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Buccoadhesive slow-release tablets of acitretin: design and 'in vivo' evaluation

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Abstract

Acitretin is an aromatic retinoid used in the treatment of buccal keratinization disorders. The local therapy has recently shown promising results. The aims of this study were to develop buccoadhesive tablets with different 'in vitro' release profiles of acitretin in order to select the types of tablets to be tested 'in vivo', to determine the saliva acitretin concentration and to verify the therapeutical efficacy. On the basis of preliminary in vivo studies the dose of 10 mg of acitretin was used. Ten different formulations of two-layer buccoadhesive tablets were considered. The inferior layer (10 mg of Carbopol® 934P:Methocel® K4M, 1:2) provided bioadhesive properties to all the tablets. The upper layer of all tablets was the slow-release matrix containing the acitretin. The release-controlling component was an hydroxypropylmethylcellulose (HPMC); lactose was used as soluble filler. Aiming to achieve a wide range of release rates, three types of HPMC with different viscosity grades were used. A good relation was found between the in vitro dissolution profile and the in vivo permanence of acitretin in the oral cavity, that was longer for the formulation containing 85% of Methocel® E5 than the formulation containing 45% of Methocel® E5. Both formulations permitted to obtain good clinical results.

Keywords: Acitretin; Keratinization disorders; Buccoadhesive tablets

1. Introduction

Acitretin is an aromatic retinoid, orally administrated in the treatment of severe chronic keratinization disorders (Fig. 1). Oral acitretin has unfavorable side effects, such as teratogenicity (Blanchet-Bardon et al., 1991). Topical application of retinoids has not been associated with fetal abnormalities (Barbagna et al., 1991) and for this reason topical dosage forms have been investigated.

Recently, an acitretin formulation, based on a mucoadhesive gel, has shown promising results in

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local therapy of buccal hyperkeratosis (Serpico et al., 1994). However, it is known that gels are difficult to retain in the mouth and large quantities of the drug can be swallowed, inducing systemic toxicity.

Among the various dosage forms, buccoadhesive slow-release tablets seem to be the most suitable in order to improve local drug delivery in the oral cavity (Smart, 1993; Nair and Chien, 1996).

Aims of this work were: (1) to develop buccoadhesive tablets with different 'in vitro' release profiles of acitretin in order to select two types of tablets to be tested 'in vivo'; (2) to determine the profile of the saliva acitretin concentrations following the administration of the two types of tablets; (3) to investigate the efficacy of the buccoadhesive tablets of acitretin in the local therapy of oral leukoplakia. This paper reports results concerning steps 1 and 2.

On the basis of preliminary in vivo studies (Serpico et al., 1994), a dose of 10 mg of acitretin twice a day was set.

Two-layer buccoadhesive tablets with different release rates were considered. The upper layer of all tablets was the slow-release matrix containing the acitretin. The inferior layer provided bioadhesive properties to all the tablets.

Two-layer tablets are more difficult to manufacture than monolithic ones, but they permit to modify the composition of the slow-release matrix without compromising the adhesive properties, that could be significantly reduced by the addition of other substances to the adhesive polymers.

Of all the available bioadhesive polymers, the acrylic acid derivatives (PAA) and hydroxypropylmethylcellulose (HPMC) are the most extensively used due to their stability and low toxicity (Junginger, 1991). PAA have better bioadhesive properties than HPMC (Mortazavi and Smart, 1994) but may cause irritation of the mucosa (Bouckaert and Remon, 1993), while their mixture has acceptable properties of adhesion and biocompatibility (Bouckaert and Remon, 1993). Because satisfactory adhesive properties are obtained using 30–40% of PAA (Ponchel et al., 1987), a fixed mixture of HPMC and PAA (2:1, w/w) was considered.

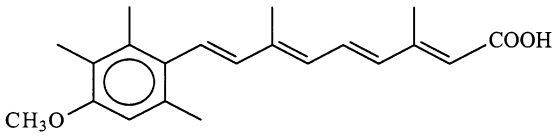

Fig. 1. Chemical structure of acitretin.

In this work 10 types of tablet were prepared with the same composition in the inferior adhesive layer, but different compositions in the upper layer.

The release-controlling component was a hydrophilic cellulose derivative; lactose was used as soluble filler. Aiming to achieve a wide range of release rates, three types of HPMC with viscosity grades of 4000, 50 and 5 cps (2% aqueous solution) were used.

The adhesion properties of the tablets were evaluated in vivo.

The release profiles of acitretin from the tablets were evaluated in vitro. On the basis of these data two formulations were selected to be used in the subsequent in vivo studies. The formulation that gave the best in vivo results has been submitted to preliminary long-term and accelerated stability tests.

Table 1
Upper layer compositions of the different buccoadhesive tablets

Formulation no.	Composition			
	Methocel®		Lactose (%)	Acitretin (%)
	Type	%		
1	E5	85	—	15
2	E5	70	15	15
3	E5	60	25	15
4	E5	45	40	15
5	E50	60	25	15
6	E50	45	40	15
7	E50	15	70	15
8	K4M	60	25	15
9	K4M	45	40	15
10	K4M	15	70	15

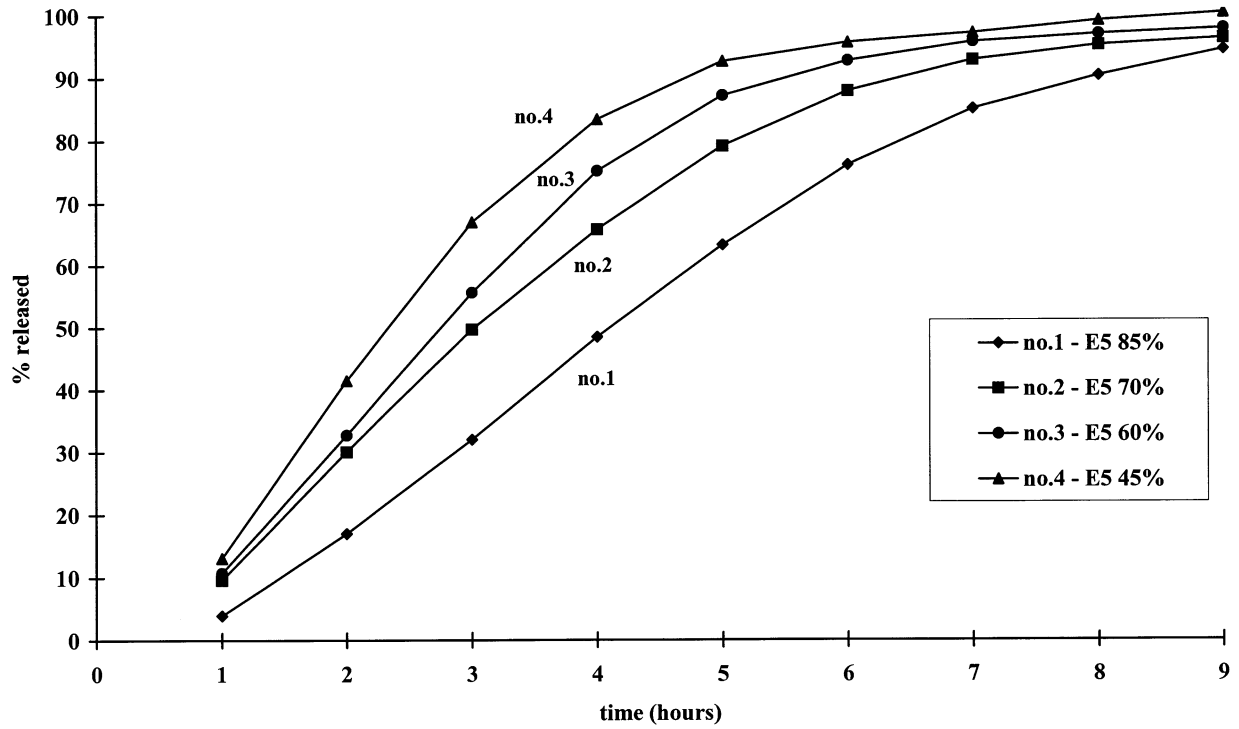

Fig. 2. Release profiles of acitretin from formulations containing Methocel® E5 in the upper layer ($n = 6$, coefficient of variations are less than 10%).

2. Materials and methods

2.1. Materials

Acitretin was a gift of Mag (Milan, Italy). Arotenoid-ethyl sulfone was a gift of Roche (Basilea, Switzerland). Carbopol® 934P (CP) was supplied by B.F. Goodrich (Brecksville, USA). Methocel® K4M Premium ($\eta = 4000$ cps, 2% aqueous solution), Methocel® E50-LV Premium ($\eta = 50$ cps, 2% aqueous solution) and Methocel® E5 Premium ($\eta = 5$ cps, 2% aqueous solution) were purchased form Dow Chemical Company (Midland, USA). Spray-dried lactose was supplied by DMV International (Veghel, The Netherlands).

2.2. Photoprotection

Because of the photodegradation and photoisomerization of acitretin, all operations were carried out under minimal light exposure (e.g. protection or yellow light).

2.3. Tablet preparation

The inferior layer (10 mg) was made of a mixture of CP and Methocel® K4M (1:2, w/w). The upper layer (66 mg), representing the controlled-release matrix of the system, contained 10 mg of acitretin and a mixture of HPMC and lactose. The compositions of the prepared tablets are reported in Table 1. The two-layer tablets were prepared by compression on an single punch compression machine (Korsch, type EK0, Frankfurt, Germany) equipped with 7-mm diameter flat punches. The tablets were 2 mm thick. The compression procedure was carried out manually. The bioadhesive material was placed in the die and then lightly tapped. The active portion of the formulation was then fed into the die and the powder compressed.

Tablet hardness was in the range of 6.5–7.5 Kp (Erweka TBM 28 hardness tester, Heusenstamn, Germany).

2.4. Drug content

The tablet was dissolved in 100 ml of methanol. Three samples were withdrawn, diluted as required and analyzed spectrophotometrically at $\lambda = 353$ nm (Spectrophotometer UV-VIS, Model DU6, Beckman, Fullerton, CA, USA). Each value represents the medium of the content of three tablets.

2.5. In vitro dissolution studies

Release experiments were conducted in a paddle dissolution apparatus (Apparatus II, USP 23) (Erweka TD6, Heusentamn, Germany) at 37 ± 0.5°C. The tablets were posed with the inferior layer attached to the bottom of the vessel. A volume of 900 ml of dissolution medium consisting of phosphate buffer, pH 7.4, with 1% Tween 80 was used in each experiment at an agitation rate of 50 rpm. The use of pH 7.4 buffer solution and the addition of the surfactant agent were necessary in order to maintain sink conditions for the release of acitretin (Spah et al., 1989), as acitretin, according to its manufacturer, is insoluble in water. Acitretin concentrations were determined spectrophotometrically at $\lambda = 353$ nm (Spectrophotometer UV-VIS, Model DU6, Beckman, Fullerton, CA, USA). At least six tablets of each formulation were tested.

2.6. In vivo bioadhesion studies

Placebo two-layer tablets (upper layer 100% Methocel® E5 stained with an inorganic dye) were attached to the gingiva, in the region of the bottom canine tooth, in six healthy human volunteers aged 20–35 years. Volunteers were allowed to drink during the study. The adhesion time, comfort, smarting sensation in mouth and irritability were assessed within 6–8 h.

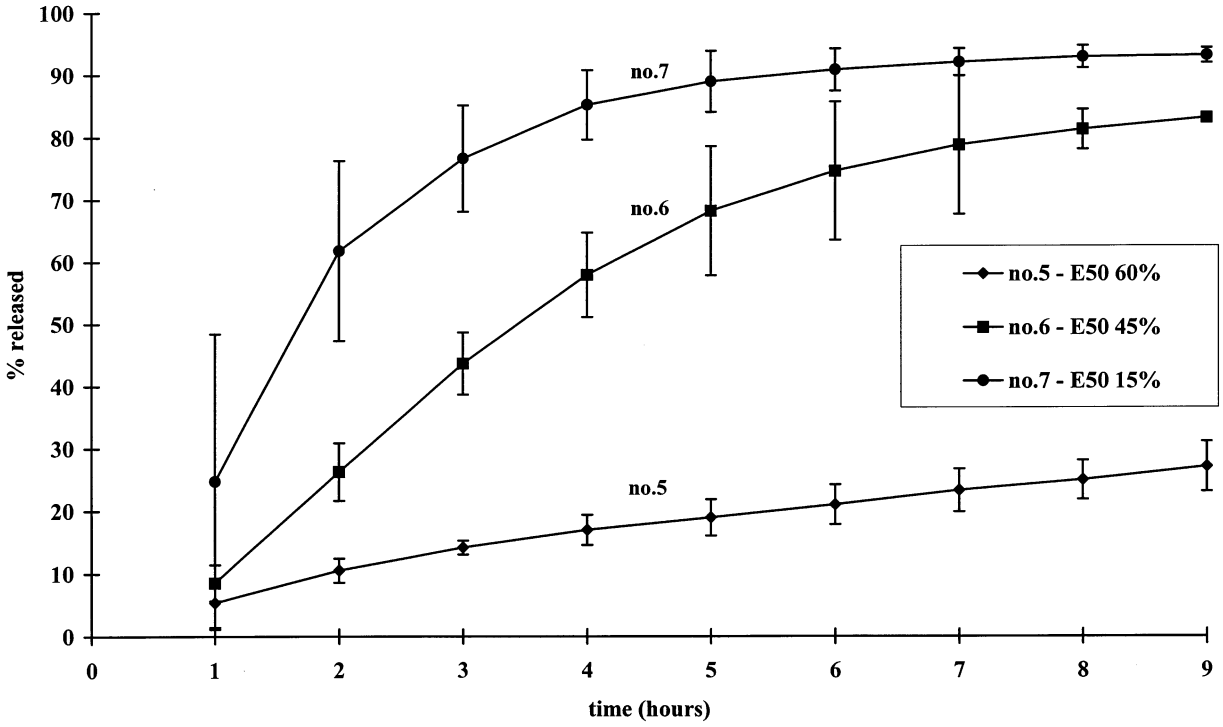

Fig. 3. Release profiles of acitretin from formulations containing Methocel® E50 in the upper layer ($n = 6$, error bars are S.D.).

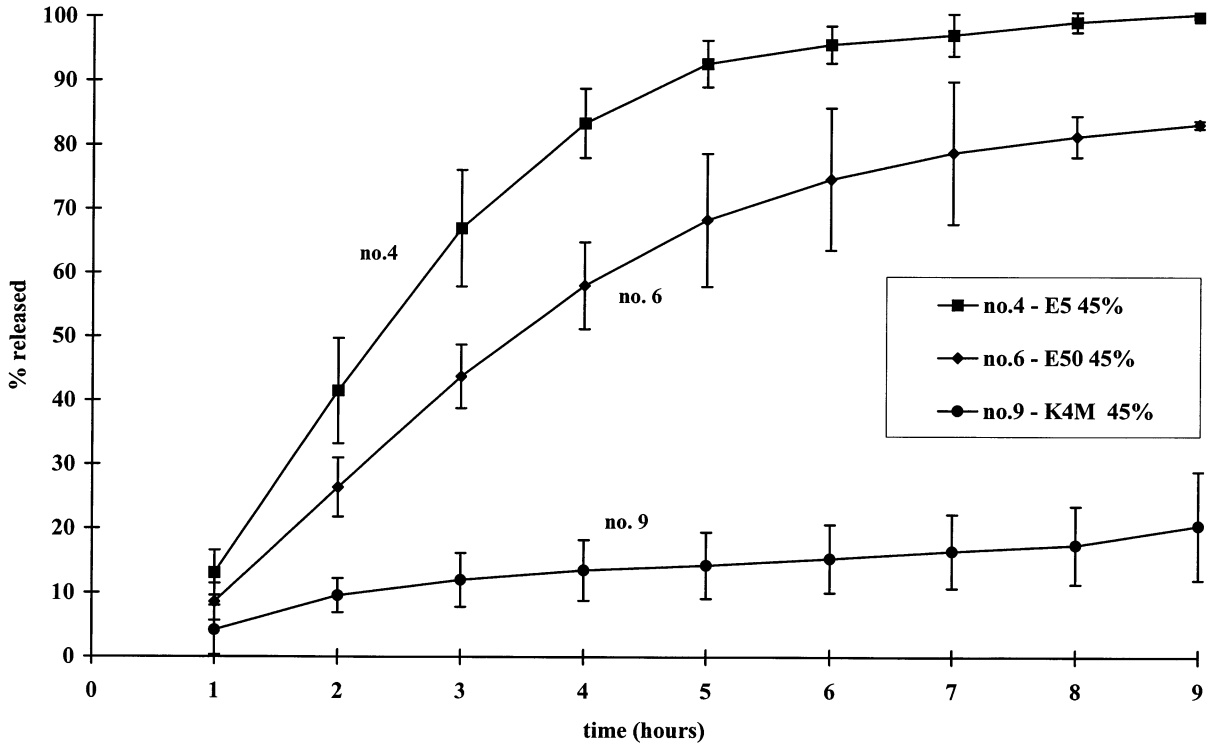

Fig. 4. Release profiles of acitretin from formulations containing 45% of different Methocel® in the upper layer ($n = 6$, error bars are S.D.).

2.7. In vivo evaluation of saliva acitretin concentration

Fourteen volunteers, 11 men and three women with oral leukoplakia, aged from 42 to 73 years (mean, 52.5), were included in the study. The study conformed to the Declaration of Helsinki, Tokyo and Venice and was approved by the Ethical Committee.

Each patient received a written form with instruction on how to use the tablets and a questionnaire to report any adverse effect (smarting sensations, dry mouth or irritability, angular cheilitis and adhesion time).

Patients were randomized into two groups, seven in each. Every group was treated with one of the two different formulations selected on the basis of in vitro dissolution study.

Saliva samples (200 μl) were collected by direct withdrawal from the sublingual area by a micropipette at 30, 60, 120, 180, 240, 300, 360 and 540 min after the administration of a tablet. No stimulation was performed before the collection. The samples were stored in plastic vials at -20°C until analysis. The statistical analysis was performed using the t-test for independent samples.

2.8. Saliva extraction

Saliva samples (200 μl) were diluted to 1 ml with Tris-buffered saline, pH 7.50. After addition of 20 μg of arotenoid-ethyl sulfone as internal standard, they were processed as follows.

2.9. Analytical method

All analyses on saliva samples were performed with a Varian liquid chromatography apparatus (Model 9010, Walnut Creek, CA, USA) equipped with a variable wavelength UV-Vis detector (Model 9050) and a peak-integration program (LC-Star). A reversed-phase Bio-Sil ODS-5S

HPLC column (Bio-RAD) 250 × 4 mm was utilized at room temperature. The mobile phase was methanol/acetonitrile (7:3) (85%, v/v) and 1.5% acetic acid in water (15%, v/v); the flow rate was 1 ml/min and the detection was performed at 350 nm (Laugier et al., 1994). Quantitative analysis was achieved by measuring the peak areas of *trans*-acitretin (retention time, 10.15 min), *cis*-acitretin (retention time, 8.17 min) and arotinoid-ethyl-sulfone (retention time, 11.56). For the all-*trans*-acitretin the peak area response was linear in the range of 10–250 ng.

2.10. Stability testing

The physical and chemical stability of the final formulation was evaluated by long-term testing conducted at 25 ± 2°C/60 ± 5% RH and accelerated testing conducted at 40 ± 2°C/75 ± 5% RH for 12 months (the stability testing; USP 23, 1995). The samples were maintained in closed amber glass bottles.

General appearance, organoleptic properties, dissolution profile and drug contents of the tablets were determined after 3, 6 and 12 months.

3. Results and discussion

3.1. In vitro drug release

The release profiles of the tablets made of different percentages of Methocel® E5 are reported in Fig. 2. Acitretin was almost completely released from all formulations in 9 h. The release profiles of formulations no. 1 and no. 4 containing, respectively, the highest and the lowest percentage of polymer were significantly different in the first 7-h period. The release was linear in the first 6 h ($r^2 = 0.9964$) only for formulation no. 1.

The release profiles of tablets prepared with different percentages of Methocel® E50 are given in Fig. 3. The three release profiles were significantly different: the formulation containing in the

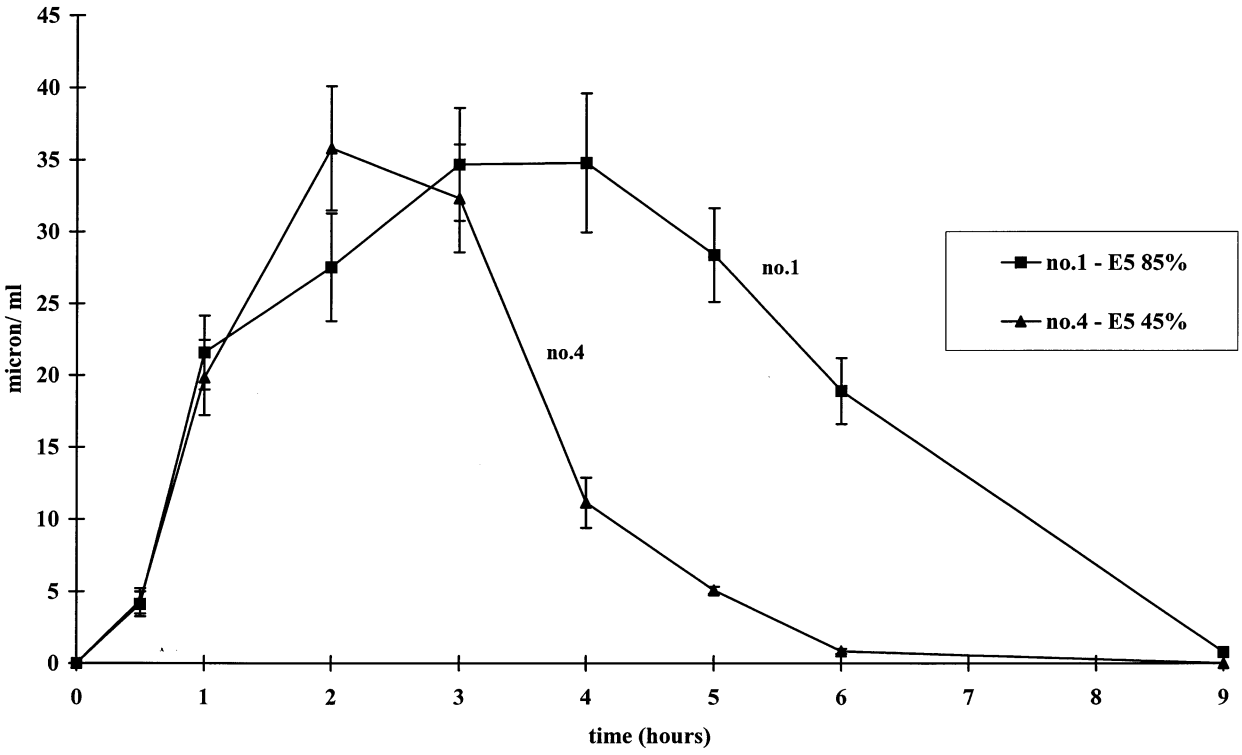

Fig. 5. Acitretin saliva concentration following administration of formulations no. 1 and no. 4 ($n = 7$, error bars are S.D.).

upper layer the lowest percentage of HPMC (Table 1, no. 7) released more that 60% of the drug in 2 h, while the one with 60% of HPMC (Table 1, no. 5) had a very slow release (less than 30% of drug after 9 h).

In all cases evident swelling phenomena were observed.

As far as Methocel® K4M was concerned, tablets containing 45% of polymer (Table 1, no. 9) swelled and no apparent erosion occurred within 5 h: acitretin was released in a very low percentage (about 20%) of the drug content after 9 h. Consequently, dissolution test of formulation no. 8 (Table 1), containing a higher percentage of Methocel® K4M, was not performed.

The upper layer was almost completely dissolved within 2 h in tablets of formulation no. 7 and no. 10 (Table 1) containing 70% of lactose. The release of acitretin from the tablets containing the three tested Methocel® was significantly different when the polymer was added in percentages greater than 15% (Fig. 4). However, as the polymers involved have different swelling and erosion characteristics (Wan et al., 1993) the in vivo behavior is not reliably predictable.

For the in vivo study formulations containing Methocel® E5 seems to be the more suitable considering that this polymer is the less swellable and this property is very important to achieve patient compliance. Consequently formulations no. 1 and no. 4, that showed significantly different release profiles, were selected.

3.2. In vivo bioadhesion study

Placebo tablets showed a good adhesion for a period of 6–8 h. No volunteers felt any uncomfortable or smarting sensation during the considered period.

3.3. In vivo evaluation of acitretin saliva concentration

The mean acitretin saliva concentrations obtained in the period following the administration of the two selected formulations are reported in Fig. 5. The maximum concentration of acitretin was almost the same with both types of tablet (35.77 μg/ml for formulation no. 4, 34.76 μg/ml for formulation no. 1) as well as the kinetics of appearance of the drug in saliva in the first hours.

The acitretin saliva concentration in patients treated with formulation no. 1 disappeared between 6 and 9 h, while in patients treated with formulation no. 4 the acitretin concentration in saliva decreased drastically after 3 h and disappeared after 5 h; thus we can presume that the release rate of the drug in vivo from formulation no. 1 was slower than that from formulation no. 4, confirming the behavior in vitro.

The amount of acitretin in the salivary compartment was almost double for formulation no. 1 with respect to formulation no. 4. This could be due to a partial boundary or absorption of the drug by the gingiva, that could be a more important phenomena when the release of acitretin from the pharmaceutical form is faster. However, the concentrations of acitretin in the tissue as well as in the plasma at the end of the administration where practically undetectable (Gaeta et al., 1997). That means that the concentrations were lower than the quantitative limit of the analytical method (10 ng).

The inter-individual variability of the drug determined in the saliva after buccal administration is generally very high, even because the collection of the sample is critical and can be made after stimulation or without stimulation and for spontaneous flow or direct withdrawal (Dittgen and Oestereich, 1989; Bouckaert et al., 1992). Confirming the validity of the sampling procedure our data showed a low variability ($0.730 < p < 0.957$).

3.4. Stability testing

The preliminary stability tests were performed with formulation no. 1 which gave significant concentrations of acitretin in the saliva for a more prolonged period than formulation no. 4. The drug content for this formulation was $97 \pm 1.5\%$.

General appearance and organoleptic properties did not change significantly in 12 months in tablets submitted to long-term testing as well as to accelerated testing.

The potency was maintained in 90% of the drug content in the long-term testing. In the accelerated conditions the degradation was much more evident and the potency was: $93.4 \pm 2.6\%$ after 3 months, $89 \pm 1.3\%$ after 6 months and $88.4 \pm 3.5\%$ after 12 months.

Dissolution profiles did not change significantly in 12 months in tablets submitted to long-term testing as well as to accelerated testing.

4. Conclusions

As far as the matrix formulations is concerned, the selected system based on hydroxypropylmethylcellulose polymers proved to have an acceptable flexibility in terms of in vitro release profiles.

Among the three different types of hydroxypropylmethylcellulose tested, Methocel® E5 represented the first choice polymer as the tablets made with this polymer showed poor swelling characteristics, which could facilitate patient compliance. As a consequence formulations no. 1 and no. 4, that showed different in vitro release rates, were chosen for clinical evaluation.

The tablets with formulation no. 4 that had a faster release in vitro remained for a shorter time in saliva in vivo.

Even if the acitretin permanence in saliva was longer for formulation no. 1 than for formulation no. 4. no clinical differences were observed between groups treated for 4 weeks with the two formulations, as reported in the paper related to our clinical data (Gaeta et al., 1997).

Side effects, such as erythema, erosion, smarting sensation or nausea, were never reported and consequently compliance was very high.

In conclusion, these buccoadhesive two-layer tablets represent a good alternative to topical gel or ointment for the buccal administration of acitretin for local therapy, as the acitretin was maintained in the salivary compartment for at least 5 h, and good clinical results were obtained with two tablets/die.

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