J. Appl. Cosmetol. 11, 71-76 (July-September 1993)

SKIN TREATMENT WITH TWO DIFFERENT GALENICAL FORMULATIONS OF RETINYL PALMITATE IN HUMANS

Thom Erling, Ph. D.
Medstat Research Ltd. P.O. Box 210 - 2001 Lillestrøm, Norway

Receveid: *May 21,1993*

Key Words: *Retinyl palmitate; Skin penetration; Galenical formulations; Skin thickness; Skin elasticity.*

Synopsis

A randomized comparative double-blind within subject study of topical administration of two different galenical formulations of retinyl palmitate (RP) α 2% W/W for 3 months in 20 females, caused significant changes in skin thickness and elasticity as well as in the persons self-evaluations of skin improvements following administration of a polysaccharide conjugated RP formulation. No significant changes were observed after administration of the conventional formulation. The results indicate that the topical application of RP can bring about biochemical changes in skin quality if the neccessary hanrips are made to enhance the topical bioavailability of RP by making the substance artificially water soluble by using a suitable "tween". Conventional RP formulations do not have a similar biological effect, probably due to lack of sufficient skin penetration. Such formulations can therefore not be expected to have clinical efficacy in the treatment of wrinkles and other aging symptoms of the skin in spite of the claims made in advertisements.

Riassunto

Su venti donne è stato condotto uno studio comparato in dopppio ceco per tre mesi utilizzando due diverse formulazioni galeniche di retinil palmitato al 2% (W/W). In una delle due formulazioni il retinil-palmitato (RP) era legato ad un polisaccaride. RP coniugato ha provocato cambiamenti significativi sia nello spessore che nell'elasticità della cute rispetto al RP semplice. Questi cambiamenti sono probabilmente legati alla maggiore biodisponibilità della RP che, legata ai polisaccaridi, diventa idrosolubile e, quindi più attiva. RP tal quale non esplica, al contrario, questa attività perchè probabilmente non penetra sufficientemente attraverso l'organo pelle. Queste formulazioni nuove che esplicano una attività chimica controllata sulle rughe possono essere utilizzate come cosmetici attivi in tutte le forme di precoce invecchiamento cutaneo.

INTRODUCTION

The antiaging effect on human skin after treatment with retinoic acid is convincingly documented in a number of open and controlled studies (1-4), From a tolerability point of view, however, some patients (4-10%) report side-effects of a dermatitis type after using retinoic acid (2). The drug may therefore be unsuitable for a number of patients for antiaging treatment. Other vitamin A derivatives, however, seem to have a better local tolerability than retinoic acid and may be preferable from a cosmetic point of view. A number of cosmetic preparations with different derivatives have been developed and launched for the treatment of the aging symptoms. The efficacy documentation for these preparations is normally limited or lacking. The adds, however, claim superb efficacy.

Satisfactory skin penetration is a prerequisite for the clinical effect of the actual substance. Different ester derivatives, mainly retinyl palmitate (RP) and acetate (RA), gave been utilized in cosmetic preparations (5). In order to make the RP biologically active, modifications of the solubility have to be carried out by using "tweens". In this way the substance is made artifically water soluble and can penetrate the skin. Similar techniques are aldo used for enhancing the biovailability of drugs after topical and peroral administration (6). RP, in an active form, has been shown in animal studies to bring about biochemical changes with both epidermis and dermis (7). Parts of the action of RP in the skin may depend on its conversion to retinoic acid (8). This coversion depends on the enzymatic cleavage of the ester bonds in the retinyl to retinoic acid (9). It has been demonstrated that skin preparations can indeed convert retinyl to retinoic acid (10). Although it has been known that RP has the potential to effect a change of skin composition this potential has only to a limited extension been investigated in persons using RP preparations for cosmetic treatment of aging symptoms. In order to investigate the importance of the galenical formulation for clinical effect, we decided to carry out a comparative clinical study of two different galenical formulations.

MATERIAL AND METHODS

The study was carried out as double-blind within subject study in 20 female persons. Each of the participants used each of the two formulations either, on the right or the left volar (protected) part of the forearm, respectively. The administration of the formulations was randomized, meaning that half of the persons used formulation A on the right arm and formulation B on the left arm and vice and versa for the other persons. The total treatment period was 3 months and the cosmetic formulations were applied b.i.d. (in the morning and the evening) during the study period.

Cosmetic formulations

The two cosmetic formulations used in the study were manufactured by Pedersens laboratorier, Vejle, Denmark, and had identical cosmetic properties and appearances. The retinyl palmitate (Roche) concentrations in both formulations were 0.2%. The stability of the two formulations has been extensively tested. In formulation A* the retinyl palmitate is stabilized and conjugated with a "tween" in form of a complex polysaccharide, while formulation B contains unconjugated retinyl palmitate. The vehicles used in the two formulations were identical.

Measurements of skin thickness and skin elasticity

The measurements of skin thickness and skin elasticity were performed by Dermascan R A using ultrasound technique and DermaflexR A instruments, respectively (Cortex Inc., Aarhus,

Denmark). Measurements were carried out initially, after 1 month and after 3 months. Measurements of skin thickness and elasticity were performed by the same person (ET) on all three occasions and measurements were performed at the mid-region of the volar part of the forearm. All measurements were done in triplicate and average value were used for statistical evaluation.

*) Formulation A is identical to the marketed preparations SinceraR (S,N) and Gloria Mundi® (DK).

Self-evaluation by the participant

Self-evaluation of skin quality by the participant was performed by using visual analog scales of 10 cms with defined endpoints "no change" snf "very pronounced change". The subject was asked to score the global change in skin quality by placing a mark on the line between the endpoints. The distance from the zero point ("no change") to the mark was used as the score for the actual patient (11).

Statistical methods

A significance level of 5% was used in the tests and two-tailed tests were applied. Mean was used for estimation of continuous and near-continuous variables, and the Student procedure was used for coustruction of confidence interval of mean. The one-sample t-test was used analysing change over time within groups. Analysis of covariance and two-sample t-test were used to compare between arms with regard to continuous variables.

RESULTS

Study population

20 female persons aged between 40 and 60 years (mean (SD) of 50,3 yrs (6,2)) were included in the study and all participants concluded according to the protocol. All participants gave their informed consent to take part in the trial after having received written and verbal information about the objectives of the study.

Efficacy parameter, skin thickness and elasticity

The results from the skin thickness measurements are shown in table 1.

Table I.

Changes in skin thickness (mm) after administration of RP for 3 months (n=20).

FORMULATION		INITIALLY	AFTER 1 MONTH	AFTER 3 MONTHS
A	Mean (SD)	0,91 (0,10)	0,98 (0,10)	1,19 (0,10)
	Range	0,69-1,23	0,68-1,26	0,87-1,32
B	Mean (SD)	0,91 (0,09)	0,93 (0,10)	0,93 (0,10)
	Range	0,70-1,10	0,7,-1,12	0,74-1,10

Following the administration of the conjugated RP formulation A an average increase in skin thickness of 31% is observed, while the change in skin thickness after treatment with the unconjugated RF (formulation B) is the negligible. The increase in skin thickness following treatment with formulation A is statistically significant ($p<0.01$) while formulation B gives no significant changes.

In table II the viscoelastic properties of the skin following administrtion of formulation A and B respectively, are presented. The results show that formulation A improve the elasticity with about 18% on average ($p<0.01$) while no significant changes can be observed after treatment with formulation B.

Efficacy parameter, self-evaluation by use of VAS

The participants were also asked to make a self-evaluation of the global effect of the two formulations on the quality of the skin (including smoothness and colour). These assessments were performed using VAS, the results from the self-evaluation il shown in table III.

A significant improvement ($p<0.01$) in skin quality is reported on the forearm treated with formulation A, while no significant effect is reported on the arm treated with formulation B. Formulation A seems to improve the smoothness and the glow of the skin, which were the two most frequent changes observed by the par-

Table II.

Changes in skin elasticity (%) after administration of RP for 3 months (N=20).

FORMULATION		INITIALLY	AFTER 1 MONTH	AFTER 3 MONTHS
A	Mean (SD)	60,4 (7,4)	67,2 (7,8)	71,5 (7,9)
	Range	45,1-80,1	49,1-80,4	50,9-84,2
B	Mean (SD)	59,9 (7,1)	60,1 (6,9)	60,2 (6,5)
	Range	44,3-70,1	45,2-71,4	44,7-70,4

Table III.

Changes in VAS scores (cms) after administration of RP for 3 months (N=20).

FORMULATION		AFTER 1 MONTH	AFTER 3 MONTHS
A	Mean (SD)	1.6 (1.0)	5.9 (0.9)
	Range	0.0 - 3.1	0.8 - 8.4
B	Mean (SD)	0.7 (1.2)	0.9 (1.3)
	Range	0.0 - 1.2	0.0 - 1.6

ticipants, after chronic treatment for 3 months. The correlation between the changes in the objective measures (skin thickness and elasticity) and the observed improvement in skin quality following treatment with formulation A is striking.

Tolerability

No of the subjects participating in the study reported any side-effects following chronic administrations of the two formulations b.i.d. for 3 months.

DISCUSSION

The results from this study are in agreement with previously reported results from animal and human studies, showing that RP in active form brings about biochemical changes with dermis and epidermis (7,12). Cosmetic preparations are generally accepted to have a minimal effect on skin biology. The proven benefits of the application of cosmetic skin preparations have usually been attributed to a moisturization effect. One particular material used in cosmetic formulations, vitamin A, is known to have systemic physiological and biochemical effects (6). The role of retinoids in the regualation of skin development has, until now, mainly been focused upon the use of retinoic acid. Retinoic acid appears to be more soluble in water and is effective without a water solubilizing vehicle (6). This probably accounts for its popularity as a local therapeutical agent. Using RP in cosmetic preparations is actually a prodrug principle as the ester has to be transformed to the acid in order to exert its clinical effect. Previous studies have pointed out that suitable "tweens" have to be used to increase the skin penetration of highly lipophilic substances. The relative oil/water solubility of the different forms of vitamin A have not been compared, but differences in this respect will affect epidermal penetration. The penetration of the palmitate can readily be made equal to that of the acid by the use of special solvents. Conjugation with a complzx polysaccaride or another suitable substance will enhance the skin penetration of the lipophilic molecule and at the same time protect the substance against oxydation (degradation).

The objective parameters measured in this study, skin thickness and elasticity, indirectly verify that formulation A, which is the conjugated RP, penetrates the skin and brings about changes in the biological systems responsible for the skin's thickness and elasticity. The unconjuated retinyl palmitate, however, does not have the same effect on these objective skin parameters, probably due to lack of on a very limited skin penetration.Our results on increase in skin thickness (31%) and elasticity (18%) are in good agreement with the results reported by Fthenakis et al (12%). In this study an improvement of skin elasticity of 23% was registered after a treatment period of 6 weeks with a 0.10% RP formulation, while skin thickness was improved by 16% after 4 weeks of treatment. No significant effects in these two parameters were registered after treatment with a simple moisturizer. The slight differences might be due to a longer treatment period (12 weeks) and a higher concentration of RP (0,2%) in our study.

Beyond doubt, active RP formulations are able to bring about changes in skin composition and morphometry with good clinical tolerability. As reported by others, the concentration of RP should probably be less than 0,5% in cosmetic preparations if erythematous reactions are to be avoided.

It can be questioned if cosmetic preparations containing plain RP should be recommended for the antiaging treatment of the skin due to lack of (or at least a vety modest) biological effect, probably due to its restricted skin penetration ability. Misleading consumers to anticapte antiaging effects after continuous use of these preparations are unetichal.

More sophisticated studies dealing directly with the skin penetration of RP and studing the vitamin A concentrations in the skin and systemic circulation, should be carried out.

References

1. **Kligman AM, Grave GL, Hirose R, Leyden KK. (1986)** Topical tretinoin for photoaged skin. *J. Am. Acad. Dermatol.* ; ? 836-59.
2. **Weiss KS, Ellis CN, Headington JT, Hmilton TA, Voorhees KK. (1988)** Topical tretinoin improves photoaged skin: a doubleblind vehicle controlled study, *JAMA* ; **259**: 527-32.
3. **Bhawan J, Gonzalez-Serva A, Nehal K, Labadie R, lufrano L, Thorne G et al. (1991)** Effect of tretinoin on photodamaged skin. *Arch. Dermatol.* ; **127**: 666-72.
4. **Rafal FS, Griffiths CEM, Cherie MRCP, Ditre M, Finkel LJ, Hamilton TA et al. (1992)** Topical tretinoin (retinoic acid) treatment for liver spots associated with photodamage. *The New England J. of Med.* ; **6**: 368-74.
5. **Jarrett A, Jackson RJ. (1980)** The effect of vitamin A on the skin and its role as a cosmetic agent. Abstract 11th International I.F.S.C.C. Congress Research and technology, Venice 23-26 Sept.
6. **Sperman RIC, Jarrett A. (1974)** Biological comparison of isomers and chemical forms of vitamin A (retinol). *Br. J. Dermatol.* ; **90:** 553-60.
7. **Connts D.F., Skreko F., McBee J., Wich A.G. (1988)** The effect of retinyl palmitate on skin composition and morphometry. *J. Soc. Cosmet. Chem.* ; **39**: 235-40.
8. **Shapiro S. (1986)** Retinoids and epithelial differentiation. In: Retinoids and cell differentiation, Michael Sherman, Ed. CRC Press, Inc. Boca Raton, Fl ; 25-29.
9. **Igosin Y. (1976)** Histochemical study of non-specific esterases, lipase and cholinesteras e in normal skin. Vestnik Dermatologii Vernerologii ; **12:** 13-16.
10. **Connor M, Smit MH. (1987)** The formulation of all-trans retinyl in hairless mouse skin. Biochem. Pharmacol. **36:** 919-24.
11. **Marks R, Edwards C. (1992)** The measurement of photodamage. Br. J. Dermatol. ; **127.** Suppl. **41;** 7-13.
12. **Fthenakis CG, Maes DH, Smith WP. (1991)** In vivo assesment of skin elasticity using ballistometry. *J. Soc. Cosmet. Chem.* **42:** 211-222