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REVIEW ARTICLE

Collagen and Collagenases: A Brief Review

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ABSTRACT

Collagen is the most important and abundant protein in the human body that is needed for a number of physiological functions such as tissue repair during wound and fracture healing. There are about thirty different types of collagen. Collagenases are enzymes that helps breakdown the deposited collagen in the muscles, joints, tissues and arteries. There are a variety of collagenases used in different dosage forms in the treatment of diseases such as wound healing, Dupytren's contracture, Peyronie's disease etc. A major part of the collagenase enzyme is used for the treatment of chronic wounds such as tumors, burns and ulcers.

INTRODUCTION

Collagen is the most abundant constituent protein found in the body of human beings. It contains one-third of the total protein from three quarter of the weight of skin and is the most prevailing component of the extracellular matrix (ECM) [1,2]. The collagen molecule is an elongated rod-like fibrous protein structure consisting of three polypeptide chains called the α-chains [3]. The α-chains are folded in such a manner that a glycine residue is present at every third position in the polypeptide chain. Each α-chain is made up of multiple triplet sequences of Gly-Y-Z in which Y and Z can be any amino acid. The Y is commonly found as proline and Z is usually present as hydroxyproline (Figure **1**). The presence of hydroxyproline in the Y position is also thought to be responsible for the stability of the helical structure [4].

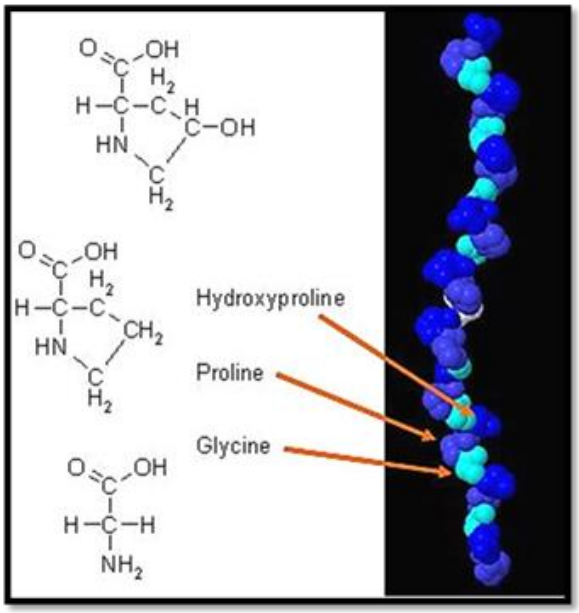

Figure 1. Conserved structural motif of three amino acid residues in collagen.

TYPES OF COLLAGEN

There are approximately thirty different types of collagen that have been identified, so far. Type I collagen is found in copious quantities in the human body along with significant amounts of Type II, III and IV [5].

- Collagen type I – found in bones, tendons and organs.
- Collagen type II – found mainly in cartilage.
- Collagen type III – found mainly in reticular fibers.
- Collagen type IV – found in the basement membrane of cell membranes.
- Collagen type V – found in hair and nails.

Collagen production is increased in conditions requiring remodeling and replacement of tissues, for example, tissue repair such as during wound and fracture healing. Collagen degradation occurs in infected tissues and specific collagenolytic enzymes may be released. The primary sources responsible for destruction of bones and cartilage are protein degrading enzymes (proteinases) that degrade collagen [3].

MATRIX METALLOPROTEINASES (MMP)

They are a class of enymes that act on ECM at neutral pH and degrade all its components. The MMP family contains 17 members which are further divided into four groups and are distinguished on the basis of their substrate specificity and size. They include stromelysins, collagenases, gelatinases and other enzymes[6]. All of these are composed of common sequence of amino acids making specific domains (Figure **2**). They are secreted as inactive proenzymes which are further activated by exposing them to mercurial compounds or by proteolysis. Zinc is present as an active center and they require calcium for its activity, while on the other hand their activity is inhibited by tissue inhibitor of metalloproteinase (TIMP) [6].

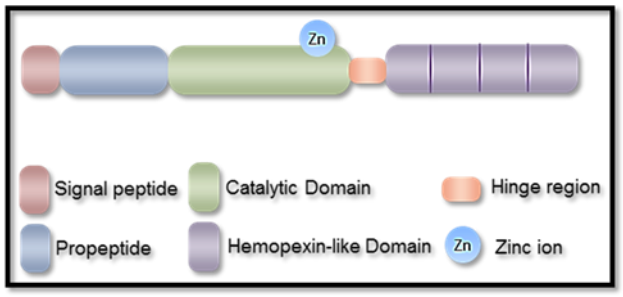

Figure 2. Schematic representation of the basic structure of an MMP.

During growth and development of the connective tissue matrix the activity of these proteinases is widely observed such as:

- Ovulation and embryo implantation [7-9]
- Embryological development [10,11]
- Angiogenesis [12,13]
- Bone turnover [14,15]
- Uterine resorption [16]
- Cervical ripening [17]

They are also used for the destruction of tissues in various diseases [6], including the following:

- Wound healing [18,19]
- Periodontal disease [6,20,21]
- Corneal ulceration [20,23]
- Tumor growth and metastasis [24-27]
- Cartilage and bone destruction in the arthrititis [28-32]
- Arteriosclerosis [33]

COLLAGENASES

Collagenases are enzymes that break down collagen which holds animal tissues together. Collagenases are efficient in removing detritus without harming the nearby healthy tissues and therefore contribute towards the formation of epithelium [34]. Interstitial collagenase is an adjunct of the MMP gene club that splits the collagen triple helix to yield distinctive ¼–¾ aggregates [35]. This ability determines a great variety of different biotechnological and medical applications of such enzymes. The specific activity of collagenases makes them particularly effective in the removal of debris [36,37].

TYPES OF COLLAGENASES

Collagenases obtained from bacteria differ from those of vertebrates in that they are more substrate specific [38]. In contrast conflicting to animal collagenases which divide collagen into its natural triple-helical anatomy, bacterial collagenases are unexampled as they can degenerate water-insoluble and water-soluble denatured collagens [39,40]. They can invade nearly all collagen types and is able to initiate numerous cleavages within its helical regions [41]. There are three distinct collagenases [42-46].

- MMP–1 (interstitial collagenase)
- MMP–8 (neutrophil collagenase)
- MMP–13 (collagenase–3)

As soon as the collagen separates apart into its fragments, they are no more stable at body temperature because of the loss of their triple helical region. The remaining polypeptide chains are then degraded by other proteinases [47,48].

THERAPEUTIC USES

The most potent collagenase is the one secreted by the anaerobic bacteria *Clostridium histolyticum*. Bacterial collagenase is a crude complex containing a collagenase referred to as clostridiopeptidase-A. It is unique among others because of its ability to attack and degrade the triple-helical collagen fibers found in connective tissues. It can be used alone as an active ingredient but on the other hand, it is also found to be performing its functions more generously with other compounds. It is indicated in a variety of diseases/conditions such as wound debridement, ulcers and burns, Peyronie's disease, Duptyren's contracture, bed wounds, lumbar disc herniation, tendonitis, tissue engineering, and urethral fibrosis (Table **1**).

DOSAGE FORMS OF COLLAGENASES

Dosage forms are pharmaceutical drug products in the form in which they are marketed and administered. They involve a combination of active drug components and excipients. Collagenases are widely used and have been incorporated in different dosage forms including creams, ointments, injections, etc. (Table **1**).

The different dosage forms available for collagenases in the market include:

I. Ointments intended for external application to the skin or mucous membranes. Collagenase ointments are available as:
   a) Collagenase Santyl® ointment is a sterile enzymatic debriding ointment which contains 250 collagenase units per gram of white petrolatum and is available in 15 and 30 g tubes.
   b) Iruxol® ointment is an enzyme preparation from *Clostridium histolyticum* with 0.6 units of clostridiopeptidase A (collagenase) and 10 mg chloramphenicol. It is available in 15 g tube.
   c) Bionect Start® is a fluid ointment for topical application containing hyaluronic acid sodium salt 0.2%, collagenase (not less than 2.0 nkat / g ointment), liquid paraffin and white Vaseline. It is available in 30 g tube and is indicated in the treatment of chronic ulcers with necrotic tissue.

II. Creams containing one or more drug substances dissolved/dispersed in a suitable base.
   a) Novuxol® is a sterile adhesive cream which is available in 30 g tube. It also contains the enzyme clostridiopeptidase derived from *Clostridium histolyticum*.

III. Injection is an infusion method of putting liquid into body with a needle or a syringe pierced through skin using a sufficient depth forced into the body. It is available as:
   a) Xiaflex® is for intra-lesional use and is available as 0.9 mg collagenase from

Clostridium histolyticum per vial. It is used to treat Dupuytren's contracture and Peyronie's disease that are caused by collagen found in the knots/cords of the hand in Dupuytren's contracture or plaques in the penis in Peyronie's disease.

b) Xiapex® is a white lyophilized powder. Each vial of powder contains 0.9 mg of collagenase from *Clostridium histolyticum*. It is indicated for the treatment of Dupuytren's contracture in adult patients with a palpable cord and treatment of adult men with Peyronie's disease with a palpable plaque and penile curvature deformity.

Liniments are fluids, semi-fluid or semi-solid preparations intended for application to skin. They may be alcoholic or oily solutions which should not be applied to broken skin. Collagenase liniment is applied on scars for skin regeneration.

Table 1. Dosage forms and therapeutic uses of collagenases.

S. No.	Other Additives / Therapies	Dosage Form	Disease Condition
1.	Fibrinolysin	Ointment / gel	Wound debridement [49]
2.	Silver sulfadiazine and polymyxin	Ointment / cream	Burns [50-53]
3.	Hyaluronic acid	Cream	Wound debridement [54-55]
4.	Hyaluronic acid	Ointment	Ulcerative lesions [56-58]
5.	-	Ointment	Burns [59]
6.	-	Ointment	Bed wounds [60]
7.	Poly(2-hydroxy methacrylate)	Hydrogel	Tissue engineering [61]
8.	Hartman's solution	Injection	Infra-orbital dark circles [62]
9.	-	Ointment	Ulcers (lower extremities) [63,64]
10.	-	Ointment	Pressure ulcers [65]
11.	Papain urea	Ointment	Limb ulcer debridement [66]
12.	-	Injection	Herniated lumbar disk [67]
13.	-	Solution	Endothelial cell harvesting [68]
14.	-	Liniment	Scar treatment [69]
15.	Lecithin	Suspension	Adipose tissue [70]
16.	Negative pressure wound therapy (NPWT)	Ointment	Chronic ulcers [71,72]
17.	Saline medical gauze (SMG)	Ointment	Diabetic foot ulcers [73]
18.	Serial sharp debridement (SSD)	Ointment	Diabetic foot ulcers [74,75]
19.	Hydrogel dressing	Ointment	Pressure ulcer [76,77]
20.	Semi occlusive dressing	Ointment	Wound debridement [78]
21.	-	Ointment	Pyonecrotic wounds [79]
22.	-	Ointment	Wound debridement [80,81]
23.	*Centella asiatica*	Ointment	Wound healing [82]
24.	-	Ointment	Dermal and decubitus ulcers [83]
25.	Tretinoin and adapalene	Ointment / cream / gel	Wound healing [84]
26.	-	Ointments and injection	Ulcers, burns, herniated discs [85]
27.	Chloramphenicol	Ointment	Ulceration of legs [86]
28.	-	Injection	Dupuytren'scontracture [87-91]
29.	-	Injection	Silicone-based operations [92,93]
30.	-	Injection	Peyronie's disease [94,95]
31.	-	Cream	Intra-abdominal adhesions [96]
32.	-	Injection	Urethral fibrosis [97,98]
33.	Oxygen-ozone	Injection	Lumbar disc herniation [99]
34.	Chloramphenicol	Mixture	Orthopedics and traumatology [100]
35.	-	Ointment	Eschar [101]
36.	-	Injection	Tumor [102]

CONCLUSION

Collagen being a very useful protein of the body can cause some serious disorders if gets deposited. Collagenase is an enzyme used for breaking down collagen and is most commonly used for the healing of wound and also used in severe diseases of bones and joints. Much research on the dosage forms of collagenases should be done in order to get rid of the problems interacting.

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