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Epidermal effects of tretinoin and isotretinoin: influence of isomerism

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Received April 27, 2005, accepted August 3, 2005

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Pharmazie 61: 453–456 (2006)

The efficacy of tretinoin is well established in the treatment of acne and photoaged skin, however as a typical side effect of tretinoin treatment most patients develop a low-grade irritant dermatitis. Since isotretinoin topical treatment usually shows much lower incidence and intensity of adverse effects than tretinoin topical treatment, histological studies are needed to scientifically evaluate the effects of isotretinoin application on epidermis and also to assess if it can be used in anti-aging products as an alternative to tretinoin. Thus, the aim of this study was to compare the effects of topical use of tretinoin or isotretinoin on hairless mice epidermis, using appropriate histopathological and histometric techniques, in order to evaluate the influence of isomerism on skin effects. For this, gel cream formulations containing or not 0.05% tretinoin or 0.05% isotretinoin were applied in the dorsum of hairless mice, once a day for seven days. Histopathological evaluation, viable epidermal and horny layer thicknesses as well as the number of epidermal cell layers were determined. Our results showed that tretinoin and isotretinoin were effective in the enhancement of viable epidermis thickness and number of epidermal cell layers, suggesting that they could be used for stimulation of cellular renewal. However isomerism influenced skin effects since isotretinoin had more pronounced effects than tretinoin in viable epidermis. In addition only isotretinoin treatment enhanced horny layer thickness when compared to the gel cream treatment.

1. Introduction

Among all vitamin A derivatives, the most effective are the vitamin A acids, tretinoin (all-*trans*-retinoic acid) and isotretinoin (13-*cis*-retinoic acid). The efficacy of topical tretinoin in acne treatment (Orfanos et al. 1997) and photoaged skin (Schwartz et al. 1991; Olsen et al. 1992, 1997; Griffiths et al. 1995; Nyirady et al. 2001) is well established; however as a typical side effect of tretinoin treatment, most patients develop a low-grade irritant dermatitis with erythema and scaling. Thus, identifying other compounds with a similar effect and improved tolerance would be of great value.

Oral isotretinoin has been used for the treatment of dermatological conditions such as gram-negative folliculitis, recalcitrant rosacea, pyoderma faciale, psoriasis, cutaneous lupus erythematosus and acne fulminans (Wysowski et al. 2002). Besides oral isotretinoin is considered the most effective agent for the treatment of acne, it is also a potent teratogen mandating strict precautions for use among women of childbearing age (Koren et al. 2004; Nau 2001; Goldsmith et al. 2004). Furthermore, Sendagorta et al. (1992) reported that topical isotretinoin was well tolerated by subjects in a study that showed the efficacy of this substance in the improvement of photodamaged facial skin.

Thus, more studies are needed to scientifically evaluate the effects of isotretinoin application on skin and also to assess if it can be used in anti-aging products as an alternative to tretinoin. These studies are important since that although tretinoin has been considered effective as an anti-aging active substance, one of its problems is excessive skin irritation (Rigopoulos et al. 2004); moreover dermatologists and pharmaceutical companies are looking for effective and less irritating active substances.

Considering that the isomerism of retinoic acid can influence its activity on the skin, it is important to study comparatively these retinoic acid isomers quantifying their effects on the epidermis.

Thus, the aim of this study was to compare the effects of topical use of tretinoin or isotretinoin on the hairless mice epidermis, using appropriate histopathological and histometric techniques, in order to evaluate the influence of isomerism on skin effects.

2. Investigations and results

2.1. Histology

The lining epithelium of the hairless mice skin is of the keratinized stratified pavement type, containing approximately two cell layers. The epidermis basal layer that did not receive treatment (control) is clearly visible resting on the basement membrane and consisting of low cells with scarce cytoplasm, and ovoid nuclei more lightly stained than the nuclei in cells of more superficial layers. The

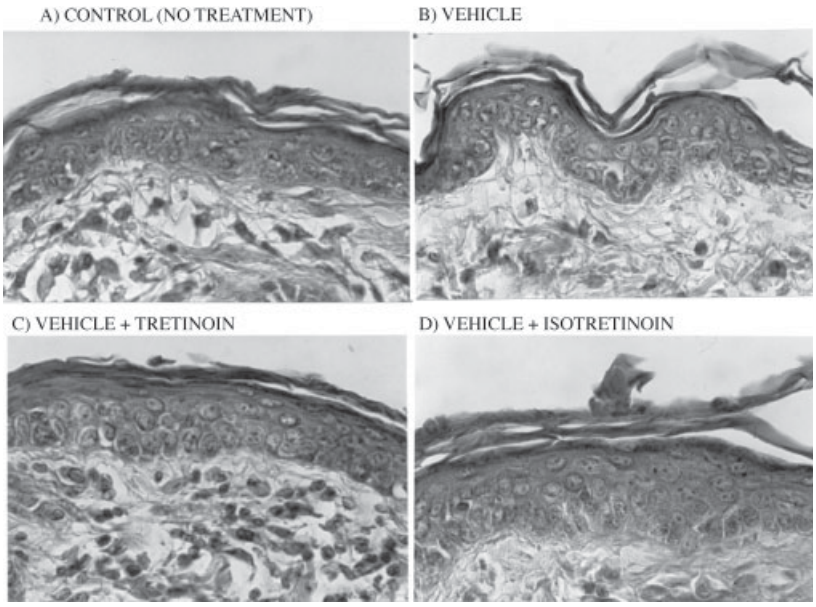

Fig. 1:
Photomicrographs of hairless mice skin. Magnification: X960, HE. A) control (no treatment), B) vehicle (V), C) V + tretinoin, D) V + isotretinoin

cells in this layer are well organized. Above spinous layer is the granular layer, whose cells contain keratin-hyaline granules in the cytoplasm. The horny layer is located in the outermost portion and consists of keratin filaments firmly adhering to the granular layer. The dermis, located immediately below the epidermis, consists of a layer of connective tissue (Fig. 1A).

The aspect of the epidermis and dermis of the vehicle (V) treated area was quite similar to that of the control area (Fig. 1B).

In tretinoin treated area, the epithelial thickness was increased and cells in the basal, spinous and granular layers were apparently more voluminous. The horny layer and dermis were similar to that observed in vehicle treatment (Fig. 1C).

In contrast, isotretinoin-containing formulations increased the thickness of both the epidermis and the horny layer. The dermis was similar to that observed in vehicle treated area (Fig. 1D).

2.2. Histometry

The statistically analyzed results for viable epidermal and horny layer thickness as well as the number of epidermal cell layers obtained in the histometric evaluation are shown in the Table and in Figs. 2–4.

Viable epidermis thickness was increased in all areas that received the formulations studied (vehicle, V + tretinoin and V + isotretinoin), when compared to the control area ($p < 0.001$). In addition, the effects of all formulations studied were statistically different among themselves ($p < 0.01$) (Table and Fig. 2) and isotretinoin induced a significant increase in viable epidermis thickness values followed by tretinoin and vehicle ($p < 0.01$).

Total epithelium thickness was also increased in all areas that received the formulations studied (vehicle, V + tretinoin and V + isotretinoin), when compared to the control ($p < 0.05$) and isotretinoin area had more pronounced effects than tretinoin area ($p < 0.05$) (Table).

The results obtained for the number of epidermal cell layers showed that the effects of all formulations studied were statistically different from the control and also

Table: Histometric values in mice after treatment with active substances and the values obtained for the control area

Parameter	Area (μm)			
	Control	Vehicle	Tretinoin	Isotretinoin
Viable epidermis thickness	14.23	19.24	23.57	24.61
	16.39	18.67	23.58	29.44
	16.53	18.98	19.54	27.15
	18.23	19.74	22.02	29.34
	17.10	20.66	25.45	28.40
Horny layer thickness	8.95	10.49	10.18	15.42
	8.66	9.09	10.62	17.82
	7.30	12.89	11.55	14.21
	10.50	9.12	9.34	13.70
	9.78	9.48	8.38	12.65
Epithelium thickness	23.18	29.73	33.75	40.03
	25.05	27.75	34.20	47.26
	23.83	31.87	31.08	41.36
	28.72	28.87	31.36	43.04
	26.88	30.15	33.83	41.05
Number of epidermal cell layers	1.52	1.83	2.58	2.99
	1.37	1.79	2.53	2.92
	1.32	1.90	2.54	2.81
	1.25	1.73	2.72	3.00
	1.25	1.70	2.93	3.05

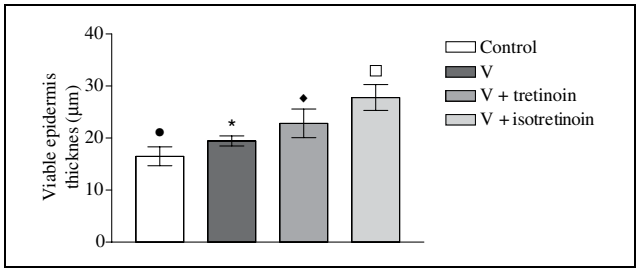

Fig. 2: Mean values of viable epidermis thickness in mice after treatment with active substances: vehicle (V), V + tretinoin and V + isotretinoin and the values obtained for the control area. Different symbols indicate statistically significant differences between treatments (Friedman test, n = 5 hairless mice, mean ± SEM, $p < 0.01$)

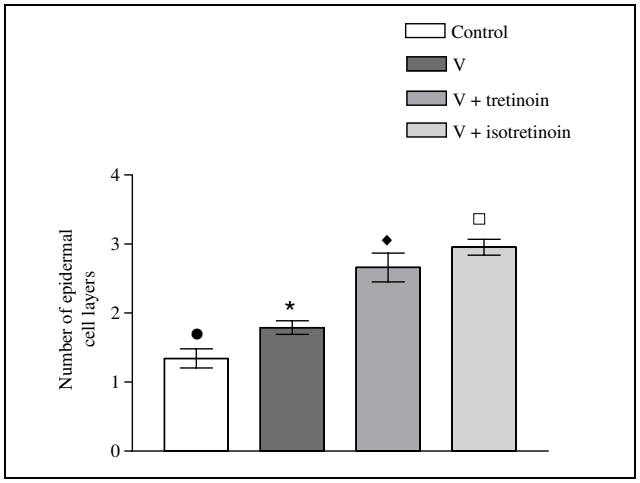

Fig. 3: Mean values of the number of epidermal cell layers in mice skin after treatment with active substances: vehicle (V), V + tretinoin and V + isotretinoin and the values obtained for the control area. Different symbols indicate statistically significant differences between treatments (Friedman test, n = 5 hairless mice, mean ± SEM, $p < 0.01$)

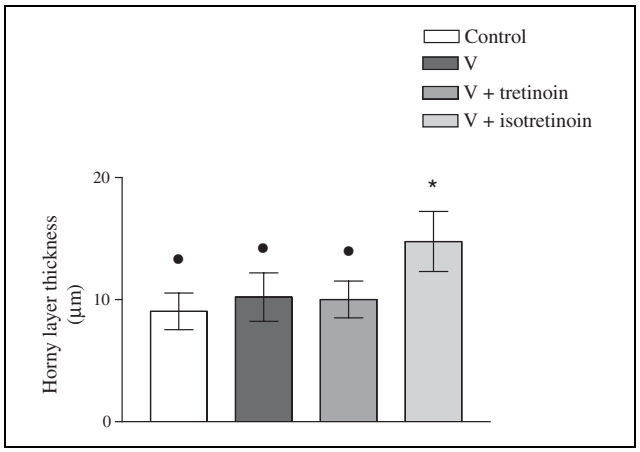

Fig. 4: Mean values of horny layer thickness in mice skin after treatment with active substances: vehicle (V), V + tretinoin and V + isotretinoin and the values obtained for the control area. Different symbols indicate statistically significant differences between treatments (Friedman test, n = 5 hairless mice, mean ± SEM, $p < 0.01$)

among themselves ($p < 0.01$) (Table and Fig. 3). Similarly to the data obtained for viable epidermis thickness, isotretinoin induced a significant increase in the number of epidermal cell layers followed by tretinoin and vehicle ($p < 0.01$).

Analysis of the horny layer showed that only the isotretinoin treatment enhanced horny layer thickness when compared to the vehicle ($p < 0.01$) (Table and Fig. 4).

3. Discussion

Topical retinoids were firstly reported for acne treatment (Rigopoulos et al. 2004); during this treatment some anti-aging effects were clinically observed in patients and since this finding, retinoids have also been used for anti-aging purposes. Among all retinoids, retinoic acid has presented the higher biological activity for acne and photoaging (Orfanos et al. 1997; Nyirady et al. 2001). In this paper, the effects of different topical retinoids (tretinoin and isotretinoin) on the epidermis were compared in order to evaluate the influence of isomerism on skin effects.

The results showed that alterations in the epidermis produced by the active substances occurred in different intensities and ways depending upon the variable studied.

Our results showed that when formulations containing the active substances were compared to the vehicle, the enhancement of viable epidermis thickness provoked by isotretinoin, which was accompanied by the enhancement of the number of epidermal cell layers, was even more evident than tretinoin effects. Thus, it can be suggested that isotretinoin is also effective in acceleration of epidermal turnover and could be employed in anti-aging formulations.

There are some studies reporting the increase in viable epidermis thickness by tretinoin, which may occur due to epidermal hyperplasia and hyperproliferation of basal keratinocytes (Olsen et al. 1992; Pepine et al. 1996; Pierard et al. 1997; Fisher and Voorhees 1996; Chapellier et al. 2002). Furthermore, other studies reported that tretinoin is effective in cellular renewal stimulation by the acceleration of epidermal turnover (Mandy 1986).

There are some studies reporting that isotretinoin has anticomedonal activity, altering the epithelization of follicles. In addition it was found that it exerts a sebum-suppressive effect, by an anti-androgenic mechanism, which is a property that is unique to oral isotretinoin and is not shared by tretinoin (Karlsson et al. 2003). However no reports were found in the literature concerning histological effects of isotretinoin in the horny layer.

In our study, only the isotretinoin treatment increased horny layer thickness, which probably occurred due to the enhancement of viable epidermis thickness and of the number of epidermal cell layers. This enhancement probably did not occur with tretinoin treatment because it increases horny layer disorganization and desquamation by decreasing cohesiveness of corneocytes (Bernerd et al. 1991).

Considering that an increase in epithelium thickness leads to an improvement of skin conditions, once aged skin usually shows a thinner epidermis, we suggest that isotretinoin is an effective ingredient that may be used in anti-aging cosmetic formulations, since it provoked a significant increase in epithelium thickness.

The results still showed that the vehicle formulation also enhanced the viable epidermis thickness and number of epidermal cell layers when compared to control area. In previous studies, our research group observed that formulations containing similar raw materials also provoked an enhancement of epidermis thickness due to intra and extracelular hydration (Silva and Maia Campos 2000). Thus, it can be suggested that this fact occurred due to hydration effects caused by vehicle formulation. This way, the skin hydration is very important to keep the normal skin condition since epidermis can be altered by many factors, such as hydration.

Concerning tretinoin and isotretinoin irritation effects, according to the histological analysis shown in this study no inflammation process was evident in both treatments since no leucocyte infiltration was observed. Our results are supported by another study indicating that tretinoin does not produce cutaneous inflammation in human volunteers (Griffiths et al. 1995).

However the main difference between these two drugs is related to adverse effects, which occur at a much lower incidence and intensity with isotretinoin than with tretinoin (Rigopoulos et al. 2004). Furthermore, Bernerd et al. (1991) reported that after 1 week treatment of rhino mice with tretinoin, some cutaneous toxic effects were noticed,

but they were normalized within two weeks. It is important to highlight that the vehicle used must be considered since tretinoin skin irritation is often linked to its hydroalcoholic vehicle. To circumvent this problems, various cream vehicles with emollient properties are now been used (Rigopoulos et al. 2004), i.e., gel cream formulations such as the one used in this study.

In summary, our results indicate that tretinoin and isotretinoin were effective in the enhancement of viable epidermis thickness and of the number of epidermal cell layers, suggesting that they could be used for cellular renewal stimulation. However isomerism influences their skin effects since isotretinoin had more pronounced effects than tretinoin in the epidermis.

Considering the differences between hairless mouse and human skin caution is needed in interpreting the results. Nevertheless, the data obtained in this study contribute to orient the use and the elucidation of possible effects of tretinoin and isotretinoin. In addition, our results showed that although the *trans* isomers are usually described as biologically more active, the *cis* isomer of vitamin A acid (isotretinoin) can also be considered active in the evaluated parameters.

4. Experimental

4.1. Test formulations

Gel cream formulations (vehicle), consisted of 2% hydroxy-ethyl-cellulose, 3% glycerin, 2% squalane, 1% hydrogenated lecithin, 0.05% butylated hydroxytoluene, 0.2% methyldibromo-glutaronitrile and phenoxyethanol, 2% propylene glycol and distilled water, were supplemented or not with 0.05% tretinoin or 0.05% isotretinoin.

4.2. Study protocol

Adult male hairless mice (HRS/J-hairless, Jackson, Bar Harbor, ME) weighing on average 30 g were used. The animals were kept in individual cages and received commercial ration (Nuvilab CR-1), as well as water *ad libitum*. This study was carried out in accordance with the "Principles of Laboratory Animal Care" (NIH).

The formulations were applied on the dorsum of the animals once a day for seven days, as follows: a) no treatment (control); b) application of the vehicle only; c) application of the vehicle with 0.05% tretinoin; d) application of the vehicle with 0.05% isotretinoin.

4.3. Histology

One week after starting the treatment, mice were euthanized by CO_2 inhalation and skin fragments were obtained and immediately immersed in a fixing solution consisting of 85 mL of 80% alcohol, 10 mL formaldehyde, and 5 mL acetic acid. After 24 h the fixed fragments were dehydrated, cleared, and embedded in paraffin. Semi serial 6 μm-thick sections were then obtained and each section corresponded to an interval of fifty sections, i.e., ten sections were obtained from the 2 mm biopsy. The sections were stained with haematoxylin and eosin for general histopathological, and histometric analysis (Maia Campos et al. 1999; Silva and Maia Campos et al. 2000; Lu et al. 1999).

4.4. Histometry

Viable epidermis and horny layer thickness as well as the number of epidermal cell layers were analyzed by using a light microscope Leica DMLB, coupled with a digital camera DC 300, using 100-fold magnification. The number of nucleated cell layers was counted at ten randomly selected locations per slide and averaged, as described previously (Lu et al. 1999). Viable epidermis and horny layer thickness were also measured in a similar manner, and the means ± SEM were calculated.

4.5. Statistical analysis

Non-parametric tests were selected for statistical analysis of the experimental data points, since they showed a non-Gaussian distribution. The paired Friedman test was used for comparison of multiple measured data points using statistical software, GMC. Differences were accepted as statistically significant at $p < 0.05$.

Acknowledgement: The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant 01/04816-8) for financial support.

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