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A COMPARATIVE DOUBLE-BLIND WITHIN SUBJECT STUDY OF THE EFFICACY AND TOLERABILITY OF TWO DIFFERENT DERIVATIVES OF VITAMIN A ON SKIN THICKNESS AND ELASTICITY: RETINOIC ACID AND CONJUGATED RETINYL PALMITATE

Erling Thom, Ph.D.*
*Medstat Research Ltd, Lillestrøm, Norway

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Synopsis

A randomized comparative double-blind within subject study of topical administration of two different Vitamin A preparations - conjugated retinyl palmitate, RP (0,2%) and retinoic acid, RA (0.025%) for 3 months in 20 females caused significant changes in skin thickness and elasticity as well as in the participants own judgment of skin improvement using visual analogue scales.
The results indicate that both galenical formulations exert a comparable effect, while the tolerability of the RP formulation was significantly better. It might be of great importance for the efficacy of the RP cream that the compound is conjugated and thus having a better skin biovalability than unconjugated RP has. Whether the clinial effect is due to metabolism of RP to RA or retinol in the skin can not be answered by this study, but should be subject to further percutaneous absorption studies. Other studies indicate that the mechanism of action for RP creams might be due to biotransformation to retinol rather than to RA.

Riassunto

Uno studio randomizzato a doppio-cieco è stato condotto per tre mesi su 20 donne utilizzando due diversi preparati a base di vitamina A: retinil palmitato coniugato (RP 0,2%) ed acido retinoico (RA 0.025%). Le due formulazioni hanno provocato cambiamenti significativi sia nello spessore che nella elasticità della pelle .
I risultati indicano che entrambe le formulazioni galeniche svolgono un'attività paragonabile, ma la tollerabilità della formulazione RP è stata significativamente maggiore. Potrebbe essere di grande importanza per l'efficacia della formulazione RP il fatto che la miscela sia coniugata ed abbia perciò una biodisponibilità cutanea migliore rispetto ad un RP non coniugato. Questo studio non ha potuto rispondere al quesito se l'effetto clinico sia dovuto al metabolismo dell'RP che si trasforma in RA o in retinolo nella pelle. Questo argomento dovrebbe essere oggetto di ulteriori studi sull'assorbimento percutaneo. Altri studi indicano che il meccanismo d'azione delle creme RP può essere dovuto alla biotrasformazione in retinolo, piuttosto che in RA.

INTRODUCTION

Data has previously been reported on the efficacy and tolerability of a skin cream containing conjugated Retinyl palmitate (RP) (1,2). The aims of these two studies were to investigate the effect of the cream on skin thickness and elasticity and in addition to study the duration of the effect after treatment was stopped. From these studies we have clearly documented that use of conjugated RP results in an increase in skin thickness and an improvement in skin elasticity. These observations have been confirmed by others (3,4) using other measuring techniques.

RP is a widely used ingredient in cosmetic formulations. Because it is the most stable of the available vitamin A esters it can be directly incorporated into an anhydrous base or the oil base of a cosmetic cream or lotion. RP is lipophilic and photostable. Ofter products containing RP are advertised as having benefical effects on the appearance of the skin. A great number of products containing RP are marketed. Previous studies suggest that enzymes present in the skin metabolize RP during skin absorption. Esterase activity hydrolyzes RP to retinol (Vitamin A), wich is oxidized in many tissues to Retinoic acid (RA) primarly by alcohol dehydrogenase. RP can therefore be classified as a prodrug where the inactive RP is transformed to active substances by metabolic biotransformation reactions in the skin.

Recently a number of studies have been carried out with RA creams showing that this compound has a positive effect on photo-aged skin (5-8). Preparations containing RA have been registered in some countries as ethical drugs for treatment of aging skin. However, one main drawback with this type of treatment is that a number of patients (4-10%) develop soreness in and miscolouring (redness) of the skin (6).

As mentioned above RA has been convincingly documented as having an effect on the treatment of the aging skin and retinol is also a compound with know pharmacological activity (9). Previous studies have shown the formation of all-trans-retinoic acids after topical application of all-trans-retinol in vivo in hairless mouse skin (10). Recently, however, a study showed that retinol was the only detectable metabolite following administration of RP both in guinea pig and human skin, while no retinoic acid was detectable (11). About 30 and 18% of topically applied RA were absorbed from an acetone vehicle by hairless guinea pig and human skin, respectively. Less than 1% of the applied dose of RP diffused from the skin into the receptor fluid. In human skin, 44% of the absorbed RP was hudrolyzed to retinol.

An essential prerequisite for an optimal penetration of the lipohilic RP into the skin is to modify the solublity of the compound by the use of tweens (12,13). The producer of the RP preparation used in this study has developed a process where RP in conjugated with a complex polysaccharide thereby making the compound water soluble. In this way the penetration of RP through the skin and into the diffusion cell receptor fluid is enhanced. The same technique has been used succesfully to enhance the bioavailability of drug substances both administered perorally as well as topically (14).

Based on the abovementioned information and our previous experiences with conjugated RP, we decided to carry out a clinical comparison on skin thickness and skin elasticity following treatment with an cream containing either RA or conjugated RP in order to gain an indirected impression of the bioequivalency of these two substances.

MATERIAL AND METHODS

The study was carried out as a randomized double-blind study in 20 females with each participant using both formulations one on the right and the other the left volar (protected) part of the forearm, respectively. The administration of the formulations was randomized, such that half of the participants used formulation A on the

right arm and formulation B on the left arm and vice a versa for the other women. The total treatment period was 3 months and administration was b.i.d. (in the morning and evening) during the study period. The study was carried out in accordance with the Declaration of Helsinki.

The investigational formulations

The two formulations used in this study were: A conjugated RP cream made by Pedersens Laboratorium, Vejle, Denmark, containing 0.2% RP and a cream containing 0.025% RA made with the same cosmetic properties and apperance as the conjugated RP cream. Formulation A is identical to the marketed preparations Sincera®, Demelle®, Luscinia® and Rebilt®. The investigational products were packed in identical packages in order to keep the study blinded.

Measurements of skin thickness and skin elasticity

The measurements of skin thickness and skin elasticity were performed by ultrasound using Dermascan A and Dermaflex instruments, respectively (Cortex Inc. Aarhus, Denmark). Measurements were carried out at baseline, after 1 month and after 3 months, 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 in triplicate and average values used for statistical evaluations.

Self-evaluation by the partecipant

At the same time as each ultrasound measures was made, participants made a self evaluation of skin quality using visual analogue scales of 10 cm with endpoints of "no change" and "very pronunced change". Subjects were 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 subject.

Statistical methods

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

RESULTS

20 females aged between 40 and 60 years (mean 51.4 yrs) were included in the study and all participants were compliant with the protocol. All participants gave their informed consent to take part in the study after having received written and verbal information about the study.

Efficacy parameters, skin thickness and elasticity

The results from the skin thickness measurements are shown in Table I. Formulation A resulted in a average increase in skin thickness of 31% compared with 33% for formulation B. This increase in skin thisckness was significant ($p<0.01$) for both formulations, while the difference between the two formulations was not significantly different.

In Table II the viscoelastic properties of the skin following administration of formulations A and B, respectively, are presented. The results show that formulation A improved the elasticity by 19% on average, compared with 20% for B.

Both formulations improved the elasticity of the skin significantly ($p<0.01$), but again the difference in elasticity improvement between the two formulations was not significant.

Efficacy parameters, self-evaluation by use of VAS

The VAS results are shown in Table III.
A significant improvement ($p<0.01$) in skin quality was reported for both forearms. The difference in skin improvement between the two formulations is, however, not significant.

Table I
Changes in skin thickness (mm) after administration of conjugated RP (A) and RA (B) creams for 3 months, respectively (N=20)

	Formulation	Initially	After 1 month	After 3 months
A	Mean (SD)	0.90 (0.12)	1.01 (0.17)	1.18 (0.15)
	Range	0.71-1.24	0.74-1.27	0.90-1.34
B	Mean (SD)	0.88 (0.15)	1.02 (0.15)	1.17 (0.17)
	Range	0.70-1.25	0.76-1.30	0.90-1.37

Table II
Changes in skin elasticity (%) after administration of conjugated RP (A) and RA (B) creams for 3 months (N=20)

	Formulation	Initially	After 1 month	After 3 months
A	Mean (SD)	59.3 (7.9)	65.2 (8.2)	70.6 (8.4)
	Range	44.3-81.0	50.2-81.2	51.3-85.0
B	Mean (SD)	60.0 (7.5)	67.2 (8.0)	71.2 (8.6)
	Range	45.4-80.8	46.4-81.6	52.4-86.0

Table III
Changes in VAS scores (cm) after administration of conjugated RP (A) and RA (B) creams for 3 months (N=20)

Formulation		After 1 month	After 3 months
A	Mean (SD)	1.8 (1.2)	6.3 (1.6)
	Range	0.0-3.5	1.0-8.3
B	Mean (SD)	1.9 (1.5)	6.5 (1.8)
	Range	0.3-3.4	1.2-8.6

Tolerability

At each visit subjects were asked if they had had any problems and/or side-effects with the treatments they had used.

Seven of the subjects reported skin soreness after using the RA formulations and 2 of these also reported a considerable redness in the skin 1/2-1 hour after application. The reported side-effects are, however, transient and none of the subjects stopped the treatment due to the side-effects. One subject reported redness of the skin following administration of the conjugated RP formulation.

The tolerability was significantly in favour of formulation A, the conjugated RP formulation. This is also revealed in the preference choice from the users (see below).

Preference choise from the subjects

On completion, subjects were asked to give a preference for one of the two creams they had used taking into account the effect, the tolerability and the cosmetic properties of the creams.

Thirteen subjects prefered the conjugated RP cream, formulation A, while 4 prefered the RA cream, and 3 subjects had no preference. The difference in preference is significantly in favour of the conjugated RP formulation.

DISCUSSION

In recent years knowledge of the effect and metabolism of vitamin A derivatives in the human skin (15,16) has been accumulating.

The results from the present study indicate that both, the 0.025% RA cream and a 0.2% conjugated RP cream bring about a comparable effect in skin composition and morphometry. No significant differences could be detected either in skin thickness or elasticity improvement between the two creams. As the tolerability of the conjugated RP is significantly better than the RA, the conjugated RP may represent a valuable alternative to RA.

As have been stressed by other investigators (3,4) it is of utmost importance that the RP is in a form allowing skin penetration. In a previous comparative study we reported a significant difference between conjugated and non-conjugated RP in the effect on the skin parameters (1). The change in solubility of the RP is probably essential for making the ester available in sufficient concentration.

To our knowledge no percutaneous absorption and metabolism studies have been carried out with conjugated RP. Such studies are needed in order to explain the mechanism of action behind the effect seen in skin parameters measured. The minimal conversion of retinol to RA may not be relevant to the application of formulations containing RP to the human skin.

Both retinol and RA can have pharmacological effects on human skin. Retinol is about 50% as potent as RA in inducing epidermal hyperplasia in the hairless mouse (11).

It has, however, been shown that retinol is formed in hairless guinea pig and human skin after topical application of RP, but if further metabolism to R occurs, it is to small to detect. Any biological response of skin treated with retinyl palmitate formulations may be due to ester hydrolysis of the parent compound to retinol. The use of RP in cosmetic formulations may result in a significantly improved delivery of retinol into the skin.

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Author Address:

Erling Thom, Ph.D.
P.O. Box 210
N-2001, Lillestrøm, Norway
*Fax: (+47)*63.81.92.50