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Recombinant enzymatic products in Dermatology

Produtos enzimáticos recombinantes em dermatologia

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ABSTRACT

Esthetic dermatology is a growing field, and different noninvasive therapies are gaining attention. The objective of this narrative review was to update current evidence-based knowledge about recombinant enzymes. The PubMed, SciELO, Cochrane Central, and Imbiomed databases were searched for meta-analyses, systematic reviews, randomized controlled clinical trials, observational registries, and preclinical data published in English, Spanish, and Portuguese. Effectivity, safety, and tolerability of recombinant hyaluronidases, lyases, collagenases, and lipases, including their combinations, were evaluated and confirmed in diverse indications. Further research could increase current knowledge on this constantly developing and promising therapeutic area.

Keywords: Lyases; Collagenases; Hyaluronoglucosaminidase; Lipase.

RESUMO

A dermatologia estética é um campo em crescimento, e diferentes terapias não invasivas estão ganhando atenção. O objetivo desta revisão narrativa é atualizar o conhecimento baseado em evidências sobre as enzimas recombinantes. Uma pesquisa bibliográfica de metanálises, revisões sistemáticas, ensaios controlados e randomizados, registros observacionais e dados pré-clínicos publicados em inglês, espanhol e português foi realizada nas bases de dado PubMed, SciELO, Cochrane Central e Imbiomed. A eficácia, segurança e tolerabilidade das hialuronidases, liases, colagenases e lipases recombinantes, incluindo suas combinações, foram confirmadas em diversas indicações. Espera-se que novas pesquisas aumentem o conhecimento sobre essa área terapêutica promissora e em constante desenvolvimento.

Palavras-chave: *Liases; Colagenase microbiana; Hialuronoglucosaminidase; Lipase.*

Review Article

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INTRODUCTION

The definition of beauty has puzzled the minds of artists, mathematicians, and beauty professionals alike for the past seven centuries, starting with Pacioli's divine proportions. Current beauty standards can be related to concepts such as youth, health, and symmetry. Progress towards these apparently simple goals may be achieved through interventions ranging from minimally invasive or noninvasive options to radical, surgical solutions, the first of which are cost-effective and fast-acting therapies, thus gaining the trust of patients and surpassing the popularity of traditional, surgical options.[1] Techniques such as cryolipolysis, radio frequency, low-level laser therapy, and high-intensity focused ultrasound are reported to offer "significant and satisfying results without any adverse side effects".[2] Nevertheless, it is worth noting that these treatment strategies differ in terms of outcomes and tolerability profiles.

A novel class of injectable solutions relies on therapeutic enzymes, which act on their target with great affinity and specificity.[3] Therapeutic enzymes are synthesized in heterologous organisms with the help of biotechnology and genetic engineering, leading to the development of recombinant DNA products.[4] Recombinant DNA technology is a technique of gene cloning that leads to the production of a defined sequence of DNA, with its subsequent propagation and amplification in a suitable host cell or expression system (bacteria, yeasts, animal cells). This process culminates with the purification steps of centrifugation and/or filtration.[4] The resulting recombinant enzymes are obtained quickly, in large quantities, and with high purity levels. Bacterial enzymes are characterized by lower immunoreactivity than those of eukaryotic origin and, therefore, by a better safety profile. The absence of glycosylation in bacterial enzymes is related to the lack of organelles (Golgi apparatus and endoplasmic reticulum in prokaryotic organisms) in which the post-transcriptional process of glycosylation takes place.[5]

The main objective of this narrative review is to update current evidence-based knowledge about recombinant enzymes in Dermatology.

METHODS

A literature search for meta-analyses, systematic reviews, randomized controlled clinical trials, observational registries, case reports, and preclinical data was performed in biomedical databases (PubMed, SciELO, Cochrane database, and Imbiomed), focusing on the use of recombinant enzymes in Dermatology; "lyase", "hyaluronidase", "collagenase", and "lipase" and their corresponding translations were defined as keywords. Publications in English, Spanish, and Portuguese from January 2011 to January 2023 were considered. All authors have reviewed the relevant contents before writing this objective, comprehensive narrative synthesis of published information.

RESULTS

Hyaluronidases and lyases

The skin's extracellular matrix (ECM) provides a structural framework and plays a role in regulating cellular proliferation, adhesion, and migration. The main components of the ECM are collagen, elastin, and proteoglycans, to which glycosaminoglycan (GAG) chains are linked. The most expressed GAG in the dermis is hyaluronic acid (HA).[6] Human hyaluronidases enzymatically degrade GAGs and hydrolyze HA, increasing both skin and connective tissue permeability. Six hyaluronidases have been identified in humans (HYAL1, HYAL2, HYAL3, HYAL4, and PH- 20).[7]

Both hyaluronidases and lyases degrade HA. Hyaluronidases are part of the broader group of lyases, which may degrade several substrates, including GAGs different from HA. It is worth noting that high-purity recombinant lyases like PB72K are characterized by a different amino acid sequence when compared with human hyaluronidases. PK72K lyase acts on local permeability and allows the reduction of edematous components at a tissue level.[6–9]

Progressive loss of HA is a hallmark of skin intrinsic aging (reduced biosynthesis of HA in fibroblasts) and extrinsic aging (progressive degradation of HA due to exogenous factors, such as recurrent and prolonged exposure to ultraviolet radiation).[6]

Local injection of HA-based fillers is the current preferred treatment for soft tissue augmentation, deep skin hydration, and facial contouring. However, this procedure may be associated with adverse events, including superficial placement leading to skin discoloration (Tyndall effect), the use of excessive product and granulomatous foreign-body reactions.[10] Hyaluronidase is used for the management of complications resulting from filler injections. Its efficacy has been demonstrated by Vartanian et al., who performed a randomized, controlled trial including 12 subjects receiving two 0.2 mL injections of stabilized HA in the proximal forearm. Up to three days after injection, skin scores were determined on a scale based on the size of dermal augmentation. Participants were randomly divided to receive 0.5 mL of 75 units of hyaluronidase or normal saline as a placebo. After one week, hyaluronidase-treated patients experienced an 80% decline in skin scores, compared to 10% among controls ($p < 0.001$). Ninety days after treatment, no palpable HA remnant was identified in 92% of subjects.[11] It is worth noting that, in contrast to calcium hydroxylapatite or poly-l-lactic acid-based fillers, HA-based filler effects may be reversed with hyaluronidase treatment.[6]

Hypertrophic scars are characterized by an altered ECM and may develop after injuries, burns, surgery, and several inflammatory processes. These scars remain a therapeutic challenge. Hyaluronidases regulate the level of HA mostly by its degradation, but the role of these enzymes in the wound healing processes is less evident. Products of hyaluronidase degradation seem to stimulate angiogenesis, contributing to wound healing. In a clinical study, treatment of hypertrophic scars with hyaluro-

nidase injections led to changes in scar consistency and a significant reduction in height, independently of their pretreatment elevation.[12] These results are consistent with in vitro and in vivo data showing that hyaluronidases accelerate wound closure in a full-thickness excisional model. This action is mainly associated with regulation of the inflammatory response by mediating pro and anti-inflammatory cytokines, lipid mediators (derived from arachidonic acid) and transcription factors. Moreover, hyaluronidase contributes to the balance between biosynthesis and deposition of collagen.[8]

Collagenases

Collagenases are part of the matrix metalloproteinases group and participate in physiological processes related to collagen biosynthesis, integrity or rearrangement. Collagen represents 30% of the total protein content of the human body.[13] The human skin expresses three collagenases that may initiate degradation of type I fibrillar collagen (MMP-1, MMP-8, MMP-13).[14] All these isoforms prompt collagenolysis by single scissions across the three chains that integrate the collagen molecule in distinct loci from the N-terminus; nevertheless, collagenase posterior action on alpha chains is poor, and subsequent collagenolysis is mainly mediated by gelatinases.[15] By contrast, collagenases synthesized by bacteria from the Clostridioides genus (clostridial collagenases), like collagenases G/H PB220, are able to induce multiple scissions in the collagen triple helix and complete collagenolysis, resulting in several small peptides.[15] In addition, clostridial collagenases reduce the expression of other fibrosis-related molecules, including fibronectin, smooth-muscle actin, and transforming growth factor beta. Notably, the presence of collagen metabolites stimulates fibroblast activity for the formation of new fibers with improved functionality.

In the skin, fibrotic responses to an injury are characterized by scar formation, with excess collagen deposition and a lack of dermal appendages. The process leading to fibrosis is still poorly understood and may be related to cell lineage reprogramming and fibroblast heterogeneity.[16] Hypertrophic and keloid scars have been associated with excessive collagen deposition and reduced native collagenase activity.[14] Keloid fibroblasts have a higher rate of proliferation, more excessive deposition of ECM proteins, and increased expression of myofibroblast biomarkers. The main drawback of keloid treatments, including surgical excision and intralesional corticosteroid injections, is the high recurrence rate.[17] As an isolated therapeutic approach, collagenases have been proposed for the management of these difficult-to--treat lesions and also for enzymatic debridement of burns.[18-20]

Lipases

Lipases catalyze the hydrolysis of triglycerides to smaller molecules (free fatty acids and glycerol). Human lipase activity is determined by modulating factors, including the metabolism of insulin, diet, and physical activity. On the contrary, bacterial lipases, such as recombinant PB500 lipase, do not require cofactors and have a broad substrate specificity.[21]

Adipose tissue accumulates in larger quantities in several areas, predominantly the abdominal and gluteal regions, thighs, periarticular region, retro-orbital areas, as well as on the face and visceral structures. Accumulation of triglycerides may lead to an increment of volume and proliferation rate of adipocytes; triglyceride hydrolysis mediated by lipase induces the formation of easily diffusible metabolites that may be eliminated by lymphatic drainage for further metabolic degradation.[14] In each area, adipose tissue accumulation is characterized by a different profile of adipokine expression.

Intradermal or hypodermic application of lipase to induce fat dilution in areas including the neck, arms, abdomen, and thighs has shown important clinical effectiveness with a good safety profile. Lipases also reduce the size of localized fat deposits or in case of post-lipoplasty imperfections, making this strategy a valuable complement or even an alternative for patients looking for minimally invasive treatments.[14] In addition, one or more injections may be considered for nonobese individuals requiring a mild reduction of adipose tissue, with lower risk than invasive procedures.[14]

Rational and clinical applications of combined enzymatic therapies

The combination of nonfunctional collagen degradation (recombinant collagenases G/H PB220), fat reduction (recombinant lipase PB500), and GAGs hydrolysis (recombinant lyase PB72K) represent a rational, pathogenic-based, and synergic strategy for approaching several esthetic concerns. In addition, the incorporation of high molecular weight HA (HMWHA) into this combination is associated with superior results. HMWHA is characterized by anti-inflammatory properties, contrasting with the potent proinflammatory activity of low molecular weight HA. HMWHA also modulates angiogenesis and cell migration, in relation to skin repair processes. Antioxidant, antiplatelet and inhibitory actions on endothelial cell proliferation and migration have also been identified.[22,23]

Combined enzyme therapy (CET) is a suitable treatment for facial rejuvenation and double chin. As a result of aging, dental alveolar regression leads to flattening of the malar region, with subsequent deepening of the nasolabial folds. This remodeling process causes an imbalance in the upper, middle, and lower thirds of the face.[24] Also linked to aging, a double chin develops due to weakening of the ligaments, loss of skin tone and flaccidity of the facial and cervical subcutaneous adipose tissue.[25,26] Therefore, several components (including connective and adipose tissues) are involved in the pathogenesis of both conditions, which may be targeted by CET.

Edematous fibrosclerotic panniculopathy (EFP) is another esthetically important condition, currently considered as a metabolic disorder of adipose tissues. EFP is probably multicausal, and related factors include connective tissue architecture, estrogen action, microvascular alterations, and genetic and

hormonal characteristics.[27] As a consequence, local hypoxia is induced, leading to a fibrotic response with thick bundles and collagen septa that finally connect the subcutaneous fat to the skin, producing the classical EFP appearance.[12] Ongoing studies and future publications will add more medical evidence on the benefits of CET in patients with EFP.

Additionally, in a clinical multicentric study conducted by Castro-García et al., 42 patients who reported 44 scarring fibrotic lesions (hypertrophic, atrophic or keloids) were treated with a combination of HA, collagenase, lipase, and hyaluronidase, administered with a blanching technique. Fibrosis was evaluated with the Vancouver score and the patients' perceptions were quantified with the Patient and Observer Scar Assessment Scale (POSAS). Prespecified visits were scheduled at day 15, 30, 45, and 60. Combined therapy with HA and recombinant enzymes was associated with relevant improvement, starting at the first control visit. These benefits included the domains of the Vancouver scale (pigmentation, lesion height, vascularization, flexibility) and POSAS (pain, itching, color, stiffness, thickness, irregularity).[28]

CET targeting several components of the MEC is a suitable strategy for treating patients with abnormal wound healing, including keloids. These pathological scars are correlated with an abnormal fibroproliferative response, in which raised scar tissue grows excessively and invasively beyond the original wound edges. Keloids may occur following a triggering stimulus (dermal injuries or inflammatory processes). Environmental factors (type of injury), anatomical location, and genetic predisposition are involved in keloid pathogenesis.[29] As previously cited, collagenases G/H PB220 degrade nonfunctional collagen fibers, while lyase PB72K leads to better tissular penetration and HMWHA modulates local angiogenesis. This enzymatic synergy addresses several patient complaints simultaneously.

Safety and tolerability

Hyaluronidase is associated with a low risk of adverse events. Reports of complications are usually linked with hypersensitivity reactions. Severe forms (facial angioedema, anaphylaxis) have been reported with an estimated incidence rate of 0.1%; nevertheless, such cases are associated with higher doses used to facilitate anesthesia. The risk of allergic reactions has significantly reduced with the use of recombinant formulations. Hyaluronidase is contraindicated in patients who have previously developed hypersensitivity reactions to bee or wasp stings.[30] Collagenase therapy is generally well tolerated. In a real-world setting, the most common treatment-related adverse events are injection site-related and typically resolve before the next treatment session.[31]

Lipase therapy is considered safe, with a good tolerability profile. By contrast, deoxycholic acid, also indicated for fat reduction, has been associated with disruption of adipocyte cell membranes and local tissue responses involving macrophage infiltration, with risk of necrosis.[32]

In the combined therapy study by Castro-García et al., 91% of participants reported local pain as an adverse event, taking into consideration the long-term history of the scarring lesions. Pain was progressively reduced during follow-up, consistently with fibrosis improvement. Local reactions were reported by 75% of patients (erythema, local pain, edema) and spontaneously resolved after 48 hours from the application. No participant withdrew from the study due to adverse events.[28] Enzymatic recombinant therapy is contraindicated during pregnancy and breastfeeding and should not be administered in case of local irritation or infection. Caution is advised in patients with autoimmune diseases and those who had recently received vaccines.

Discussion and conclusion

Esthetic Dermatology is a growing field, and different noninvasive therapies are gaining attention. Enzymes obtained through recombinant DNA technology are safe and effective as injectable products, yielding progressively beneficial results. These enzymes may be used as stand-alone products or as part of recombinant CET, with indications ranging from localized excessive adipose tissue to keloid scarring. Different published and ongoing studies have confirmed the effectivity of recombinant CET with diverse indications, including facial rejuvenation, double chin, hypertrophic scars, and EFP. While current minimally invasive and surgical treatment strategies have variable outcomes and several tolerability issues,[3] recombinant enzymes represent a safe treatment when administered by trained healthcare professionals. It is concluded that enzymatic therapy represents an important advance in the cosmetic field, taking into consideration that these pharmaceutical products act on their targets with great affinity and specificity. It is expected that further research will increase current knowledge of this constantly developing and promising therapeutic area. ●

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