

Original Articles

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Nutraceutical compound increases collagen, elastin and hyaluronic acid synthesis

Composto nutracêutico aumenta a síntese de colágeno, elastina e ácido hialurônico

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ABSTRACT

Introduction: The use of nutraceutical products in order to attenuate signs of skin aging has been proposed in the literature. The stimulus to the synthesis of substances that support the dermis is one of the mechanisms linked to this effect.

Objective: To evaluate the effectiveness of a nutraceutical compound containing lutein, lycopene, vitamin C and manganese for the synthesis of collagen, elastin and hyaluronic acid.

Methods: An in vitro study was carried out based on the culture of human fibroblasts treated with the investigated product in different non-cytotoxic concentrations. The quantification of the elastin and collagen was performed with the assistance of spectrophotometric measurements. Hyaluronic acid was measured using an immunoenzymatic method.

Results: Cell cultures treated with the different concentrations of the product showed a significantly higher amount of synthesized collagen, elastin and hyaluronic acid as compared to the untreated culture ($p < 0.05$).

Conclusions: The use of the nutraceutical compound containing lycopene, vitamin C, lutein and manganese has shown in vitro efficacy for stimulating the synthesis of collagen, elastin and hyaluronic acid, components that are crucial for providing the dermis' supporting structure, being responsible for the skin's firmness and elasticity.

Keywords: Dermatology; Dietary Supplements; Skin Aging

RESUMO

Introdução: O uso de produtos nutracêuticos com a finalidade de atenuar os sinais do envelhecimento da pele tem sido proposto na literatura. O estímulo à síntese de elementos de sustentação da derme é um dos mecanismos relacionados a esse efeito.

Objetivo: Avaliar a eficácia de um nutracêutico contendo luteína, licopeno, vitamina C e manganês na síntese de colágeno, elastina e ácido hialurônico.

Métodos: Estudo realizado in vitro, através de cultura de fibroblastos humanos tratadas com o produto investigado em diferentes concentrações não citotóxicas. A quantificação de elastina e colágeno foi determinada através de medidas espectrofotométricas enquanto que a de ácido hialurônico foi determinada por método imunoenzimático.

Resultados: As culturas celulares tratadas com as diferentes concentrações do produto apresentaram quantidade sintetizada de colágeno, elastina e ácido hialurônico significativamente maior quando comparadas com a cultura não tratada ($p < 0,05$).

Conclusões: O uso de nutracêutico contendo licopeno, vitamina C, luteína e manganês demonstrou eficácia *in vitro* no estímulo à síntese de colágeno, elastina e ácido hialurônico, elementos essenciais na estrutura de sustentação da derme e responsáveis pelas características de firmeza e elasticidade da pele.

Palavras-chave: dermatologia; suplementos dietéticos; envelhecimento da pele

INTRODUCTION

The interest of dermatology for oral nutrients supplementation aimed at controlling the cutaneous aging process has been increasing substantially.

Modern life, especially in large urban centers, imposes a condition that favors inadequate ingestion of nutrients, with an impact on the skin's and adnexa's health.¹ Excessive work, stressful routines, inadequate sleeping periods, and diets based on industrialized foods with high levels of carbohydrates and fats, as well as reduced content of vitamins and trace elements, are conditions that lead to the development of a picture described as "hidden hunger", meaning a borderline deficiency of certain nutrients, nevertheless without the clinical evidence of malnutrition.²

In the process of skin aging, in addition to the intrinsic cellular functional decline that is common to all organs, there are extrinsic factors, such as ultraviolet radiation, which can intensify the aging process via a complex biological mechanism that affects the various skin layers, is special the dermis' connective tissue.³ Alterations in the components of the extracellular matrix responsible for the supporting structure of the dermis⁴ – elastin, collagen and hyaluronic acid – induce the loss of viscoelasticity in the cutaneous tissue, with reduction of firmness and elasticity, clinically culminating with the emergence and accentuation of wrinkles, furrows and flaccidity.⁵

In the pathogenesis of photoaging, reactive oxygen species have a central role, consuming and damaging enzymatic and non-enzymatic antioxidant systems of the skin, destabilizing molecules and triggering chain reactions, causing damage to membranes and structural proteins.³ One of the primary events in reactive oxygen species (ROS) induced photodamage is the activation of transcription factors – such as the nuclear factor kappa B (NFkB) and the activator protein-1 (AP-1). These factors are involved in the regulation of the expression of several genes responsible for inflammation, tissue remodeling, oncogenesis, apoptosis and many degenerative processes associated with aging.^{6,7} Baseline levels of matrix metalloproteinases (Matrix Metalloproteinases – MMPs) are higher in aged skins as compared to younger skins, with the activation of AP-1 leading to an increase in the levels of MMPs, with greater collagen and elastin degradation.⁵

Recent evidence has shown that diets with a high content of vegetables, fruits and grains may reduce the risk of numerous diseases, with this benefit being linked to the presence of antioxidant substances.⁸

Antioxidants act on different levels protecting organisms against free radicals and are the first defense mechanism aimed at preventing their formation, particularly by inhibiting chain reactions with iron and copper.

In addition, antioxidants are capable to intercept free radicals generated by the cellular metabolism or exogenous sources, preventing their action on lipids, amino acids, polyunsaturated fatty acids' double bond, and DNA bases, avoiding cellular structural damage.⁹

Carotenoids and vitamin C stand out among nutrients with antioxidant action intended for the prevention and treatment of cutaneous aging. They are not synthesized by the body and should be acquired through diet or oral supplementation.¹⁰

Vitamin C is a powerful free radicals neutralizer. Its use, either topically or orally, has been proposed in programs aimed at mitigating the aging process.⁵

Previous studies have demonstrated the benefit of using carotenoids in the prevention and treatment of damages caused by sunlight and photoaging.¹⁰ Oral supplementation with lycopene and lutein has been evaluated within a program of aging prevention and treatment, with encouraging results.¹¹

In this way, the objective of the present study was to evaluate the efficacy of a nutraceutical product containing lycopene, lutein, vitamin C and manganese in the synthesis of the skin's structural supporting elements, using human fibroblast culture models.

METHODS

Ethical aspects

The experimental conditions adopted – the use of human cells under optimum cultivation conditions – are in line with the current methodologies applied, accepted and validated by the international scientific community. The human cell cultures used in the present study were commercially acquired from qualified and certified international companies.

Methodological procedures

Culture of human fibroblasts

Human fibroblasts (Clonetics, Cambrex / Lonza, USA) were commercially obtained, grown and expanded in culture medium containing 90% RPMI-1640 and 10% fetal bovine serum (GIBCO Life Technologies, Baltimore, USA), plus antibiotic solution 0.02 µg/mL gentamicin (Sigma Chemical St. Louis, USA) and 0.25 µg/mL amphotericin B (GIBCO Life Technologies, Baltimore, USA), having been seeded in 75cm² vials (Nunc, USA) and kept in a humidified incubator (Thermo Fisher) with 5% CO₂ atmosphere at 37 °C. The culture medium was changed every 48 hours up until the cells had 70-80% confluence, being subsequently trypsinized and counted in Neubauer's chamber for determination of cell density. After having been counted, the fibroblasts were established by the sowing of 1.5x10⁵ cells/well or 1x10⁴ cells/well in plates containing 6 or 96 wells (Nunc, USA), respectively.

For the determination of the non-cytotoxic concentrations of the nutraceutical compound, a preliminary cytotoxicity trial was performed using the XTT method (data not shown). Twenty-four hours after the initial seeding, the cell cultures were treated with three non-cytotoxic concentrations of the nutraceutical compound (0.065 mg/mL, 0.0325 mg/mL and 0.0163 mg/mL) for 72 hours. Subsequently, the culture's supernatant was collected for evaluation of the proposed parameters.

Evaluation of the collagen, elastin and hyaluronic acid

The extracellular matrix elements were measured in the fibroblast culture's supernatant using commercially available kits. The levels of collagen and elastin were determined by colorimetric trial (Biocolor, Belfast, Ireland), while hyaluronic acid levels were quantified by immunoenzymatic trial (ELISA sandwich) (R&D Systems, USA). The data obtained from the quantification of collagen, elastin and hyaluronic acid were expressed in pg/mL, mg/mL and ng/mL, respectively, and computed based on the standard curve's reference values.

Statistical analysis

For the characterization of the statistical data, a parametric method for analysis of variance (P) (ANOVA) was applied followed by a multiple comparison test, termed Dunnett. In all groups studied, those whose P values were less than 0.05 were considered statistically significant.

Studied product

The test product contains lycopene, vitamin C, manganese and lutein.

RESULTS

Evaluation of elastin synthesis

The nutraceutical product led to an increase in the synthesis of elastin in culture of human fibroblasts, incubated for 72 hours with the product, when applied at concentrations of 0.065, 0.0325 and 0.0163 (mg / mL), at 27%, 22% and 21%, respectively, when compared to the Basal Control group (Graph 1). The result was statistically significant ($p < 0.05$) at the concentration of 0.065 mg/mL.

Evaluation of collagen synthesis

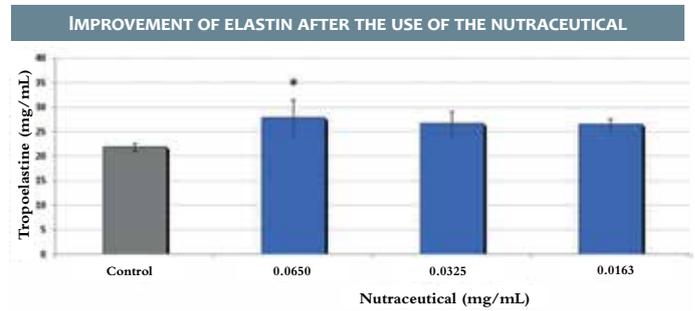
The nutraceutical product was capable to significantly increase ($p < 0.05$) collagen levels at concentrations of 0.0650, 0.0325 and 0.0163 (mg/mL), yielding an increase of 9.49, 9.75 and 10%, 12%, respectively, *vis a vis* the Basal Control, when applied in culture of human fibroblasts for a period of 72 hours (Graph 2).

Evaluation of hyaluronic acid synthesis

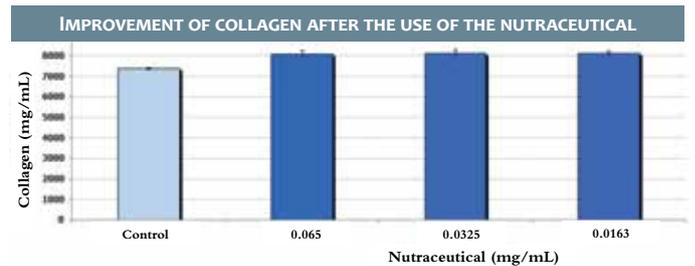
The nutraceutical product increased the concentration of hyaluronic acid in culture of human fibroblasts, incubated for 72 hours with the product, when applied at concentrations of 0.065, 0.0325 and 0.0163 (mg/mL), at 53%, 29% and 11%, respectively, when compared with the Basal Control group. The result was statistically significant ($p < 0.05$) for the 0.065 and 0.0325 mg/mL concentrations. (Graph 3)

DISCUSSION

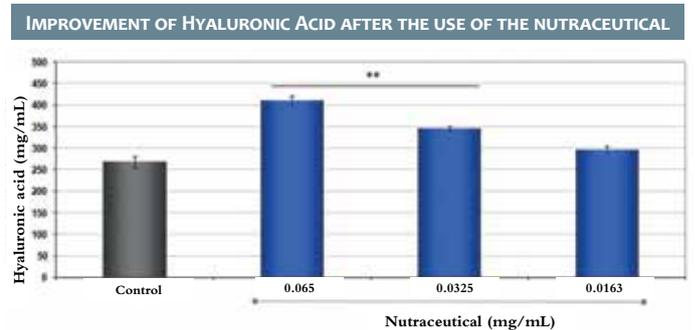
The increase in life expectancy has raised concerns about the development of preventive and restorative measures for the aging process.¹⁷ Measures already recognized as effective for the prevention and treatment of cutaneous aging include the adequate use of sunscreens¹⁸ and cosmetic products with ther-



GRAPH 1: Results regarding the quantification of elastin in the control fibroblast culture (untreated) and in that treated with the nutraceutical product in different concentrations



GRAPH 2: Results regarding the quantification of collagen in the control fibroblast culture (untreated) and in that treated with the nutraceutical product in different concentrations



GRAPH 3: Results regarding the quantification of hyaluronic acid in the control fibroblast culture (untreated) and in that treated with the nutraceutical product in different concentrations

apeutic action (cosmeceuticals)^{10,19} acting primarily through antioxidant and hydration.

More recently, evidence of the nutritional impact on the skin has been presented.⁴

A previous study¹⁹ evaluated the clinical efficacy of an oral supplement based on marine protein, concentrated acerola, concentrated grape seed extract, and tomato extract. After 360 days of use, the volunteers treated with the product had clinical evidence of improvement in the signs related to skin aging. These signs included the improvement of wrinkles, solar melanoses, hydration, lushness and improvement of the general ap-

pearance of the skin, associated to the ultrasonic improvement in the density of both photoprotected and photoexposed skin.

Among the agents studied, vitamin C and carotenoids – in particular lycopene and lutein – have been linked, especially in associations, to benefits for improving the quality of the dermis' supporting structure.^{9,10} In 2006, Heinrich et al.¹¹ evaluated the use of oral supplementation with carotenoids containing lycopene and lutein, demonstrating improvement in clinical parameters such as density, skin thickness, roughness and scaling. The mechanism by which this action takes place is not yet completely established, however it is believed to involve these elements' strong antioxidant properties, reducing the oxidative stress and its destructive effects on cellular structures, such as fibroblasts.⁵ Nevertheless, the effects of the association of vitamin C, lycopene, lutein and manganese assessed in the present study seems not to be restricted to the inhibition of the degradation of ROS-induced collagen and elastin. The results showed that there was a significant increase in the synthesis of collagen, elastin and hyaluronic acid, three essential components of the extracellular matrix that are responsible for the dermis' supporting structure.

The present study's findings may derive from the associated effects of lutein and lycopene, which are important agents with anti-inflammatory and antioxidant action;¹² of manganese, a necessary element for optimal activity of the enzymes involved in the synthesis of glycosaminoglycans,¹³ and vitamin C, a cofactor in the hydroxylation of proline and lysine, essential amino acids in the process of collagen synthesis and capable to inhibit the accumulation of degraded elastin.¹⁴

Lutein is able to modify the extracellular matrix's remodeling that occurs after exposure to ultraviolet radiation, through a beneficial effect on the regulation of metalloproteinases, in addition to inhibitory effects on cell loss, cell membrane damage and elastin expression.⁵

In addition, lutein increased the synthesis of hyaluronic acid in *in vitro* studies by increasing the expression of hyaluronan synthetase,³ which can justify the improvement in parameters such as skin roughness, since hyaluronic acid plays an important role in the maintenance of cutaneous hydration, anti-oxidation, as well as acting as a signaling molecule in response to skin damage.^{15,16}

It is inferred from the stimulus to the synthesis of collagen and elastic fibers, and hyaluronic acid that the nutritional association presented is capable of positively interfering in the skin aging process, particularly in maintaining the dermis' viscoelasticity properties, thus allowing a greater firmness and elasticity of the skin.

CONCLUSION

The use of an association containing vitamin C, lutein, lycopene and manganese has demonstrated the ability to stimulate the synthesis of collagen, elastin and hyaluronic acid in a fibroblast culture model, contributing to the improvement of the dermis' supporting structure. As a result, it can slow down the skin aging process. ●

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