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Therapeutic applications of collagenase (metalloproteases): A review

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ABSTRACT

Non-invasive therapeutic methods have recently been used in medical sciences. Enzymes have shown high activity at very low concentrations in laboratories and pharmaceutical, enabling them to play crucial roles in different biological phenomena related to living organism, especially human medicine. Recently, using the therapeutic methods based on non-invasive approaches has been emphasized in medical society. Researchers have focused on producing medicines and tools reducing invasive procedures in medical. Collagenases are proteins which catalyze chemical processes and break the peptide bonds in collagen. Collagen may be generated more than the required amount or produced in unsuitable sites or may not degrade after a certain time. In such cases, using an injectable collagenase or its ointment can be helpful in collagen degradation. In both *in vitro* and *in vivo* tests, it has been revealed that collagenases have several therapeutic properties in wound healing, burns, nipple pain and some diseases including intervertebral disc herniation, keloid, cellulite, lipoma among others. This review describes the therapeutic application of collagenase in medical sciences and the process for its production using novel methods, paving the way for more effective and safe applications of collagenases.

1. Introduction

Matrix metalloproteinases (MMPs) comprise a group of zinc endopeptidases that have the ability to cleave the peptide bounds in the extracellular matrix (ECM) [1]. Peptidase enzymes can be divided into two groups: endopeptidase and exopeptidase. Endopeptidases, which are the focus of the present review, are divided into six major families, including metalloproteinase, aspartic, serine, cysteine, glutamic and threonine peptidases [2] (Figure 1). Most molecular structures of metalloproteases have been shown to use zinc but some others require cobalt. There are two subgroups of metalloproteinases, metalloexopeptidase or metallocarboxypeptidase and metalloendopeptidases, which include a disintegrin and metalloproteinase proteins and MMPs. MMPs can be comprised of collagenases (MMP-1, MMP-8, MMP-13, and MMP-18) (Table 1), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, MMP-11, and MMP-17), matrilysins (MMP-7 and MMP-26), membrane type (MMP-14, MMP-15, MMP-16, MMP-24, and MMP-25) and other types (MMP-12, MMP-19, MMP-20, MMP-21, MMP-22, MMP-28, and MMP-29) [3]. Collagenase was identified for the first time in 1962 [4]. Collagenase as well as MMP-13 and MMP-18 are the enzymes that break down four types of collagen (I, II, III, and IV), and only protease enzymes are able to hydrolyze the triple-helical domain of collagen under various physiological conditions. Interstitial collagen fibrils resist degradation by most proteinase enzymes. Only MMP-1, MMP-2, and MMP-3 could start the degradation of an intact, triple-helical collagen and of collagen types I, II, and III into one- and three-quarter sections. MMP-1 and MMP-13 preferentially cleave collagen types II and III, respectively [5], while collagenases have a covering that is a part of substrate specificity. MMP-8, however, degrades type I collagen three times more potently than MMP-1 or MMP-13. After initial cleavage, fibrillar collagen fragments become susceptible to further degradation by various MMPs such as MMP-2, MMP-3, and MMP-9 [6]. At the moment when the collagen is cleaved into smaller pieces, the endogenous enzymes help

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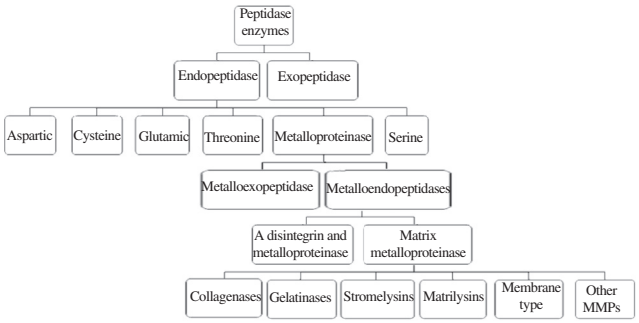

Figure 1. Peptidase enzymes diagram.

in breaking down the fibrous substance. Since collagenase does not harm the cell membrane, it has been greatly used in cell dispersion, tissue separation, and cell culture in laboratories for many years. For instance, *Clostridium histolyticum* (*C. histolyticum*) has been approved by the Food and Drug Administration (FDA) as a drug that breaks down the tough cords in Dupuytren and is widely used in human cell isolation, wound healing [7], and wound debridement [8].

The data for isolation and characterization of three types of collagenase enzymes from both microbial cells and animal tissues are available. Pathogenic microorganisms, mainly *C. histolyticum*, have been reported as microbial collagenase resources [9]. These collagenases could split each polypeptide chain of collagen into multiple sites [10]. It is known that they function as an exotoxin and disrupt connective tissue metabolism due to the hydrolysis of collagen in the host cells [11]. The recombinant bacterial collagenases are capable of hydrolyzing both water-insoluble native collagens and water-soluble denatured collagens [12]. In contrast to microbial collagenases that have been studied with focus on a single species, tissue collagenases seem more accessible to be isolated and characterized from a variety of tissues in different animals. Since tissue collagenases are digestive enzymes, they are commonly isolated from the digestive tracts of various fish and invertebrates. Collagenase is a unique enzyme used for the treatment of particular diseases, and excessive collagen aggregation can induce disorder in the etiology of organelles. Collagen is the major protein of the extracellular filamentous tissues such as tendons, skin, cartilage, and blood vessels, as well as the organic component of teeth, bones, and the cornea. Recently, two types of collagenase have been identified. The first type of collagenase is synthesized by some microorganisms. For example, *C. histolyticum* is a bacterium that causes gas gangrene. It produces the collagenases that can degrade the polypeptide chain of collagen over 200 states and hydrolyze the collagen in the c-terminal bonds. In addition, it can denature the connective-tissue barriers of the organism. Hence, the biological process of connective tissues depends on collagenase [13]. The second type of collagenase is synthesized by mammals. It can break down collagen triple helix from a certain point and make tropocollagens A and B, which include 3/4, and 1/4 of tropocollagen molecules, respectively. These broken fragments are then converted into gelatin chains. In addition to the collagenase, physical factors such as temperature can also separate tropocollagen polypeptide chains and produce the gelatin chains, which can be degraded by different proteases. One-third of the amino acid of collagen is composed of glycine, proline and hydroxyproline. Unlike mammal's collagenase, bacterial collagenase, which is one of the factors of bacteria virulence, attacks the different parts of the collagenal helix and breaks it down. Furthermore, there are collagenases in the invasive strains of *C. histolyticum* and *Pseudomonas aeruginosa*. Collagenases are more often used for the separation of tissue cells in medical investigation. These enzymes are successfully applied to remove and relocate the insulin gland cells (for diabetic patients) [14]. Using collagenases, one can also isolate intact liver parenchyma cells, fat cells, and adrenal [15]. After being isolated, these cells can be used for growing cells [16]. Collagenase has also been applied in G-banding technique to study human chromosomes. Today, collagenase has been employed as a treatment and seems to be able to replace some invasive treatments for diseases, in which excessive collagen deposition causes disorders in some physiological functions of body systems [17,18].

Regarding the sequencing of nucleic acid and the functions of protein, there are differences between the bacterial and vertebrate collagenases. The most commonly used microbial collagenase in medical products has been obtained from *C. histolyticum* [8–19]. Collagen forms one-third of the protein in the human body; however, the change in its production or degradation can be a source of problems. Collagen may be generated more than the amount which is required or produced in unsuitable places, or not degraded after the appropriate time. In such cases, the use of an injectable form of collagenase or its ointment can be helpful in the degradation of obstructive collagen. In the continuation of our discussion, some human diseases that can be treated with collagenases are described.

2. Methodology

In the present review, we performed a literature search using scientific sites such as ScienceDirect, Elsevier, Springer, PubMed, and Google Scholar. The search comprised the keywords collagenase, therapeutical, and disease. The references

Table 1
Classification of collagenases and its substrates.

Row	Enzyme	Matrix metalloproteinase	Group	Substrate
1	Collagenase 1	MMP-1	Collagenases	Collagens 1, 3, 7, 8, 10, gelatin, L-selectin, interleukin-1, entactin, ovostatin, MMP-2, MMP-9, proteoglycans, aggrecan
2	Collagenase 2/Neutrophil collagenase	MMP-8	Collagenases	Collagens 1, 3, 5, 7, 8, 10, fibronectin, gelatin, aggrecan
3	Collagenases 3	MMP-13	Collagenases	Collagens 1, 4, 9, 10, 14, fibronectin, MMP-9, gelatin, plasminogen, aggrecan, perlecan osteonectin
4	Collagenases 4	MMP-18	Collagenases	Type I collagen

found in the search were later conferred with details on the models or bio-assays used for examining the collagenase against diseases. In this review, interest has been focused on experimental studies performed on therapeutics.

3. Scientific evidence of collagenase therapeutic applications

3.1. Dupuytren's disease

Dupuytren's disease is an abnormal thickening of the tissue caused by the immoderate deposit of collagen tissue, mainly types I and III. In this disorder, one or more fingers of the hands, especially the small one, bent(s) into the palm and its function is reduced. The disease, in many cases, happens to the old white men, and it is likely a hereditary disease [20,21]. Collagenase from *C. histolyticum* source is a new cure for Dupuytren's muscle contraction, which was approved by FDA [22]. Before production of *C. histolyticum*, surgery operation was the only treatment for this disease [23]. To evaluate the long-period efficacy of collagenase *C. histolyticum*, this method is very beneficial to the patients [21]. Collagenase injection is more efficient than surgical fasciotomy [24], has less and milder side effects and exhibits a better total reduction of Dupuytren's contracture, which leads to higher patient satisfaction [25]. *In vitro* studies on the influence of clostridial collagenases on Dupuytren's cords [26] and on Dupuytren's disease fibroblasts [27] have demonstrated that *Clostridium* collagenases can efficiently digest ECM of Dupuytren's disease cords without inducing significant cytotoxicity or structural damage to non-collagenous tissue elements. Furthermore, it has been indicated that *Clostridium* collagenases controlled cellular spreading, attachment and proliferation [27].

3.2. Peyronie's disease

Peyronie's disease, known as induration penis plastica is categorized as a connective tissue disorder that involves in the growth of fibrous plaques in the soft tissue of the penis, and it affects 5% of men in the world [28,29]. The disease is caused by the deposition of excessive collagen, in which the penis is tilted to one side, damaged and failed to function properly. However, there are some methods for treatment of this disorder. Collagenase *C. histolyticum*, known as Xiaflex, is a drug approved by the FDA for treatment of Dupuytren's contracture and as an injectable medicine for treatment of Peyronie's disease. The drug has been reported to work by breaking down the extra collagen in the penis that causes Peyronie's disease [30].

3.3. Wound healing

Experimental investigations have shown that collagenase enzymes in the wound healing process can increase the proliferation, the angiogenesis, and the migration of dermal cells [31]. Collagenase enzymes remove the living process of necrotic tissues in the wound area but does not damage to the health tissue. However, the formation of granulation and epithelial cells are essential. Sometimes the healing process of bedsores and colloidal scars is not carried out properly, and the wound site becomes a suitable place for the growth of infectious agents. In these situations, collagen must be reduced and controlled. However, it is not easy to do this because collagen is not easily affected by proteases. In 2004, the value of *Lucilia sericata* larvae in the treatment of wounds was recognized by both USA FDA and the UK Prescription Pricing Authority so that sterile maggots can now be officially prescribed [32].

3.4. Burns

Burns are an important public health problem in the world. According to official statistics, 265 000 deaths occur per year due to burns alone [33]. Most deaths are caused by hot liquids, steam, electrical burns, and other forms of burns that global statistics are not available. Over 96% of fatal fire-related burns happen in low- and middle-income countries. In addition to those who die, millions more are left with life-long disabilities and deformity, often with resulting stigma and rejection [34]. Unlike serious burns, wound healing in partial-layer burn wounds is treated with collagenase ointment [35]. Many people are annually burned alive or died due to their injuries or infections. The standard treatment of burned tissues by collagenase has already been approved by the FDA [22]. Recently an investigation has indicated that collagenase ointment heals burn wound faster than the standard treatment of burns. In one study, 78 burn patients treated by collagenase *C. histolyticum* ointment were compared to 41 patients whose tissues around burn wounds were removed surgically [36]. The use of collagenase provides a short-time hospitalization and shortens the overall need for surgery and blood transfusions in partial-layer burns. Therefore, collagenase can be considered as the first treatment option to remove scars in infants having a partial-thickness burn wound without infection [37].

3.5. Glaucoma

Glaucoma disease is the major reason for permanent blindness. Approximately 60.5 million people suffered from this illness in 2010, with an estimated increase of about 79.6 million by 2020. To control the disease, therapies that decrease intraocular pressure are common, irrespective of the type of glaucoma. Collagenase injection is a method for treatment of glaucoma [38]. The use of collagenase in the production of a drug helps to treat intraocular scarring and fibrosis that happen after glaucoma filtration surgery as well as after the implantation of set on implants. Glaucoma occurs when the fluid drainage channels of the eye are closed because of abnormal production of collagen. Almost 40% of blindness is caused by this disease. Using collagenase to treat glaucoma was patented in 2013 [38].

3.6. Intervertebral disc herniation

One of the factors in low back pain is the aggregation of collagen tissue and the reduction in the distance between the vertebral spines. Investigations have shown that injection of enzymes such as collagenase can be an improvement in indications of disc herniation [39,40]. Nowadays, more research is being performed on this issue [39–41]. In a study by Zhang *et al.*, 29 patients with perpetual low back and sciatic pain were injected with intradiscal collagenase at a single abnormal disk space. After conservative treatment for 2 months and resting for 2 weeks in bed, 6 patients (21%) obtained complete pain relief, while 12 (42%), 6 (21%), and 1 patients got noteworthy, moderate, and slight pain relief, respectively [39].

3.7. Debridement

Debridement is the elimination of unhealthy tissue from a wound for improvement of healing. This procedure, which is a vital factor in wound bed preparation, can be carried out by surgical, chemical, mechanical, or autolytic removal of the tissue. In clinical activities, various techniques are commonly used for debridement. Many studies have confirmed that collagenase is a safe and an effective choice for debridement of cutaneous lesion and burn wounds [42,43]. One of the most well-known applications of microbial collagenases in the health industry is related to the use of the collagenase *C. histolyticum* in the enzymatic debridement of wounds and other injuries, where the removal of devitalized tissue is necessary [44]. Debridement is the removal of foreign material as well as devitalized or contaminated tissue from a wound bed. This process is applied to the treatment of pressure ulcers, leg ulcers, wounds and burn wounds, and chronic, non-healing, or indolent wounds considered to be stalled in the inflammatory phase of wound healing [44]. Management of these chronic non-healing ulcers or wounds is a difficult clinical problem [45], and the search for effective therapeutics has been a key target for many years [46]. In fact, enzymatic debridement has been suggested as a therapy more than half a century ago and has been continuously applied till the current days [47]. In several studies, enzymatic methods were compared with surgical/mechanical methods as well as between several enzymes. The results were controversial. Some studies indicated that wound debridement is more effective than enzymatic procedures [48] but others suggested that surgical methods give faster results. However, enzymatic procedures seem to be slightly more effective when the substrate is eschar as opposed to fibrin slough. The use of *Clostridium* collagenase is considered as a capital gain in wound debridement. It helps to avoid the complications of surgery and also limit the progress and enlargement of the necrotic tissue. Enzymatic debridement decreases the number of surgical debridement and the duration of hospital stay [8,36,43,49–53].

3.8. Degradation of human retained placenta

In an experimental study, placenta samples were covered with bacterial collagenase solution at several concentrations. Human placenta and equine placenta collagens have the most sensitivity to bacterial collagenase degradation. Average collagenase activity found by the release of hydroxyproline (a major part of collagen) from human was 1.6 times. However, in equine placenta, it was three times greater than that of bovine. When injected into complete placenta, the collagenase digested placenta smoothly within 6–12 h. Placenta was changed to liquid over night although umbilical blood vessels resisted bacterial collagenase degradation. Bacterial collagenase was highly effective in the decomposition of human placenta collagen. Intraplacental injections of bacterial collagenase via umbilical cord arteries may help to detach retained placenta in women [54–58].

3.9. Cartilage repair

When using injectable collagenase to repair cartilage, a significant increase is occurred in chondrocyte cell compactness in ulcer. Treatment with collagenase after preparing a high quality purified enzyme caused cartilage repair, perhaps by increasing the cell density at cartilage lesion edge. Therefore, to have a better healing, surgical operation with the aim of promoting the renovation of particular cartilage defects may be beneficial before treating with these enzymes [56–58].

3.10. Nipple pain

It has been well documented that breastfeeding has enormous benefits for both mother and baby; however, nipple pain is a common cause reported by adult female for the early termination of breastfeeding. Some studies have compared several treatments that prevent or treat nipple pain [59,60]. One of these treatments is the application of injectable bacterial collagenase for treatment of nipple pain [61].

3.11. Keloid

Keloidal scar induces as the result of an excessive growth of granulation tissue (collagen type 3) at the place of a cured skin injury, which is not quickly substituted by collagen type 1. Keloids are steady elastic lesions or bright stringy nodules that can change their color from pink to red or dark brown. Keloids should not be confused with hypertrophic scars, which are raised scars that do not grow beyond the boundaries of the original wound [62]. Collagenase pursuited by compression seems to be a secure and decently effective care for keloid disease [63].

3.12. Vitrectomy

Another use of collagenase is the elimination of fibroproliferative tissue in specific vitrectomy cases. It has been determined that satisfactorily purified collagenase can create extensive degrade of scar tissue after a 10-, 15- or 30-minute incubation. There has been no phenotypic damage to scar cellular sections or to the internal restricting membrane of the retina [64]. A 45-minute exposure of retinas before damaged by photocoagulation to collagenase enzyme also did not result in morphologic witness of injury [65,66].

3.13. Cellulite

Cellulite or adiposis edematosa is the hernia of subcutaneous fat within fibrous connective tissue that indicates skin dimpling, and the symptoms often are on the pelvic region, lower limbs, and abdomen [67]. Cellulite occurs in most sexually mature females [68]. Bacterial collagenase is an effective enzyme for the treatment of cellulite.

3.14. Chronic total occlusions (CTO)

Clostridial collagenases have been used for the treatment of CTO in non-human models [69]. CTO is a coronary problem [70]. It includes a lot of degrees of fibroma, atheromatous emboli, and thrombus, relies on the blockage handle and its time period and it happens in nearly 30% of the patients with this disease. CTO is usually used to be cured by surgery or less regularly percutaneous intervention. In most cases, despite the ischemia upraises using percutaneous intervention as a treatment for CTO, surgery is preferred. However, percutaneous intervention is limited for inability to cross the CTO with leader wires because of the attendance of occlusive fibrotic plaques [69]. The combination of percutaneous intervention with collagenolytic

therapy increases the success rate of CTO treatment. Collagen tissue is the major structural ingredient of atherosclerotic plaques and the use of a collagenolytic enzyme to the plaques prior to leader wire crossings results in plaque breakdown and therefore to a significant 2-fold betterment [71]. Clinical trials of collagenase have currently revealed that in human manners, application of topical delivery of collagenase enzyme into coronary whole occlusion is possible and safe [72]. However, more clinical trials are still necessary to prove exactly the efficacy of this technique in humans; the result is very promising.

3.15. Gene delivery

Recently, collagenases have been used in genetic therapy. Actually, the simplest and safest technique for gene delivery can be the implementing of decorated nucleic acids into cells and tissues [73]. However, gene therapy technique suffers from one disadvantage, which is the low efficiency of gene expression [74]. Macromolecules, such as monoclonal antibodies and DNA vectors, broadly show low activity in solid neoplasm for gene therapy [75]. The application of collagenase (purified or mixed with hyaluronidase) indicates transfection efficiency in the spreading of metastases, particularly in abnormal swelling of any part of the body with a high segment of ECM [76]. It has also been proved that this method is effective in the bony muscle and liver tissue [77], wherein in addition to a raise in the transfection ratio, it causes an increase in the transfection homogeneity [77].

3.16. Uterine fibroid

A uterine fibroid is a leiomyoma that is secreted from the flat muscle layer of the uterus. The disease is often multifarious, and if the uterus contains too many leiomyomata to count, it is mentioned as propagate uterine leiomyomatosis. Having approved by FDA as a drug that does not influence nervous system or blood vessels, *C. histolyticum* collagenase has been evaluated as a suitable treatment for this disease [78]. Diversity of collagen types and amounts within personal fibroids can lead to various reactions and require supplementary investigations. The injection of *C. histolyticum* collagenase has potential to be used for the treatment of fibroids [79].

4. Conclusions

Therapeutic methods based on non-invasive approaches have recently been emphasized in medical community. Researchers have focused on producing medicines and tools that reduce invasive procedures in medical practice. Enzymes have important capacity in pharmaceuticals activities due to their highly selective character and high specific at very low concentrations. True collagenases cleave helical regions of collagen molecules in fibrillar form under various physiological conditions of pH and temperature. However, it is known that gelatin and the non-helical regions of collagen molecules could be degraded by numerous mammalian proteases, including pepsin, trypsin, chymotrypsin, papain, and other tissue enzymes. The study of collagenases started at the end of last century, followed by the isolation of an extracellular enzyme, namely *Clostridium* and then by identification and characterization of a number of other collagenases of both bacterial and mammalian origin. Until recently, the production of true collagenases by bacteria has been considered to be confined to only a few species [19–37] such as clostridia and a small number of other organisms, notably a strain of *Vibrio alginolyticus* (formerly *Achromobacter iophagus*). Unlike animal collagenases enzyme that split collagen in its native triple-helical structure [80], collagenases from bacteria differ from those of vertebrates, which demonstrate broader substrate specificity [81]. Regarding its recently proposed application, collagenase enzyme appears to be a convenient and a cheap medication for the treatment of burns, wound healing, and some other diseases in near future. However, it seems to be produced and used as a drug in clinics due to gaps in data and needs for further research. In the current review, all available and relevant published papers pertaining to therapeutic application of collagenase in human diseases were used. Human diseases and collagenases have been the center of this review, while role of collagenases in the treatment of more specific diseases that excessive collagen deposition is the main problem were emphasized [1,2]. Furthermore, collagenases can be applied in the isolation of liver parenchymal as well as fat and adrenal intact animal cells [5,6] and in cell culture after their separation. To sum up, this review describes the therapeutic application of collagenase in medical sciences and the process for its production using novel methods, which paves a way for more effective and safe applications of collagenases. There are some hope that future investigations can develop methods and processes to produce collagenase with new origins such as *Lucilia sericata*, which is non-pathogenic and very important to wound healing.

Conflict of interest statement

We declare that we have no conflict of interest.

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