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Improved acitretin delivery through hairless mouse skin by cyclodextrin complexation

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Abstract

The effects of four cyclodextrins, i.e., 2-hydroxypropyl-β-cyclodextrin, randomly methylated β-cyclodextrin (MβCD), γ-cyclodextrin and 2-hydroxypropyl-γ-cyclodextrin, on the aqueous solubility of acitretin were investigated. Of the four cyclodextrins tested MβCD displayed the largest effect. The aqueous solubility of the drug could be increased even further by pH adjustment. Thus, the solubility of acitretin in an aqueous pH 7.5 buffer solution containing 23% (w/v) MβCD was determined to be 1.96 mg/ml. Acitretin is insoluble in water. The flux of acitretin through hairless mouse skin was determined to be 0.032 mg h^{-1} cm^{-2} from a pure propylene glycol vehicle but 0.201 mg h^{-1} cm^{-2} from an aqueous MβCD containing vehicle.

Keywords: Acitretin; Complexation; Cyclodextrin; Permeability; Solubility; Topical administration

Etretinate (Tigason®), an aromatic retinoid, has been used for systemic treatment of severe forms of psoriasis and other dermatological disorders. However, its clinical usefulness is limited due to its potential teratogenicity and other side effects. The major active metabolite of etretinate is acitretin and several clinical trials have shown that it is as effective as etretinate against psoriasis. The main advantage of acitretin over etretinate is its short terminal half-life of 50–60 h compared to 120 days for etretinate (Pilkington and Brogden, 1992). When administered systematically, acitretin has the same spectrum of side effects as etretinate, nevertheless, it is believed that topical administration of the drug may reduce or even eliminate these side effects (Surber et al., 1991). However, the physicochemical properties of acitretin, such as its low solubility in most vehicles and photolability, may limit its flux from a topical formulation (Lehman et al., 1988). Topical acitretin formulations have lacked clinical efficacy, possibly due to an inadequate drug concentration at the target site within the skin (Surber et al., 1993). It is possible that the clinical efficacy of acitretin can be improved by increasing its dermal delivery from topical formulations. Previously, we have shown that it is possible to

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increase the transdermal delivery of drugs by cyclodextrin complexation (Loftsson et al., 1991). In the present study the effects of several cyclodextrins on the aqueous solubility and transdermal delivery of acitretin were investigated.

Acitretin was supplied courtesy of F. Hoffmann-La Roche Ltd (Basel, Switzerland). 2-Hydroxypropyl-β-cyclodextrin of molar substitution 0.6 (HPβCD), randomly methylated β-cyclodextrin with degree of substitution of 1.8 (MβCD), γ-cyclodextrin (γCD) and 2-hydroxypropyl-γ-cyclodextrin of molar substitution 0.6 (HPγCD) were obtained from Wacker-Chemie (Munich, Germany). All other chemicals used were commercially available products of special reagent grade. The hairless mice (C3H/Tif hr/hr) were obtained from Bommice (Denmark).

The quantitative determination of acitretin was performed on a HPLC component system consisting of a ConstaMetric 3000 solvent delivery system operated at 1.50 ml/min, a Merck-Hitachi AS4000A autosampler, a Beckman Ultrasphere ODS 5 μm (4.6 × 150 mm) column and a Spectra-Physics SP 8450 UV/Vis variable-wavelength detector operated at 340 nm. The mobile phase consisted of methanol, tetrahydrofuran and water (700:5:295), containing 0.015% (w/v) tetrabutylammonium hydrogen sulfate, and the retention time was 3.2 min. Due to the photolability of acitretin, all experiments, in both the solubility studies and the skin permeation studies, were performed with protection from light.

Solubilities were determined by adding an excess amount of acitretin to aqueous cyclodextrin buffer solutions (0.4 M acetic acid/sodium acetate (pH 5), 0.02 M sodium dihydrogen phosphate/disodium hydrogen phosphate (pH 7.5) and 0.05 M sodium hydrogen carbonate/disodium carbonate (pH 9)). The suspensions formed were sonicated in an ultrasonic bath for 3 h. After equilibration for at least 3 days at room temperature (22–23°C) the suspensions were filtered through a 0.45 μm membrane filter (Millex-HV filter units from Millipore, USA), diluted with a mixture of methanol and water (7:3) and analysed by HPLC. The 3 day equilibrium was considered sufficient, since further equilibration of the suspensions did not result in any additional drug precipitation.

The skin permeability of acitretin was determined in vitro. Female hairless mice were killed by cervical dislocation, and their full-thickness skins removed and placed in Franz diffusion cells of type FDC 400 15FF, diameter 1.5 cm (Vangard International Inc., USA), containing 12.3 ml receptor phase. The receptor phase consisted of isotonic aqueous 0.02 M phosphate buffer (pH 7.5) containing 5% (w/v) MβCD, 0.3% (w/v) Brij-58 and 0.4% (v/v) formaldehyde. The receptor phase was stirred with a magnetic bar. The receptor chamber was kept at 37°C by circulating water through an external jacket. The composition of the donor phases is shown in Table 1. 2 ml of the donor phase was applied to the skin surface (the stratum corneum) and the donor chamber covered with parafilm. Samples of receptor

Table 1
Composition of the donor phases

Donor phase number	Acitretin (mg/ml)	MβCD (% w/v)	Vehicle (% v/v)			
			Propylene glycol	Oleic acid	Tocopherol	Buffer [a]
1	4	0	95	0	5	0
2	4	23	95	0	5	0
3	4	23	49.5	0	1	49.5
4	4	23	47	5	1	47
5	4	23	0	0	0	100
6	2	23	0	0	0	100

[a] Aqueous 0.02 M phosphate buffer, pH 7.52.
The solubility of acitretin in the vehicles was somewhat less than 2 mg/ml and, thus, it always formed a suspension in the vehicles.

phase were removed from the cells at 12-h intervals for 3–4 days and replaced with fresh buffer solution. The samples were kept frozen until analysis by HPLC. Each experiment was repeated at least three times and the results reported are the mean values ± standard error of the mean (SE).

According to its manufacturer, F. Hoffmann-La Roche, acitretin is insoluble in water and its solubility in propylene glycol is less than 0.2 mg/ml. However, the solubility of acitretin in 94% (v/v) ethanol had been determined to be 0.7 mg/ml, 1.5 mg/ml in chloroform and 15 mg/ml in dimethyl sulfoxide. This indicates that the high lipophilicity of the drug is the main cause of its low aqueous solubility and that its solubility can possibly be increased by complexation with a water-soluble cyclodextrin. Also, acitretin is a carboxylic acid and, therefore, its aqueous solubility is pH dependent. Thus, the combined effect of various cyclodextrins and pH on the aqueous solubility of acitretin was investigated (Table 2). At pH 5, where acitretin existed mainly in the unionised form, the aqueous solubility was low and of the three cyclodextrins tested at this pH only γCD had a detectable effect on the solubility. Significant solubilisation was observed at pH 9, where acitretin existed only in the ionised form but this high pH is unacceptable for dermatological preparations. Lowering the pH to 7.5 resulted in acceptable but somewhat less solubilisation. Of the four cyclodextrins tested MβCD had the greatest solubilising effect and, therefore, this cyclodextrin derivative was selected for the skin permeation studies.

Table 2
Effect of cyclodextrins and pH on the aqueous solubility of acitretin at room temperature

Cyclodextrin	(% w/v)	pH	Solubility (mg/ml)
HPβCD	25	5	less than 0.01
	25	7.5	0.583
	25	9	0.923
MβCD	25	5	less than 0.01
	25	7.5	1.96
	25	9	2.29
	50	9	3.35
γCD	12.5	5	0.078
	12.5	7.5	0.101
	12.5	9	0.137
HPγCD	25	9	0.497

Table 3
Flux (mean ± SE) of acitretin through hairless mouse skin

Donor phase number	Flux ($\mu g\ h^{-1}\ cm^{-2}$)	Ratio [a]
1	0.032 ± 0.007	1.0
2	0.027 ± 0.002	0.8
3	0.120 ± 0.004	3.8
4	0.090 ± 0.010	2.8
5	0.051 ± 0.001	1.6
6	0.201 ± 0.035	6.3

[a] The flux of acitretin from the donor phase/the flux from donor phase number 1.

The effect of MβCD and the vehicle composition on the transdermal delivery of acitretin was investigated in vitro (Table 3). The flux of acitretin from a propylene glycol vehicle (donor phase no. 1) was only about 0.03 $\mu g\ h^{-1}\ cm^{-2}$. Addition of MβCD to the propylene glycol vehicle had little or no effect on the flux. When about half of the propylene glycol was replaced by water (donor phases no. 3 and 4) a 3–4-fold increase in the flux was observed. Addition of oleic acid lowered the flux possibly by interfering with the complex formation between the drug and MβCD. Oleic acid is a well known penetration enhancer which usually increases the transdermal delivery of drugs by interacting with the skin barrier (Cooper, 1984; Loftsson et al., 1989). When all propylene glycol was replaced by water the flux was somewhat lowered (donor phase no. 5). However, maximum flux of acitretin through the skin was achieved when the amount of acitretin in the donor phase was reduced to 2 mg/ml (donor phase no. 6). In this donor phase only about 10–15% of the drug was in the solid form and the flux through the skin was over 6-fold larger than from a pure propylene glycol vehicle. It is possible that the larger excess of the drug in donor phase no. 5 resulted in a smaller flux due to interference by the undissolved drug particles at the vehicle-skin interface. These results show that it is possible to increase significantly the dermal and transdermal delivery of acitretin by cyclodextrin complexation.

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