Combination of antioxidants, plant enhancer VDR synthesis and NB-UVB therapy for vitiligo skin care

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INTRODUCTION

Vitiligo is a skin condition occurring in all human races, affecting 1-2% of the general population, regardless of sex. It may occur in people of all ages and can be divided into two types of manifestation:

- congenital hypopigmentation (albinism), which is genetically conditioned,
- acquired vitiligo, which is caused by such external (environmental) factors (psychological, hormonal and physical stress).

Vitiligo is a systemic, idiopathic autoimmune disease. It manifests itself as numerous patches of depigmented skin which vary in size. One of the causes of the formation of vitiligo patches is the loss of melanocytes (skin cells producing the pigment melanin), which causes depigmentation and leads to the development of irregular hypopigmented areas of skin. These may form on the skin of the face and body. They are most often found on elbows, knees, wrists, as well as fingers and the back of hands. Dysfunction or damages in skin cells mitochondria (e.g. by free radicals formation) could be also one of the reason of vitiligo disease development.

Vitiligo as a pigmentation disorders characterized by loss and/or dysfunction of epidermal melanocytes is a psychologically devastating disease with a significant impact on life quality, particularly in female patients. People with vitiligo struggling with lack of effective therapies. There is no cure and existing therapies often yield suboptimal results. It's due to a problem of incomplete understanding of the disease ethology. However proper and long-lasting skin care with certain cosmetic products can improve skin condition and raise quality of life and most of all effectively support medical treatment of vitiligo.

AIM OF THE STUDY

Here we demonstrate an efficacy of cosmetic product containing mixture of antioxidants (thioproline, rosmaric acid, naryngenin) and plant extract stimulating VDR synthesis in combination with NB-UVB therapy for vitiligo skin.

MATERIALS AND METHODS

DPPH radical scavenging method

The antioxidant activity of the tested compounds was determined using the 2,2-diphenyl-1- picrylhydrazyl (DPPH·) radical scavenging method. The examination of reactivity with DPPH is very useful for providing basic information on the scavenging ability and structure-activity relationships of formulas. The reaction of compounds with DPPH was measured by Electron Paramagnetic Spectroscopy. EPR spectra of reaction mixture were measured after 15min. A DPPH-scavenging ability unit was recalculated as the amount of scavenged DPPH [mg] per 1ml added substance.

Clinical trials

Tested product (O/W emulsion) contained tioproline, rosmaric acid, naryngenin and *Cichorium intybus* plant extract as an active ingredients. INCI: Aqua, (Water), Glycerin, Sodium Polyacrylate, Dimethicone, Prunus Persica (Peach) Kernel Oil, Dimethicone/Vinyl Dimethicone Crosspolymer, Cyclopentasiloxane, Hydroxyacetophenone, Trideceth-6, Butylene Glycol, **Naringenin**, Silica, PEG/PPG-18/18 Dimethicone, **Cichorium Intybus (Chicory) Root Extract**, Ethylhexylglycerin, **Timonacic**, Pentylene Glycol, Dihydroxyacetone, Carbomer, Lecithin, Polysorbate 20, Atelocollagen, Helianthus Annuus (Sunflower) Seed Oil, **Rosmarinus Officinalis (Rosemary) Leaf Extract**, Hydroxyethylcellulose, Sodium Chondroitin Sulfate, Phenoxyethanol, Methylparaben.
28 patients with vitiligo were enrolled for the trials. The patient's age were: 10 - 72 (av. 39 years), 34 females i 4 males, in different phototype (mostly II according Fitzpatric). They use emulsion every evening for a period of 3 months. Each patient was used: NB-UVB therapy only or cream only or combination of NB-UVB + cream in three different lesions for 3 months. Researchers, in three independent dermatological clinics, were estimating the localisation and area of vitiligo lesion in %. They also measured the size of patches in *mm*.
The VASI score was estimated and clinical evaluation of skin condition in 10-point analogues scale was assessed (skin elasticity and dryness), as well as patient's self-evaluation.

NB-UVB therapy tretament proccess

Starting dose	Continuation of treatment	Comments
70% MED (minimal erythema dose) **200 mJ/cm² (I-II phototype)** **300 mJ/cm² (III-IV phototype)**	Increased by 100 mJ/cm² (or by 20-30%) every 2 weeks (the appearance of painless erythema dose radiation should not be increased, painful erythema necessitates discontinuation of therapy until symptoms disappear, re-treatment should be started at max. 50% of the last dose)	The recommended frequency radiation: 3 times a week (permissible: 2-4 times a week) In the case of irradiation need to three visits per week, giving 36 irradiation within 3 months of study.

RESULTS AND DISCUSSION

- Vitiligo melanocytes produced many biologically active proteins among the senescence-associated secretory phenotype (SAPS), such as **interleukin-6 (IL-6)**, matrix metallo proteinase-3 (MMP3), cyclooxygenase-2 (Cox-2), insulin-like growth actor-binding protein-3 and 7 (IGFBP3, IGFBP7), *(Bellei B et all. Vitiligo: a possible model of degenerative diseases. PLoS One. 2013;8(3):e59782).* The expression of mRNA for tumor necrosis factor (TNF)-alpha and **interleukin-6 (IL-6)**, two pro-inflammatory cytokines with an inhibitory effect on pigmentation, was increased in the epidermis from vitiligo biopsies, whereas their expression was practically undetectable in the skin of control subjects (*Moretti et all. Keratinocyte dysfunction in vitiligo epidermis: cytokine microenvironment and correlation to keratinocyte apoptosis. Histol Histopathol. 2009 Jul;24(7):849-57).* Our study showed that the complex consisting of two molecules: rosmaric acid and thioproline **synergistically inhibits IL-6 production** (according to Kull's equation), (Fig. 1, 2). The strongest effect was observed for combination of 7mM thioproline and 60 mg/l rosmaric acid (*M Pasikowska, R Dębowska, B Marczak, I Eris. Protective role od anti-inflammatory complex on humen skin cells. Journal of Investigative Dermatology, 133: S31-S31, 2013/5/1).* Tested complex had also good DPPH scavenging properties (*Dębowska R, Pasikowska M, Bazela K, Zawada K, Tyszczuk B and Eris I. Protective effect of tioproline and its enhancement by other active ingredients on UV-induced skin cells damage. Journal of Investigative Dermatology, 133: S1-S311, 2013).*

- We suggested that the melanocytes in patients with vitiligo could be in a dysfunctional state and the formation of free radicals cause cellular and **mitochondrial toxicity**. These free radicals could be removed by the antioxidants and **mitochondrial stimulating agents** present in the emulsion, turning the melanocytes functional and starting the production of melanin in the area of the vitiligo (*Rojas-Urdaneta JE, Poleo-Romero AG, Evaluation of an antioxidant and mitochondria-stimulating cream formula on the skin of patients with stable common vitiligo. Invest Clin. 2007 Mar;48(1):21-31).* Naryngenin included in tested emulsion had a **regulatory effect in mitochondria** membrane potential after UVA, reduced ROS and oxidative skin protein damages after UVA, induced of skin antioxidant defence system (MnSOD), protected mitochondrial proteins against UVA and had **regulatory effect on mitochondrial biogenesis** after UVA (details published at ESDR congress, 2016). Moreover it's also **inhibited IL-6 production**. Final emulsion had very high DPPH scavenging properties (Table 1).

- The VDR-mRNA expression was significantly decreased in lesional and nonlesional skin of vitiligo patients compared to controls (*Doss et al.: Vitamin D Receptor Expression in Vitiligo. Indian J Dermatol. 2015 Nov-Dec;60(6):544-8). Cichorium Intybus* (Chicory) root extract **stimulated VDR synthesis** by 32%, an effect similar to that produced by calciriol, the active form of vitamin D (data obtained by Silab).
- Clinical test with vitiligo patients (3 months, every evening emulsion application) showed that **53% of patients responded to NB-UVB + emulsion treatment**, manifested in repigmentation up to 70% (aver. 30%) or erythema, 9% responded to cream application and 18% for NB-UVB treatment (Table 2). Skin elasticity raised by 16% and skin dryness decreased by 24% (dermatologists analogues scale). In self-evaluation (Fig. 3) emulsion moisturized skin (100%) and restored its comfort (81%).

Table 1. DPPH scavenging properties of emulsion containing mixture of antioxidants (thioproline, rosmaric acid, naryngenin) and plant extract stimulating VDR synthesis .

Emulsion	RPF (10^{21} DPPH/1g emulsion)
1671	**4,19**

Table 2. Results of clinical trials

Patient assignment	NB-UVB	Emulsion	Emulsion + NB-UVB
	Number of patients with skin repigmentation after 3 months of test		
Summary	5 out of 18 patients using light therapy (28% of test subjects) Average degree of repigmentation 11%	3 out of 28 patients using the emulsion (11% of test subjects) Average degree of repigmentation 12.5%	**15 out of 28 patients using the emulsion in combination with light therapy** (53% of test subjects) Average degree of repigmentation 30%

Figure 1. Secretion of IL-6 and viability of cells incubated in presence of 5mM TioP and various concentrations of RA (15, 25, 40, 60 mg/l), measured with ELISA test and MTT assay respectively, after exposure to UVB

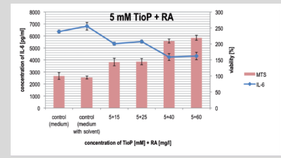


Figure 2. Secretion of IL-6 and viability of cells incubated in presence of 7mM TioP and various concentrations of RA (15, 25, 40, 60 mg/l), measured with ELISA test and MTT assay respectively, after exposure to UVB..

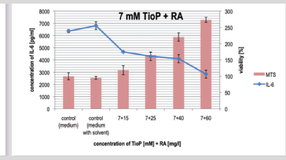
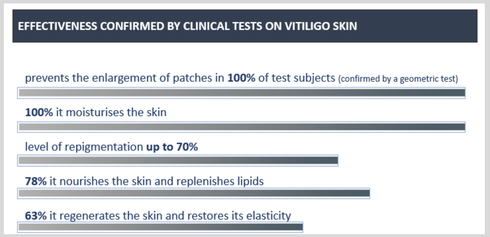


Figure 3. Clinical assessment and self-evaluation od tested emulsion



CONCLUSIONS

Rosmaric acid, tioproline as well as naryngenin revealed synergetic anti-inflammatory activity towards IL-6, which can be immunological factor of disease intensity. Cosmetic emulsion containing mixture of antioxidants and plant extract stimulating VDR synthesis can effectively restore vitiliginous skin condition and what is more enhance medical treatment with NB-UVB therapy. We suggested that the melanocytes in patients with vitiligo could be in a dysfunctional state and the formation of free radicals cause cellular and mitochondrial toxicity. These free radicals could be removed by the antioxidant and mitochondrial stimulating elements present in the emulsion, turning the melanocytes functional and producing melanin in the vitiligo area.

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