ELSEVIER

International Journal of Pharmaceutics 111 (1994) 111–116

international journal of pharmaceutics

Research Papers

Evaluation by laser Doppler velocimetry of the attenuation of tretinoin induced skin irritation by β-cyclodextrin complexation

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Received 1 February 1994; accepted 15 March 1994

Abstract

An in vitro study was performed to determine the amount of all-*trans*-retinoic acid (RA) (tretinoin) released from a dermic gel using Franz diffusion cells; the release of free retinoic acid and of its inclusion compound in β-cyclodextrin (β-CD) from the same gel formulation was thus compared. We also studied the effect of inclusion on RA-induced skin irritation using a non-invasive method: laser Doppler velocimetry; the cutaneous blood flow was measured after the application of RA-containing gels. The results obtained in vitro demonstrated release of free RA and of its inclusion compound from dermic gels; those obtained in vivo clearly indicate that β-CD complexation is particularly useful for reducing RA-induced skin irritation.

Key words: Laser Doppler velocimetry; Tretinoin; all-*trans*-Retinoic acid; β-Cyclodextrin; Franz diffusion cell; Skin irritation; Dermic gel

1. Introduction

Owing to its action on keratinisation, all-*trans*-retinoic acid (RA) is successfully used in the treatment of psoriasis and acne (Kligman et al., 1969). Kligman et al. (1986) have observed neovascularisation and thickening of the epidermis, derm and collagen fibers after the topical administration of an RA-containing cream. These results make it possible to use this molecule in the curative or preventive treatment of dermal modifications of senile skins or prematurely aged skins through too excessive exposure to ultraviolet rays. However, its repeated use causes considerable skin irritation as early as at the first application (Burge, 1989).

RA has antiproliferative and differentiating properties that have been used to check some malignant growths in man: cutaneous metastatic melanoma (Levine and Meyskens, 1980) and Kaposi's sarcoma (Bonhomme et al., 1991).

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SSDI 0378-5173(94)00090-R

The numerous drawbacks of RA, including its local irritating effect, poor stability in the presence of air and light and insolubility in water (less than 0.5 mg/ ml), led us to include this molecule in β-CD via the coprecipitation method (Amdidouche et al., 1989).

The aim of the present work was to assess in vivo skin irritation induced by two gels, one containing free RA and the other containing RA complexed with β-CD.

Nevertheless, before performing this study, it was necessary to ensure that RA could be released from its inclusion compound and thereby be bioavailable on the skin from its galenic form. With this aim in mind, the in vitro release of free RA and of its inclusion compound was investigated using Franz diffusion cells equipped with synthetic membranes (Franz, 1978); subsequently, the skin irritation induced by free RA and its inclusion compound was compared using a non-invasive technique: laser Doppler velocimetry (LDV). This technique allows assessment of the skin irritating effect related to an increase in cutaneous blood flow.

This technique has been used previously by different authors to measure the vascular dilatation induced by the local administration of different molecules. For example, Guy et al. (1983) use this method to assess the percutaneous penetration of the vasodilatator methyl nicotinate.

This non-invasive method has also been employed by Kholi et al. (1987) to evaluate the cutaneous irritating effect of different solvents (ethanol, propanol, etc.) and the vasodilatator hexyl nicotinate. Likewise, Freeman and Maibach (1988) have demonstrated and quantified skin irritation induced by patch tests with 2% sodium lauryl sulfate.

2. Materials and methods

2.1. Products

Silastic® membranes were provided by Dow-Corning Corp. (Midland, MI, U.S.A.), tretinoin (retinoic acid) by Produits Roche (Paris, France), β-CD by Roquette-Frères (Lestrem, France), Volpo 20® by Croda (Trappes, France), Klucel HF® by Aqualon (Rueuil-Malmaison, France), potassium phosphate by Osi (Paris, France), sodium chloride, ammonium acetate and acetic acid by Prolabo (Paris, France) and butylhydroxytoluene (BHT) by Sipca (Paris, France). All the solvents used were analytical reagent grade.

2.2. Gel formulations

The gels formulas are listed in Table 1. Klucel HF® is a hydroxypropylcellulose which has the property of forming hydroalcoholic gels. BHT is a antioxidant preservative used to prevent the oxidation of tretinoin.

A 0.05% concentration of RA was chosen according to the solubility of this molecule in the hydroalcoholic gels prepared and on the basis of its usual concentration in the dermic forms that are already on the market.

2.3. In vitro studies

Franz diffusion cells comprise two chambers (donor and receptor). A Silastic membrane is intercaled between the two chambers.

Polydimethylsiloxane (Silastic®) membranes are non-porous polymerized membranes through which molecules can pass via a passive diffusion mechanism (Nakano et al., 1976; Bottari et al., 1977; Di Colo et al., 1980; O'Driscoll et al., 1990).

Prior to commencing the test, the membranes were cut into 6.26 ± 0.2 cm^2 discs, washed with a

Table 1
Gels formulas (quantified in g)

Constituents	Preparation		
	Vehicle	Gel 1	Gel 2
Tretinoin		0.05	
BHT	0.05	0.05	0.05
Inclusion compound			1 [a]
Klucel HF®	3	3	3
95% alcohol	30	30	30
Distilled water	66.95	66.90	65.95

[a] Namely 0.05 g tretinoin.

hot soapy solution, rinsed with distilled water, immersed for 1 h in the receptor solution and then intercalated between the two chambers.

A 95.2 ± 4.4 mg sample of gel was spread out uniformly over the entire membrane surface. Throughout the experiment, the donor chamber was kept open. The receptor chamber was filled with the receptor solution: isotonic phosphate-buffered saline pH 7.3–7.4 (PBS) with 1% Volpo® (a non-ionic surfactant); Volpo was used to ensure the solubility of RA (Lehman, 1988).

The receptor solution was maintained at 37°C by water circulating within a glass jacket around the lower chamber and was continuously stirred by a teflon-coated magnet rotated by an external magnet mounted on a 600 rpm motor.

The cells were protected from light for the duration of the experiment.

A 0.15 ml portion of the receptor solution was removed 30, 80, 140, 200, 320, and 500 min after the beginning of the test and replenished each time by 0.15 ml of PBS buffered solution. The samples were analysed by high-performance liquid chromatography (HPLC) with UV spectrophotometric detection at 350 nm.

2.4. Assay of tretinoin by HPLC

The operating conditions were as follows: column – Spherisorb ODS2 C18, 5 μm particle size, 150 × 4.6 mm i.d. (phase separation); injector – Sedex 100 Sedere (Vitry-sur-Seine, France); detector – Spectromonitor 3100X LDC Analytical (Orsay, France); integrator – Shimatzu-Chromatopac C-R 5 A; pump – Constametric 3000 (Orsay, France).

The wavelength was set at 350 nm. The mobile phase, water-acetonitrile-tetrahydrofuran (16 : 76 : 8 v/v), was prepared fresh each day by mixing 760 ml acetonitrile, 80 ml tetrahydrofuran, 80 ml water, 1.6 g ammonium acetate and 5 ml acetic acid. Chromatography was performed at ambient temperature at a flow rate of 1 ml/min (Verweij et al., 1985).

A calibration curve was drawn by diluting a tretinoin solution. The retention time of tretinoin was 3.8 min.

2.5. Laser doppler velocimetry

2.5.1. Method

Laser Doppler velocimetry (LDV) is based on Doppler's principle. Light from a 632.8 nm He-Ne laser was transmitted through the skin with normal incidence by a quartz optical fiber. The beam penetrated the skin to a depth of 0.15–1 mm.

The laser light is reflected by erythrocytes moving in the dermal capillaries. The difference in frequency between the incident and reflected light is recorded by the apparatus. The output signal is plotted vs time. The available laser energy only permits study of the dermal blood flow.

The keratolysis induced by RA results in an increase in the cutaneous blood flow linked to skin irritation. The variations in cutaneous blood flow were measured after the application of the vehicle alone and RA-containing gels (gels 1 and 2).

2.5.2. Subjects

The study was carried out on nine informed adult volunteers (three men and six women) (mean age 27 years) free from dermatological diseases. Clinical approval was obtained for the experiments. Subjects rested for at least 15 min before the assay to acclimatize to the environment (21 ± 2°C; RH 55 ± 10%).

The different preparations (5 mg cm^{-2}) were applied to three delineated areas (9 cm^2) on the skin of the ventral surface of the forearm. A fourth area was selected as an untreated control site.

The RA-containing gels or the vehicle alone were randomly applied to the right forearm. The preparations were uniformly spread out with a gloved finger, each application being followed by a 3 min massage meant to facilitate the penetration of the gel. The application was repeated for 10 days except at weekends.

A preliminary experiment was performed to determine the most suitable recording time which was finally set at 6 min for each site. The physiological baseline was determined for each subject on the untreated control area, the recordings then being made for the three treated areas.

The variation in amplitude of cutaneous blood

flow was measured after the application of each preparation vs the vehicle alone. This variation is proportional to the irritant effect of these preparations.

The results were obtained by measuring the maximal heights of the peaks of the dermal blood flow plotted from the physiological baseline.

The attenuation percentage $A(\%)$ of the irritating effect is expressed by the following equation:

$$A\% = [(h_1 - h_2) \times 100]/h_1$$

where h_1 is the maximal amplitude of the blood flow recorded on the area treated with gel 1 (containing free RA) and h_2 denotes the maximal amplitude of the blood flow recorded on the area treated with gel 2 (containing the inclusion compound).

3. Results and discussion

3.1. Release tests

The results obtained in vitro are presented in Table 1. The in vitro tests clearly demonstrate the release of free RA and of its inclusion compound from the reference gel.

On inspection of the release curves of RA in the receptor solution, plotted vs time (Fig. 1), one can observe greater diffusion through the Silastic membrane for the free RA than from its inclusion compound.

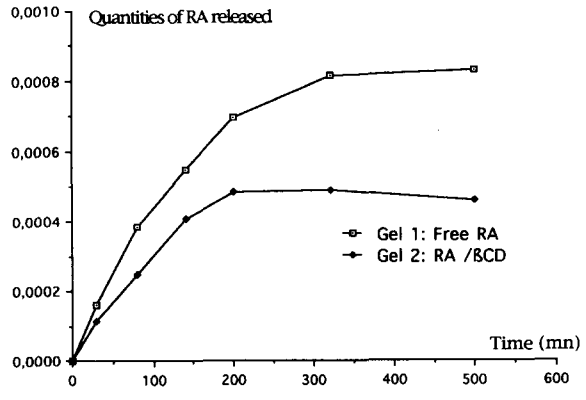

Fig. 1. In vitro study: release of RA (mg/cm^2) from gels 1 and 2.

Table 2
Results of LDV measurements

Subjects	h_1	h_2	A (%)
1	7	6	14
2	9.66	2	79
3	12.6	2	84
4	0.66	0	100
5	2	0	100
6	4	0	100
7	4	0	100
8	13	0	100
9	21	0	100
Mean	8.21	1.11	86.33
SD	6.52	2.027	

8 h after the beginning of the diffusion test, the release rates were 10% for the included RA and 20% for the untreated RA.

The hydrophilic properties of β-CD prevent this molecule from passing through the Silastic membrane. Consequently, the included RA must leave the cyclodextrin before passing through the membrane. As a result, released active molecules, as they diffuse through the membrane, can lead to progressive dissociation of the inclusion compound.

During a study of the β-CD effect on the transport of benzoic acid and phenobarbital through a Silastic membrane, O'Driscoll and al. (1990) also observed a decrease in the release rates of these molecules due to their inclusion in β-CD.

3.2. Laser Doppler velocimetry (LDV)

The results obtained by using the LDV technique on nine subjects are given in Table 2. The results were analysed using the paired Student's t-test. There is a significant difference ($p = 0.013$) in blood flow intensity between the two areas. The lowest intensity was measured on the area treated with the inclusion compound.

The attenuation of the irritation induced by including RA in β-CD is above 85% (Fig. 2).

Several authors have observed a decrease in the irritation effect caused by different molecules on their inclusion in β-CD.

After achieving the complexation of phenylbu-

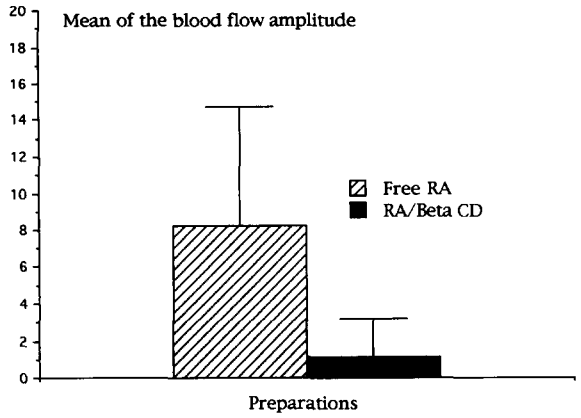

Fig. 2. Attenuation of RA-induced skin irritation by β-cyclodextrin complexation.

tazone in β-CD, Nambu et al. (1978) observed a clear-cut improvement in the reduction of stomach irritation by this molecule when taken orally.

Uekama et al. (1982) have studied the effect of three cyclodextrins (β-CD, γ-CD and α-CD) on the primary irritation of guinea pig skins, caused by prochlorperazine (PCP). Their results show that the inclusion of PCP in β-CD and γ-CD reduces primary skin irritation, with better efficiency for β-CD. In contrast, α-CD enhances the irritation, the effect increasing with PCP concentration.

The authors have assumed that these effects could be related to the stability constant of the different complexes. These constants amount to 34000 M^{-1} for the β-CD-PCP complex, 6100 M^{-1} for the γ-CD-PCP complex and only 170 M^{-1} for the α-CD-PCP complex.

Hoshino et al. (1989) have investigated the reduction in the dermal photosensitive potentiality of chlorpromazine (CPZ) after its inclusion in β-CD and dimethyl-β-CD. The results demonstrated good efficiency of the two guest molecules, with dimethyl-β-CD being the better of the two. The authors made the following assumption: β-CD and dimethyl-β-CD would keep CPZ from penetrating the skin by forming slow-diffusing complexes with this molecule and, as a result, would prevent photochemical reactions between CPZ and the biomacromolecules present in the skin; this would result in the non-formation of the photoantigens responsible for CPZ's cutaneous toxicity.

4. Conclusion

The study has demonstrated a clear reduction in RA-induced skin irritation on complexation with β-CD. The results presented here confirm previous data from several authors who studied the effect of β-CD on the irritating potentiality of different molecules. The use of LDV allows the non-invasive assessment of this property of β-CD on human subjects.

By improving the local tolerance of RA significantly, the formulas developed could lead to better observance and, as a result, to greater efficiency of the course of treatment.

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