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AN INNOVATIVE COSMECEUTICAL WITH SKIN WHITENING ACTIVITY: NOTE I

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Summary

Hyperpigmentation is a skin disturbance affecting many people all over the world.
Among the different bleaching cosmetic products, the most commonly used active ingredients are hydroquinone, azelaic acid, kojic acid, ellagic acid, rucinol, arbutin and different vitamin C derivatives.
In fact, vitamin C is widely known to have a suppressing effect on melanic pigmentation, but because of its easy decomposition, a variety of stabilized vitamin C derivatives have been developed and commercialized.
The main problem of these derivatives is their difficulty to deliver the stratum corneum (SC) for acting specifically on functioning melanocytes with active synthesis of melanin.
The aim of this study was to control the combined activity of arbutin extract, hexyl-decanoyl-ascorbic acid (VC - IP) and magnesium l-ascorbyl-2-phosphate (VC - PMG), to suppress melanic pigmentation (product A).
At the same time, we wanted to control the depigmenting activity and the product stability of the l-ascorbic-acid, included in a kojic-based cosmetic formulation utilizing a new two-chamber dispenser (SYMBIO), which allows to keep vitamin C separately from the other ingredients (product B).
Skin absorption-potential through the skin of the cosmetic vehicles and active ingredients were controlled by the dansyl-chloride methodology, stripping the SC at different levels.
Clinical evaluation of the obtained lightening effect was performed on 40 randomized female volunteers over a period of 3 months by the clinical score and the Minolta Chromameter CR 200 methods.
The topical application of both the products (A and B) was effective in lightening the skin of the majority of the treated patients, showing to have a remarkable penetrability degree and a mean reduction of the skin hyperpigmentation from 30% to 45% ($p<0.05$) from baseline in the active groups, and from 5% to 15% ($p<0,05$) in the placebo group in perfect agreement with the results obtained by the use of both the Chromameter and the score methodology.

L-ascorbic acid-based formulation was superior of about 20% ($p<0.05$) to VC-PMG-based in restoring to normal the hyperpigmentation skin disorders, such as chloasma and senile freckles.
Both the formulations were well tolerated during the study term.

Riassunto

L'iperpigmentazione rappresenta un'anomalia cutanea che colpisce molte persone in tutto il mondo.
Tra i principi attivi inseriti nelle creme cosmetiche depigmentanti, i più noti ed utilizzati sono l'idrochinone, l'acido azelaico, l'acido cogico, l'acido ellagico, il rucinolo, l'arbutina e diversi derivati della vitamina C.
Infatti, è risaputo come la vitamina C, o acido l-ascorbico, svolga un effetto "sbiancante" nei confronti della pigmentazione melanica, ma, data la sua estrema instabilità chimica, vengono di solito utilizzati i suoi derivati.
Il maggiore problema dei derivati è la difficoltà di penetrazione per permettere loro di raggiungere i melanociti ed agire sulla produzione di melanina.
Scopo di questo studio è stato quello di verificare l'attività svolta dall'azione combinata dell'estratto di arbutina, del magnesio-ascorbil fosfato (VC-PMG) e dell'esil decanoil ascorbato (VC - IP) quali depigmentanti cutanei (prodotto A).
Nello stesso tempo si è voluta controllare sia la stabilità nel tempo che l'azione svolta dalla vitamina C associata con una base cosmetica contenente acido cogico (prodotto B).
Per evitare i noti problemi di incompatibilità tra i due principi attivi, legati alla loro instabilità chimica, è stato utilizzato uno speciale contenitore, il SYMBIO, che permette alla vitamina C di essere fisicamente separata, fino all'erogazione del prodotto, sia dal cogico che dall'ossigeno che la ossiderebbe in tempi molto brevi.
La penetrazione cutanea del prodotto cosmetico, è stata verificata mediante la metodologia dello stripping-test e l'utilizzazione del cloruro di dansile come marker.
La valutazione dell'effetto depigmentante è stata valutata sia clinicamente, mediante punteggio, che con l'uso del Chromameter CR® 200 su un gruppo randomizzato di 40 donne volontatrie, per un periodo di tre mesi.
Entrambi i prodotti A e B, hanno dimostrato di possedere un buon potere depigmentante riducendo l'intensità del colore bruno dal 30% al 45 % circa ($p<0.05$) rispetto ai valori di partenza.
La formulazione a base di vitamia C si è dimostrata più attiva di circa il 20% ($p<00.5$) rispetto a quella basata sull'uso del derivato VC - PMG.
Entrambe le formulazioni hanno rivelato un buon potere di penetrabilità ed un'ottima tollerabilità cutanea.

INTRODUCTION

Hyperpigmentation is a skin disturbance affecting many people all over the world (1). The problem of bleaching the pigmentation is not easy to solve.
Among the different treatments, there is the prevalence in using cosmetic products containing bleaching agents. Among them, the most commonly used are hydroquinone, azelaic acid, kojic acid, arbutin, ellagic acid, rucinol and different vitamin C derivatives (2-8).
In fact, vitamin C is widely known to have a suppressing effect on melanic pigmentation, but because of its fast decomposition, a variety of stabilized vitamin C derivatives have been developed and commercialized.
The main problem of these derivatives is their difficulty to deliver the stratum corneum for acting specifically on functioning melanocytes with active synthesis of melanin.

AIM

The aim of this study was to control the combined activity of arbutin extract and magnesium l-ascorbyl-2-phosphate (VC - PMG) and hexyldecanoyl-l-ascorbic acid (VC-IP), to suppress melanic pigmentation (Product A).
As it is known VC-PMG is a stable compound soluble in water and easily hydrolyzed to l-ascorbic acid (ASC) by skin phosphatase (9). Moreover, VC-IP as lipophilic compound of ascorbic acid rapidly uptaken in the cell, may represent a long life ASC enrichment (10).
At the same time we wanted to control the depigmenting activity and the product stability of the l-ascorbic-acid, included in a kojic-based cosmetic formulation utilizing a new two-chamber dispenser (SYMBIO®), which allows to keep vitamin C separately from the other ingredients (Product B) (11).
As a matter of fact, it's not possible to mix in the same composition l-ascorbic acid and kojic acid because their stability is linked to completely different pH.
Moreover, l-ascorbic acid tends to oxidize very easily if included in normal cosmetic vehicles, even if they are kept at very low pH.

MATERIAL AND METHODS

Cosmetic Preparation

To control the depigmenting efficacy of the Product A, based on arbutin extract (2% w/w), magnesium l-ascorbyl-2-phosphate and hexyldecanoyl-l-ascorbic acid (w/w), a cosmetic preparation was formulated using a gel based on hydrophobically modified hydrophilic polymers.
For the Product B was used a two-chamber dispenser (SYMBIO®). The smaller chamber airfree was filled up with a b-carotene protected emulsion of l-ascorbic acid (content 50 % w/w) which equals to 5% of the entire emulsion.
The second chamber, represented by the traditional container, was filled up with the same emulsion based on hydrophobically modified hydrophilic polymers, and containing kojic-acid (2% w/w) as secondary depigmenting agent. Both the chambers operated by a single actuator only (Fig. 1), according to Edens et al. (11).

Patients Enrolment

40 female volunteers, aged between 32 and 47, were selected from outpatient at two dermatological in-offices.
Eclusion criteria, included the use of topical AHAs, topical or systemic antibiotics and /or retinoids, irritants or hormonal treatments for 4 weeks, preceded the studies.
Known or suspected hypersensitivity to the used chemicals, pregnancy or lactation and the use of oral contraceptives were more reasons for exclusion. All the patients were informed concerning the purpose and the possible consequences of the study, according to the informed consent

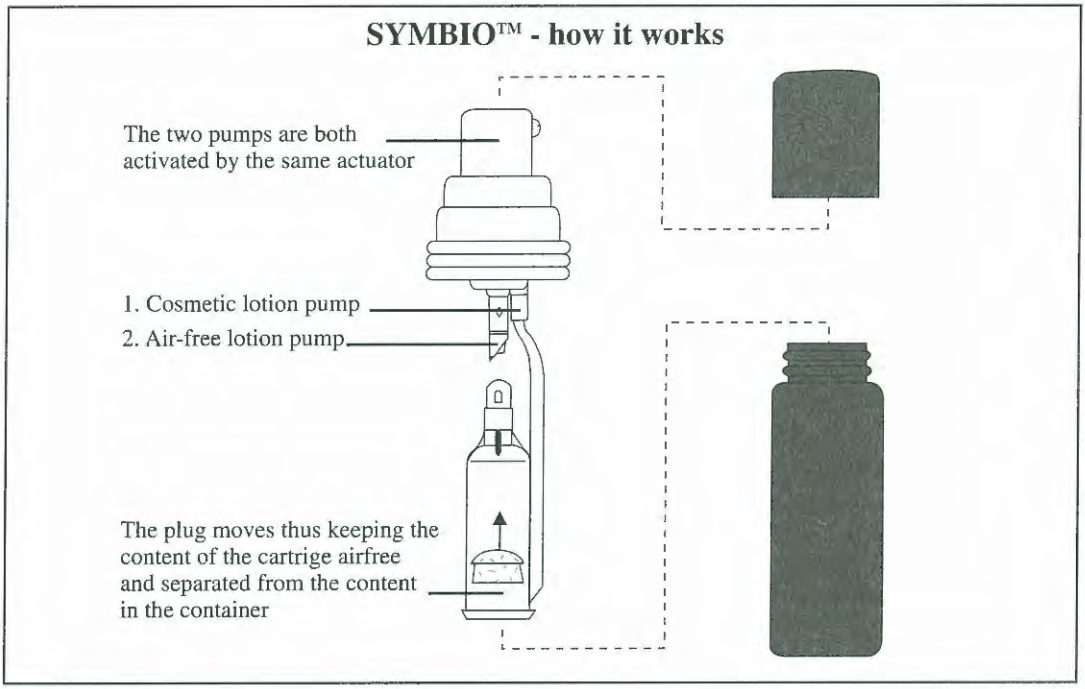

Fig. 1 - SYMBIO™

guidelines, and included in the study only after signing the consent form.

Whitening Effects

It was performed a double-blind comparison study in which it was evaluated the treatment effects of Product-A and Product-B to bleach melanic hyperpigmentation of all the volunteers with ephelides, chloasma and senile freckles. Volunteers were randomly divided into four groups of 10 individuals:

GROUP 1	CREAM A
GROUP 2	CREAM B
GROUP 3	VEHICLE A
GROUP 4	VEHICLE B

After the baseline evaluation, all the volunteers applied the given cream twice a day for a three month-period on the whole face, after cleansing with a dermatological lotion (MAVIGEN® LATTE). The effectiveness of the total bleaching of the pigmentation was monthly judged by two distinct methods: clinical score (0-10) controlled by an expert dermatologist, and color analysis of the hyperpigmentations controlled on the entire face and by the Chromameter R (Minolta CR-200, Tokyo Japan), according to Kameyama et al. (12).

If the brightness index number of color-difference meter increased more than 3.0, it was defined as effective, an increase of 2.0 to 3.0 was defined as slightly effective; an increase less than 1.0 was defined as not effective.

The correlation between the results obtained for the whitening activity from the two methods has been analyzed also.

The obtained results are reported on Table I, Table II and Fig.2.

Skin Absorption-Potential

To control the absorption-potential through the

Tab. I

WHITENING ACTIVITY OF I-ASCORBIC ACID AND I-ASCORBYL-2-PHOSPHATE COMPARED TO THEIR OWN VEHICLES (3 MONTHS PERIOD)

n= 40 t= 22°C RH= 50%

DISEASE	N° OF CASES	APPLIED PRODUCT	EFFECTIVE	FAIRLY EFFECTIVE	SLIGHTLY EFFECTIVE	NOT EFFECTIVE
CLOASMA	4	PRODUCT A ASCORBYL Ph	2	2	0	0
	4	VEHICLE A	0	0	1	3
	4	PRODUCT B ASCORBIC Ac.	3	1	0	0
	4	VEHICLE B	0	0	0	4
SENILE FRENKLES	4	PRODUCT A ASCORBYL Ph	2	2	0	0
	4	VEHICLE A	0	0	0	4
	4	PRODUCT B ASCORBIC Ac.	3	1		0
	4	VEHICLE B	0	0	0	4
EPHELIDIES	2	PRODUCT A ASCORBYL Ph	0	0	2	0
	2	VEHICLE A	0	0	0	2
	2	PRODUCT B ASCORBIC Ac.	2	0	0	0
	2	VEHICLE B	0	0	0	2

Tab. II

WHITENING EFFICACY OF I-ASCORBIC ACID AND I-ASCORBYL-2-PHOSPHATE COMPARED TO THEIR OWN VEHICLES (3 MONTHS PERIOD)

n= 40 t= 22°C RH= 50%

DISEASE	N° OF CASES	PRODUCT B I-ASCORBIC ACID	PRODUCT B I-ASCORBIC ACID	VEHICLE A	VEHICLE B
CHLOASMA	16	4	4	4	4
SENILE FRECKLES	16	4	4	4	4
EPHELIDES	8	2	2	2	2
TOTAL	40	10	10	10	10
GLOBAL OBTAINED RESULTS					
EFFECTIVE		4	8	0	0
FAIRLY EFFECTIVE		4	2	0	0
SLIGHTLY EFFECTIVE		2	0	1	0
NOT EFFECTIVE		0	0	9	10

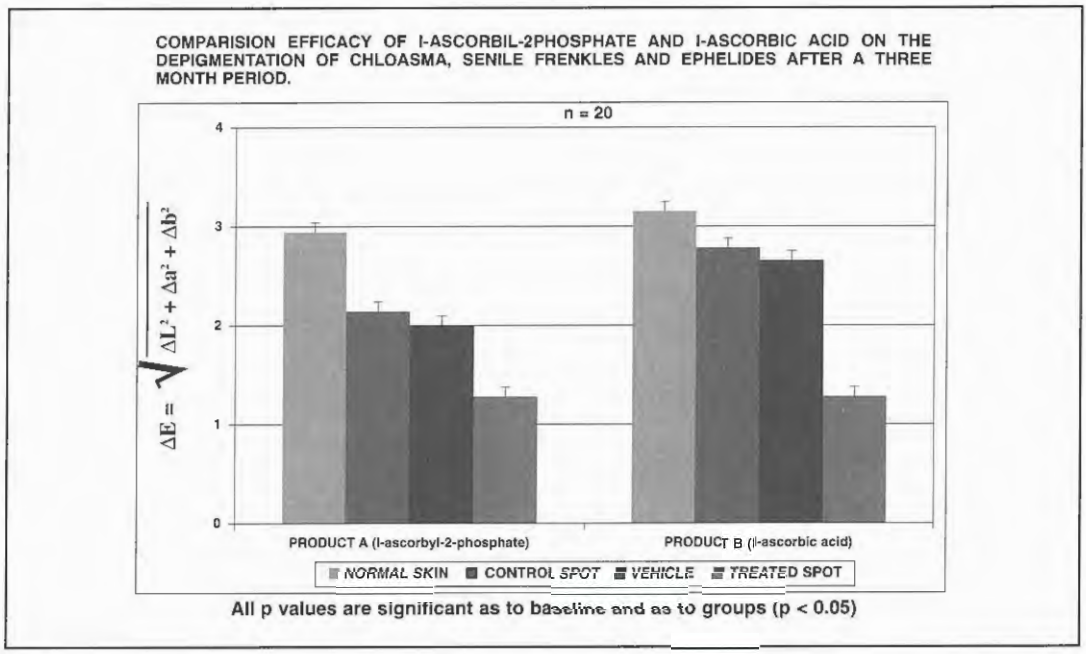

Fig.2

skin of the two whitening creams used (A and B) in comparison with their own vehicles, the dansyl chloride labelling technique was used, according to Ridge et al. (13)

Prior to treatment, the stratum corneum was labelled. According to our studies (14-15) a 5% of dansyl chloride finely triturated was added into the four formulations (Product A, Product B, Vehicle A and Vehicle B) and applied to right or left volunteers' volar forearm under semi-occlusive dressing for 24 hours. Subsequently removed with soft tissue paper, weighed any excess material and cleansed the area with the cleansing lotion (MAVIGEN® LATTE), the surface layers of the epidermis were removed by stripping with 15 successive strips of an adhesive tape (Sellotape®). By practice an expert technician may obtain successive layers of the stratum corneum , each just one cell thick.

On all the obtained SC- layers, the level of fluorescence was controlled by UV illumination, using an arbitrary scale of 0-8 (14), and the level of ascorbate was detected by high-performance liquid chromatography (HPLC), according to Darr et al. (16)

The obtained results are reported on Fig. 3.

Statistical Analysis

A two-tailed student's t test for paired series was used to analyze the differences between the values obtained before the treatment and after 1,2 and 3 months of treatment (clinical scores and chromameter analysis).

The differences were considered significant when $p<0.05$. The correlation coefficient r and its threshold of significance were calculated by linear regression analysis, using the means of the values obtained for all the subjects at each control visit, in order to determine the correlation between the results recorded by the scoring method, and those obtained by the Chromameter® analysis (17-18).

RESULTS AND DISCUSSION

Clinical Efficacy

The clinical whitening efficacy of both the products is fairly evident on Tables I-II and in Fig. 2.
As it is clearly observable, both l-ascorbic acid-based product and l-ascorbyl-phosphate based product showed to have high efficacy on chloasma, on senile freckles and on ephelides (Tab. 1). But if we compare tab. I to tab. II, we can observe how the efficacy of the l-ascorbic acid based product seems to be higher. That is more clear in Fig. 2, from which we can see how ascorbic acid-based product is more effective of about 20% ($p<0.05$).
All the obtained results appeared significantly higher ($p<0.05$) if compared both to the starting values and to the vehicles' activity.
Moreover, a significant correlation exists between the means of the clinical obtained scores for the whitening activity, and the means of the corresponding values obtained by the Chromameter® : 2= -0.95, p= 0.02.

Percutaneous Absorption

The control by electron microscopy of the different skin layers obtained by using Sellotape® applied 15 times, and the residual fluorescence control allowed us to verify the percutaneous absorption degree obtained by using the two different cosmetic vehicles (A and B). Both the vehicles and the active creams showed to have a remarkable penetrability degree, verified by the residual flourescence found on the epidermis after having stripped completely the horny layer.

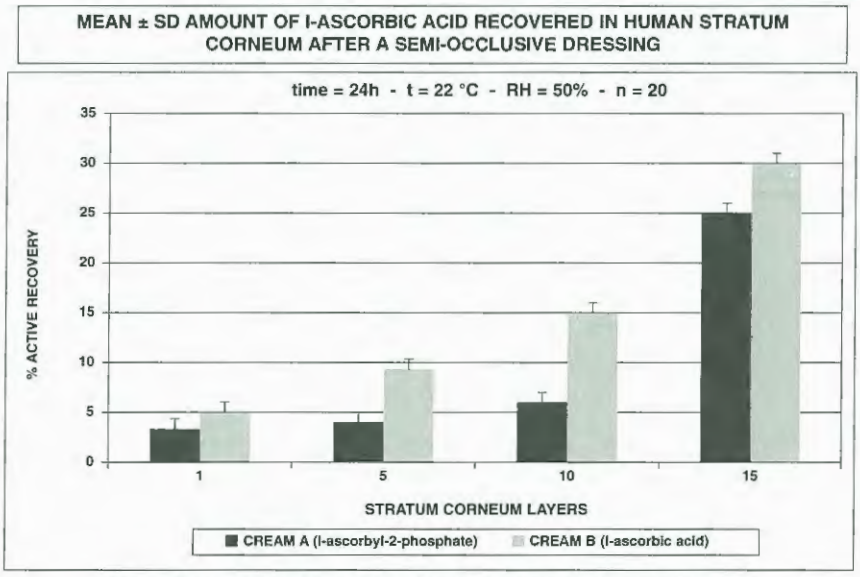

Fig.3

In fact, we found out through the biopsy how the fluorescence appears not only on the different skin layers but on the entire epidermis also.
Concerning ascorbic acid used as it is (Product B) or ascorbic acid linked to hydrophilic and lipophilic groups (Product A), its determination on the different skin layers (Fig. 3) give us the opportunity to demonstrate how the first one has a penetrability higher then the second, as already proved by other authors (19). As a matter of fact, 30% of l-ascorbic acid (Product B) has been found at the level of the granulous layer after a unique application and in a time of just 24 hours.
Moreover, vitamin C found in the residual product recorded on the soft tissue paper, resulted to be about 5% of the total quantity of l-ascorbic used, while the phosphate derived resulted to be about 10%.
Comparing the residual ascorbic acid with the one found at the different levels of the horny layers, we can desume how about its 60% seems to have reached the melanocytes after only a 24-hour-application. On the contrary, the absorption of the ASC compounds derived seems to be lower of about 20%.
From these considerations, the different activity demonstrated by the two different products seems to be clarified.

CONCLUSION

From these first data, we can assume that both l-ascorbic acid and magnesium-2-ascorbyl-phosphate and hyxyldecanoyl-l-acorbic acid may be considered effective whitening agents for hyperpigmentary disorders in a three-month-therapy, but only if proper carriers are used. When well vehicled, l-ascorbic acid alone seems to have a higher and quick efficacy. But to mavintain "active" the l-ascorbic acid, it is necessary to use protected cartridges in order to avoid a fast oxidative degradation, as we have shown already in a previous work (11).
Finally, what is interesting to underline is that VC-IP, being rapidly uptaken but slowly released as free l-ascorbic acid, may be considered a reposary agent with slow whitening activity.
Moreover, combining quick releasing compounds, as VC-PMG, with slow releasing ones, as VC-IP, it seems possible to ameliorate the whitening activity of cosmetic products specifically studied for the cutaneous hyperpigmentations

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