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Influência da suplementação com colágeno hidrolisado no metabolismo da matriz extracelular e proliferação de fibroblastos dérmicos humanos derivados de áreas fotoprotegida e fotoexposta, cultivados em monocamada e equivalente dérmico

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RESUMO

Zague V. Influência da suplementação com colágeno hidrolisado no metabolismo da matriz extracelular e proliferação de fibroblastos dérmicos humanos derivados de áreas fotoprotegida e fotoexposta, cultivados em monocamada e equivalente dérmico. [Tese (Doutorado em Biologia Celular e Tecidual)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2015.

Nos últimos anos tem havido interesse crescente na relação entre nutrientes e compostos bioativos de alimentos e seus benefícios para a pele. O colágeno hidrolisado (CH) é um destes compostos que vem sendo estudado, demonstrando potencial na melhora dos sinais clínicos do envelhecimento cutâneo. Seu uso como suplemento alimentar vem de longa data, porém poucos estudos têm endereçado os efeitos subjacentes do CH sobre a biologia celular e molecular de células da pele que poderiam elucidar os achados de melhora clínica. Este trabalho investigou, pela primeira vez, a influência do CH na modulação do metabolismo e proliferação de fibroblastos dérmicos humanos (FDHs) derivados de regiões anatômicas acometidas pelo envelhecimento cronológico (fotoprotegida) e fotoenvelhecimento (fotoexposta), cultivados em modelo de monocamada. Explantes de pele de mama e de pele de pálpebra foram utilizados para obtenção das culturas primárias de FDHs de área fotoprotegida e fotoexposta, respectivamente. Além disto, foram investigados os efeitos da suplementação com CH na secreção de colágeno tipo I, em modelo de cultura 3D de equivalente dérmico, derivado de matriz produzida exclusivamente por FDHs. Os achados deste trabalho comprovaram que o tratamento com CH não influenciou a proliferação celular dos fibroblastos derivados de ambas as áreas, porém modulou expressivamente o metabolismo dos FDHs cultivados em monocamada, elevando o conteúdo do precursor e da proteína principal da matriz dérmica, pró-colágeno I e colágeno I, respectivamente. Estes efeitos foram confirmados no modelo de equivalente de derme humana. O aumento do conteúdo de colágeno nas culturas foi atribuído à estimulação da biossíntese e diminuição do metabolismo de colágeno I, por meio a inibição da atividade de metaloproteinases de matriz (MMP) 1 e 2. A modulação do colágeno hidrolisado no metabolismo celular não diferiu entre as células derivadas de áreas protegida ou exposta ao sol. No entanto, concentrações menores de CH foram suficientes para estimular as células de área fotoexposta, sugerindo efeitos mais pronunciados do CH nestas células. Acreditamos que este estudo é uma contribuição importante para compreensão dos efeitos biológicos do CH nas células da pele e viabilidade do seu uso como ingrediente funcional de suplementos alimentares.

Palavras-chave: Colágeno hidrolisado. Peptídeos bioativos de colágeno. Suplemento alimentar. Fibroblastos dérmicos humanos. Metabolismo da matriz extracelular. Proliferação. Equivalente dérmico humano. Matriz dérmica. Envelhecimento cutâneo. Fotoenvelhecimento. Cultura primária.

ABSTRACT

Zague V. Influence of collagen hydrolysate supplementation on extracellular matrix metabolism of human dermal fibroblasts derived from sun-protected and sun-exposed body sites, cultured in monolayer and dermal equivalent models. [PH. D. Thesis (Cell and Tissue Biology)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2015.

In recent years, there has been increasing interest in the relationship between nutrients and food-derived bioactive compounds and their benefits to the skin. Collagen hydrolysate (CH) is one of these compounds has been studied, demonstrating potential for improvement of skin aging clinical signs. Its use as a food supplement has a long history; however, few studies have addressed the underlying purpose of CH on the cellular and molecular biology of skin cells that could elucidate clinical improvement findings. This study investigated, for the first time, the influence of CH on the extracellular matrix metabolism and proliferation of human dermal fibroblasts (HDFs) derived from chronological aged (sun-protected) and photoaged (sun-exposed) body sites, cultured in monolayer in vitro model. Breast and eyelid skin explants were used to obtain HDFs primary cultures of sun-protected and sun-exposed sites, respectively. Moreover, CH effects on the secretion of type I collagen were investigated in dermal equivalent 3D model derived from dermal matrix produced exclusively by HDFs. The findings of this study demonstrated that CH treatment did not affect cellular proliferation of either cell cultures, but notably modulated cell metabolism in monolayer model, increasing the content of dermal matrix precursor and main protein, procollagen I and collagen I, respectively. These effects were confirmed in the human dermal equivalent model. The increase in collagen content in the cultures was attributed to stimulation of biosynthesis and decreased collagen I metabolism through inhibition of metalloproteinase activity (MMP) 1 and 2. Modulation of CH in dermal metabolism did not differ between cells derived from sun-protected and sun-exposed areas, although lower concentrations of CH seemed to be enough to stimulate sun-exposed-derived HDFs, suggesting more pronounced effect in these cells. We believe that this study presents an important contribution to understanding the biological effects of CH in skin cells and viability of its use as a functional ingredient in food supplements.

Keywords: Collagen hydrolysate. Bioactive collagen peptides. Food supplements. Human dermal fibroblasts. Extracellular matrix metabolism. Proliferation. Human dermal equivalent. Dermal matrix. Skin aging. Photoaging. Primary culture.

1 INTRODUÇÃO

Nos últimos anos tem ocorrido interesse crescente nos benefícios da suplementação oral com nutrientes e compostos bioativos de alimentos. Alguns destes compostos têm mostrado capacidade de modular funções celulares e metabolismo da pele, sendo potencialmente eficazes para amenizar e/ou retardar os sinais do envelhecimento cutâneo. O colágeno hidrolisado (CH) está entre os compostos bioativos que têm sido estudados por seus potenciais benefícios biológicos sobre o metabolismo da pele. Contudo, até o momento, poucos estudos têm sido endereçados na investigação dos efeitos subjacentes à suplementação com CH na biologia celular e molecular de fibroblastos dérmicos.

Os fibroblastos são considerados o principal tipo celular que sintetiza matriz extracelular (MEC) nos tecidos conjuntivos. O processo de envelhecimento natural e fotoenvelhecimento da pele causam alterações na morfologia celular, potencial proliferativo, biossíntese de proteínas da MEC e resposta a fatores de crescimento e citocinas. Consistente com a ideia de que fibroblastos cultivados *in vitro* têm regulação coordenada e síntese de MEC característicos de seus locais de origem, com o melhor de nosso conhecimento, nada se sabe sobre os efeitos potenciais do CH sobre o metabolismo e proliferação de cultura de fibroblastos dérmicos de diferentes locais anatômicos. Estimulados pela possibilidade de aquisição de novos conhecimentos nesta área, nós investigamos os efeitos do CH na modulação de respostas celulares e moleculares de fibroblastos dérmicos derivados de áreas fotoexposta e fotoprotegida, cultivados em modelo de monocamada. Além disto, foram investigados os efeitos da suplementação com CH na secreção da principal proteína da MEC, colágeno tipo I, em modelo de cultura tridimensional (3D) de equivalente dérmico derivado de matriz produzida exclusivamente por fibroblastos dérmicos humanos. Acreditamos que este estudo é uma contribuição importante para compreensão dos efeitos biológicos do CH nas células da pele e viabilidade do seu uso como ingrediente funcional de suplementos alimentares.

7 CONCLUSÃO

Tendo em vista que (a) fibroblastos de pele humana derivados de diferentes sítios anatômicos podem exibir padrões de transcrição e metabolismo distintos e característicos, e que (b) o uso oral de CH tem sido muito difundido como promissor na prevenção e tratamento dos sinais de envelhecimento da pele, nossos resultados nos permitiram concluir que:

i. A suplementação com colágeno hidrolisado não atuou na modulação da proliferação de fibroblastos dérmicos humanos derivados de ambas as áreas fotoprotegida e fotoexposta.

ii. A suplementação com colágeno hidrolisado modulou positivamente o metabolismo de fibroblastos dérmicos humanos derivados de áreas fotoprotegida e fotoexposta, por (a) estimular a biosíntese do precursor e da proteína colágeno tipo I e (b) inibir a atividade das enzimas MMP-1 e -2.

iii. Concentrações menores de colágeno hidrolisado foram suficientes para estimular o metabolismo das células derivadas de área fotoexposta, em relação ao de fibroblastos de área fotoprotegida, sugerindo efeitos mais pronunciados do CH naquelas células.

iv. A suplementação com colágeno hidrolisado elevou o conteúdo de colágeno tipo I em modelo tridimensional de equivalente de derme humana, obtida exclusivamente por fibroblastos dérmicos humanos, comprovando a resposta do tecido dérmico frente ao tratamento com CH próximo a condições *in vivo.*

v. A suplementação com colágeno hidrolisado fornece, além dos aminoácidos como nutrientes construtores, peptídeos reguladores da atividade celular.

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