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Oral and/or Topical Hydrolyzed Collagen Does Not Influence CD44, Elastin, and Col1A Gene Expression in Aged Skin of Postmenopausal Women

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ABSTRACT

Background: Skin aging in women is accentuated after menopause, mainly in photo exposed areas. Upper arm aging shows cutaneous atrophy, senile purpura, pigmentary alterations, and stellate pseudoscars. Oral and topical hydrolyzed collagens have been used to try to revert signs of skin aging, despite the paucity of clinical studies to support its indication.

Objective: to evaluate whether the use of oral and/or topical hydrolyzed collagen alters gene expression of collagen 1A, elastin, and CD44 in postmenopausal women.

Methods: double-blind, randomized, placebo-controlled study. Postmenopausal women with signs of skin aging on forearms were randomized to oral hydrolyzed collagen 5 g/day or matching placebo and also to a topical serum of 2.5% hydrolyzed collagen or matching placebo once a day for six months. Skin biopsy and RNA extraction were performed in a standardized area of the forearms and real-time PCR assay was used to evaluate gene expression (collagen 1a, elastin, and CD44). The change in the expression of Col1A, CD44, and elastin comparing pretreatment and after six months (pre/post) was expressed as the fold change (CI 95%) through bootstrap estimation (5,000 resamplings).

Results: Seventeen forearms from fourteen women, aged 60–80 years (mean 66, SD 5.5 years), were assessed. After comparing data from baseline and after six months, the only statistically significant result related to oral intervention and Col1A expression, but the placebo group performed better (p = 0.019). No significant difference was observed for each intervention or their comparison for all the other efficacy parameters. The fold change (CI 95%) was 0.95 (0.64-1.32) for oral and for topical collagen, 1.11 (0.64-1.60) for CD44, 1.74 (0.42-3.58) and 2.53 (0.70-4.55) for Col1A, and 0.72 (0.30-1.22) and 1.83 (0.56-3.41) for elastin.

Limitations: This was a small study in a single center in Brazil; results cannot be inferred to premenopausal women with no signs of aging skin.

Conclusions: In postmenopausal women, oral and/or topical collagen peptides have no benefits regarding gene expression of collagen 1A, elastin, and CD44 on aged forearm skin after six months of intervention, and, therefore, their clinical use should be critically reviewed.

Keywords: Skin aging, Photo aging, Hydrolyzed collagen, Collagen peptides, CD44, Collagen 1, Elastin

INTRODUCTION

Intrinsic or chronological aging is a physiological process that results in dermal atrophy and skin thinning. Extrinsic aging is associated with environmental factors such as sun exposure, air pollution, smoking, and poor nutrition, resulting in laxity, skin texture alterations and loss of elasticity. There are two main alterations in extrinsic aging: elastosisabnormal elastic tissue in deep dermis) and reduction of type I collagen due to increased degradation and decreased synthesis. The molecular mechanisms in skin aging include oxidative stress, DNA damage, telomere shortening, and micro RNA regulation, advanced glycation end product accumulation, genetic mutations, and inflammaging [1].

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Hyaluronic acid is able to start specific signaling events due to CD44 and RHAMM (receptor of HA-mediated motility) [2]. Reduced expression of CD44 in the skin is one of the discussed mechanisms of skin fragility in aging and dermatoporosis [3].

Some studies have shown that hormone replacement therapy could increase skin thickness in postmenopausal women [4-7]. Oral hydrolyzed collagen (HC) might increase skin hydration and elasticity, collagen content and density of the dermis [8-13]. Some topical treatments, such as glycolic and retinoic acid, are useful to improve skin atrophy and fragility [14]. Topical HC formulations are yet to be clinically studied.

The aim of this study was to evaluate whether the use of oral and/or topical hydrolyzed collagen could alter gene expression of type 1 collagen, elastin, and CD44 in postmenopausal women with signs of skin aging on the forearms.

METHODS

Study Population: Postmenopausal women, aged over 60 years, with mild signs of aging of the forearms were eligible. Hormone replacement therapy, chemotherapy, immunosuppression, active infectious or inflammatory lesions in the forearms, the presence of photodermatosis and recent (3 months) oral or topical treatments or procedures for skin aging of the forearms were exclusion criteria. The study was conducted in the outpatient clinic of a public hospital in São Paulo, Brazil, from June 2016 to April 2018. All patients signed an informed consent form.

Randomization and Blinding: double-blind, randomized, placebo-controlled study. The allocation was concealed from the investigator and participants through numbered packs with the treatments inside.

Intervention: Patients were oriented to avoid sun exposure and use physical photo protection during the study and were randomized according to oral and topical treatments, through a computer software. Oral treatment was either oral placebo (Po, maltodextrin) or hydrolyzed collagen (HCo, collagen peptides, 5g) once a day for 24 weeks. Topical treatment was either topical placebo (Pt, vehicle serum) or hydrolyzed collagen (HCt, 2.5% collagen peptides serum), three pumps at night on each forearm for 24 weeks. The topical HC formulation was developed by UNIFESP's Institute of Environmental, Chemical, and Pharmaceutical Sciences and used the same collagen peptides as the oral version in a serum containing polyacrylamide & C13-14 isoparaffin & laureth-7 (3.5%), vegetable glycerin (5%), isononyl isonanoate (3%), phenoxyethanol, and parabens (0.5%). The oral HC was a combination of different and specific collagen peptides of type I collagen, with a molecular weight of 2kDa, from bovine and porcine origin.

For RNA extraction and qPCR analysis for col1A, CD44, and elastin, a 2-mm skin biopsy of the dorsal surface of each forearm was performed (7 cm from the antecubital fold on the midline).

For the total RNA extraction of the skin tissues, a RN easy mini kit reagent (QIAGEN®, Hilden, Germany) was used. A spectrophotometer (NanoDrop® Lite, Thermo Fisher Scientific®, Waltham, MA, USA) was used to quantify the RNA in each sample by determining the absorbance ratio at 260 and 280 nm. A 0.5-µg piece from the total extracted RNA of each sample was used for the synthesis of cDNA using the Superscript III reverse transcriptase kit with random hexamer primers and thermocycler Veriti (Thermo Fisher Scientific®, Waltham, MA, USA), following the manufacturer's instructions. The cDNA was frozen at −20°C until tested. The qPCR assay reaction was performed using the Evagreen (Solis Biodyne) on the thermocycler apparatus Quantstudio 5 (ABI Applied Biosystems®, Foster City, CA, USA), following the manufacturer's instructions. The Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, NM_002046 - Efficiency curve 95.848%) was used as an endogenous control for each sample. The following genes were analyzed: CD44 (NM_000610 - Efficiency curve 97.98 %), COL1A1 (NM_000088 - Efficiency curve 90.027%), and ELN (NM_001278939 - Efficiency curve 91.693%). Reactions were made in triplicate for each gene, including the endogenous control. The ΔCT values were obtained by the difference between the CT values of the target genes and the GAPDH gene. The $2^{-\Delta LN}$ method [15] was used to calculate the gene expression for each target gene.

The change in the expression of Col1A, CD44, and elastin from pretreatment to after six months (pre/post) was expressed as the fold change (CI 95%) through bootstrap estimation (5,000 resamplings).

RESULTS

Fourteen women with mild signs of skin aging aged 60-80 years (mean 66.4 ± 5.5years) were included. Among these, qPCR extraction was successful in 17 of the 28 forearms. Eight patients used Po, and six used CHo. Regarding topical interventions in each forearm, nine patients used Pt, and eight used CHt.

The results of gene expression profile are described in **Table 1**.

DISCUSSION

Oral or topical collagen did not increase gene expression of relevant dermal components after six months of intervention.

This study corroborates the findings of a randomized, double-blind, factorial study to evaluate efficacy and safety of topical and/or oral hydrolyzed collagen in women with stage 1 dermatoporosis in which no significant difference was observed for each intervention, nor their comparison, for all efficacy parameters: clinical and quality of life scores,

dermal elasticity, thickness and echogenicity, and histologic and immunohistochemical markers [16].

Table 1. Fold change of qPCR gene expression regarding oral and topical interventions for CD44, type 1 collagen, and elastin.

	CD44		Collagen 1		Elastin	
	Oral	Topical	Oral	Topical	Oral	Topical
Hydrolyzed collagen (HC)	0.95; (0.64-1.32)	1.11; (0.64-1.60)	1.74; (0.42-3.58)	2.53; (0.70-4.55)	0.72; (0.30-1.22)	1.83; (0.56-3.41)
Placebo	1.02; (0.71-1.37)	0.88; (0.68-1.10)	3.20; (1.33-5.11)	2.50; (0.79-4.55)	2.08; (0.83-3.50)	1.10; (0.43-1.84)

Critical studies of clinical efficacy of oral supplements in skin aging are key to balance the flooding of options in the market and the public search for the "pill of youth". The results of this study might contribute to spare patients from the financial impact of ineffective treatments.

This was a small study in a single center in Brazil.

CONCLUSION

Oral and/or topical collagen peptides have no benefits regarding gene expression of type 1 collagen, CD44, and elastin on forearm skin with mild signs of aging after six months of intervention in postmenopausal women.

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