholic gel and to its usual concentration in commercially-available anti-photoageing dermic forms. It should be noted that, in the aqueous gel, tretinoin is only dispersed.

2.3. *IN VITRO* GEL RELEASE STUDIES

The release of tretinoin from the gels was determined at 37 °C using Franz diffusion cells. A silicone membrane (Silastic®, Dow Corning, Sophia Antipolis, France) was used as a barrier for the diffusion of the vehicle [11]. A 0.1 g sample of gel was spread over the donor side of the membrane. The receptor phase, comprising

Table I. Gel formulae (% w/w)

Constituent	Gel 1	Gel 2	Gel 3	Gel 4
Tretinoin	0.01		0.01	
Inclusion compound		0.075		0.075
Ethanol 95%			60	60
Hydroxypropyl cellulose	2	2	2	2
Distilled water	appropriate amount			

Gels 2 and 4 were prepared with the three following inclusion compounds:
Gels 2a and 4a: β-cyclodextrin/tretinoin
Gels 2b and 4b: hydroxypropyl β-cyclodextrin/tretinoin
Gels 2c and 4c: dimethyl β-cyclodextrin/tretinoin

phosphate buffer saline pH 7.3 to 7.4 (PBS) containing 1% Oleth 20 was stirred with a magnetic bar. Oleth 20 was used to solubilize tretinoin in the receptor solution maintained at 37 °C.

The donor chamber was kept open throughout the experiment. Samples of 0.20 mL were removed from the receptor phase at 1, 2, 4, 6 and 8 h after the beginning of the experiment and replenished each time by the same volume of receptor solution. The samples were analyzed by HPLC with UV spectrophotometric detection at 350 nm as described previously by Amdidouche et al. [11].

3. Results and Discussion

The release profiles of tretinoin (mg/cm^2) plotted versus time from gels containing either tretinoin or its complexes are shown in Figure 1 for ethanolic gels, and in Figure 2 for aqueous gels.

As might be expected, the release of free tretinoin from the two reference gels is quite different. In the hydroalcoholic gel (gel 3), the release of free tretinoin is faster, and almost 100% of the drug has diffused after 8 h. The free tretinoin dissolved in the hydroalcoholic gel diffuses more rapidly through the silastic membrane than the tretinoin from the inclusion complex.

In contrast, in the aqueous gel (gel 1), the release curve of free tretinoin is dramatically low, and only 48% of the drug has diffused through the silastic membrane 8 h after the beginning of the assay. This slow release could be explained by the poor solubility of tretinoin in water ($\approx$8 μg/100 mL), and thus, under our conditions, tretinoin is only dispersed in the aqueous gel vehicle.

For inclusion compounds (gels 2 and 4), the hydrophilic properties of cyclodextrins prevent their diffusion through the lipophilic membrane. However, the complexes are progressively dissociated to release free tretinoin which can then diffuse through the membrane.

For hydroalcoholic gels (4a, b and c), this two-stage phenomenon leads to the slower release of the included tretinoin compared with the free dissolved drug. In

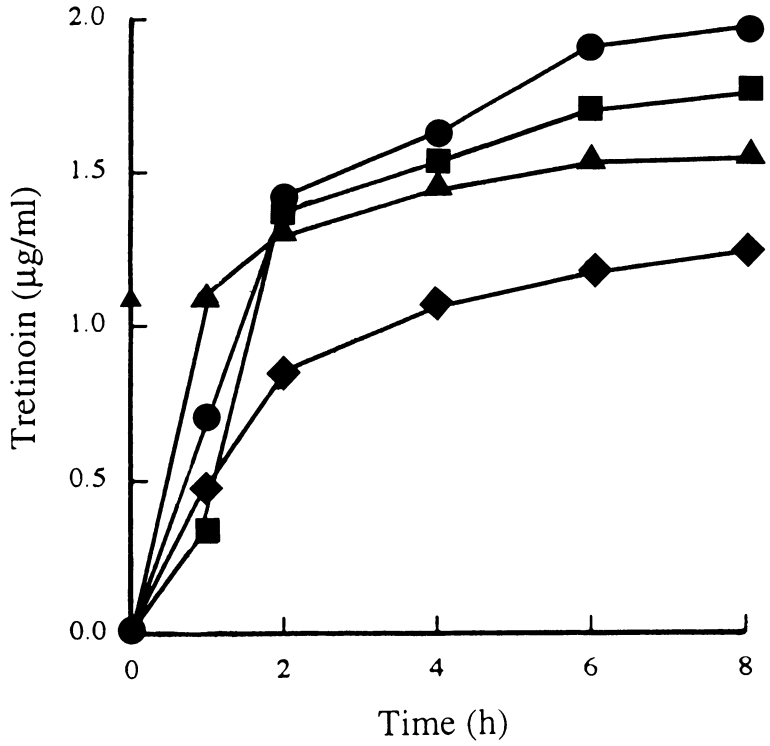

Figure 1. Release profiles of tretinoin from hydroalcoholic gels, in phosphate buffer at 37 °C. ●: Tretinoin, ▲: β-cyclodextrin, ♦: dimethyl β-cyclodextrin, ■: hydroxypropyl β-cyclodextrin.

Table II. Water solubility of tretinoin and cyclodextrin inclusion compounds (Excess of product in 5 mL of water at 20 ± 2 °C for 24 h)

Tretinoin	≈8 μg/100 mL
Tretinoin/β-cyclodextrin:	27 mg/100 mL
Tretinoin/hydroxypropyl β-cyclodextrin:	93 mg/100 mL
Tretinoin/dimethyl β-cyclodextrin:	640 mg/100 mL

contrast, for aqueous gels (2a, b and c), the release rate of tretinoin is significantly improved by complexation. This improvement may be attributed to two parameters: on the one hand, to the better solubility of the complexes in the aqueous phase which has been previously determined [10] and summarized in Table II, and, on the other hand, to the dissociation of dissolved inclusion compounds leading to free molecules of tretinoin able to diffuse through the membrane.

After 8 h, almost all the drug (96%) is released from the tretinoin/hydroxypropyl β-cyclodextrin inclusion (gel 2b). The diffusion through the membrane is of the same order as the diffusion of free tretinoin from the hydroalcoholic gel. Lower

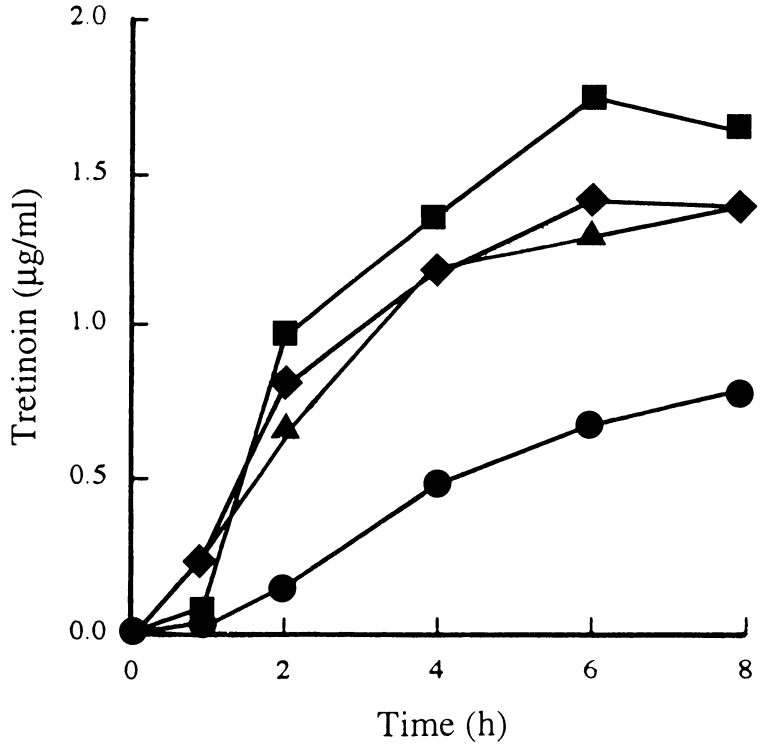

Figure 2. Release profiles of tretinoin from aqueous gels, in phosphate buffer at 37 °C. ●: Tretinoin, ▲: β-cyclodextrin, ♦: dimethyl β-cyclodextrin, ■: hydroxypropyl β-cyclodextrin.

diffusion rates are observed for β-cyclodextrin (gel 2a) and dimethyl β-cyclodextrin (gel 2c) compounds. However, the release of tretinoin after 8 h reaches 89%.

Arima et al. [12] found that the *in vitro* release of 4-biphenylacetic acid (BPAA) from a hydrophilic base was enhanced by complexation with hydrophilic cyclodextrins. These authors observed an order of β-cyclodextrin $<$ dimethyl β-cyclodextrin and hydroxypropyl β-cyclodextrin similar to tretinoin. They attributed the enhanced release of BPAA obtained by dimethyl β-cyclodextrin and hydroxypropyl β-cyclodextrin to their surface-active properties which could interfere with the stability constants of the complexes and the thermodynamic activity of the drug in the gel [13].

Our data are also in good agreement with Arima et al. [14] in their studies concerning the enhanced release of ethyl 4 biphenylacetate. The release of this poorly water-soluble drug is dramatically enhanced in the hydroxypropyl β-cyclodextrin inclusion compound.

For tretinoin, as well as for the other two drugs mentioned above, the slower release from a hydrophilic base observed with the dimethyl β-cyclodextrin complex could be explained by the improved solubility of these compounds in the vehicle

which may change their partition coefficients and thus the affinity of the active drugs to the hydrophilic phase. It is worth noting that, even if the tretinoin release from hydroxypropyl β-cyclodextrin in aqueous gel is slightly slower than that observed with either free tretinoin or the hydroxypropyl β-cyclodextrin inclusion compound in hydroalcoholic gels, it remains of the same magnitude. This point is very important because, by inclusion in hydroxypropyl β-cyclodextrin, tretinoin can be incorporated into aqueous vehicles which are better tolerated by the skin than the usual hydroalcoholic forms.

4. Conclusions

The present data indicate that cyclodextrin inclusion of tretinoin could enhance the release of this compound in an aqueous non-alcoholic vehicle. The rate of release of tretinoin is not only dependent on the stability constant of the inclusion complex but also on other factors such as binding properties to the vehicle and the partition coefficient between the vehicle and the membrane. Among cyclodextrin derivatives, hydroxypropyl β-cyclodextrin complexation may be a useful tool to improve the topical bioavailability of tretinoin.

References

1. A.M. Kligman, G.L. Grove and J.J. Hirose-Leyden: *J. Am. Acad. Dermatol* **15**, 836 (1986).
2. B. Shroot: *J. Am. Acad. Dermatol.* **15**, 748 (1986).
3. S. M. Burge: *Pharmacol. Ther.* **40**, 75 (1989).
4. L. Bonhomme, G. Fredj, S. Averous, A.M. Srekely, E. Ecstein, B. Trumbic, P. Meyer, J.M. Lang, J.L. Misset, and C. Jasmin, *Ann. Oncol.* **2**, 234 (1991).
5. C.M. Papa, *Acta Dermato.Vener.* **74**, 128 (1975).
6. D. Amdidouche, H. Darrouzet, D. Duchêne, and M.-C. Poelman, *Int. J. Pharm.* **54**, 175 (1989).
7. M. Otagiri, T. Fujinaga, A. Sakai, and K. Uekama: *Chem. Pharm. Bull*. **32**, 2401 (1984).
8. K. Uekama, M. Otagiri, A. Sakai, T. Irie, N. Matsuo, and Y. Matsuoka, *J. Pharm. Pharmacol.* **37**, 532 (1985).
9. H. Okamoto, H. Komatsu, M. Hashida, and H. Sekaki: *Int. J. Pharm.* **30**, 35 (1986).
10. P. Montassier, D. Duchêne, and M.-C. Poelman: *Int. J. Pharm.* **153**, 199 (1997).
11. D. Amdidouche, P. Montassier, M.-C. Poelman, and D. Duchêne: *Int. J. Pharm.* **111**, 111 (1994).
12. H. Arima, H. Adachi, T. Irie, and K. Uekama: *Drug Invest.* **2**, 155 (1990).
13. K. Uekama and T. Irie: 'Pharmaceutical applications of methylated cyclodextrin derivatives', in D. Duchêne (ed.), *Cyclodextrins and Their Industrial Uses*, Editions de Santé, Paris, 1987, p. 393.
14. H. Arima, H. Adachi, T. Irie, K. Uekama, and J. Pitha: *Pharm. Res.* **7**, 1152 (1990).