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REVIEW

Tryptase and Exogenous Trypsin: Mechanisms and Ophthalmic Applications

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Abstract: Ocular injuries caused by inflammation, surgery or accidents are subject to a physiological healing process that ultimately restores the structure and function of the damaged tissue. Tryptase and trypsin are essential component of this process and they play a role in promoting and reducing the inflammatory response of tissues, respectively. Following injury, tryptase is endogenously produced by mast cells and can exacerbate the inflammatory response both by stimulating neutrophil secretion, and through its agonist action on proteinase-activated receptor 2 (PAR2). In contrast, exogenously introduced trypsin promotes wound healing by attenuating inflammatory responses, reducing oedema and protecting against infection. Thus, trypsin may help resolve ocular inflammatory symptoms and promote faster recovery from acute tissue injury associated with ophthalmic diseases. This article describes the roles of tryptase and exogenous trypsin in affected tissues after onset of ocular injury, and the clinical applications of trypsin injection.

Keywords: trypsin, tryptase, inflammation, proteinase-activated receptor 2, ophthalmology, therapeutic

Introduction

As in other mammals, the human eye is a sensory organ that converts light stimuli into signals that are transmitted to the brain to produce vision. The colour, shape and depth of field of real-world objects are detected by rod and cone cells in the retina, situated at the back of the eye. Approximately 10 million colours can be distinguished by the human eye,[1] which is the most intuitive and essential organ of the body because it allows people to perceive external reality, and is the most direct and essential indicator of their inner state. Therefore, maintenance of normal eye function and appearance is vital to the well-being of patients. Among the most common ophthalmic diseases, conjunctivitis and uveitis are mainly treated with medication, whereas cataracts, glaucoma, retinal detachment and rupture injuries are usually remedied through surgery. During the course of disease development and recovery, inflammation, injury and repair all take place.[2–4] Therefore, development of treatments that target these processes and their mechanisms in the eye is a timely and essential research area.

Tryptase is produced by mast cells in response to inflammation, can contribute to disease pathogenesis.[5,6] Although endogenous trypsin has similar effects, it is primarily associated with pancreatic-related disease and does not play a major role in systemic inflammation and injury.[7] Exogenous trypsin, however, paradoxically displays anti-inflammatory and anti-infective properties, promoting damage repair.[8,9] Although the clinical therapeutic role of exogenous trypsin in pancreatic, lung and orthopedic diseases has been well studied, it has not been as widely investigated in the ophthalmic context.[10–12] Here, the roles and mechanisms of tryptase and exogenously applied trypsin in ophthalmic diseases are reviewed and investigated, and the clinical applications of exogenous trypsin injection are also discussed.

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Tryptase

Source and Action Mechanism of Tryptase

At sites of inflammation, mast cells secrete tryptase, which are members of the same family of enzymes as trypsin. The granules found in the secretory vesicles of mast cells can be additionally classified into two major types: one containing mainly tryptase, and the other containing chymotrypsin, cathepsin G and carboxypeptidases in addition to trypsin-like enzymes.[13] Mast cells can therefore be regarded as the central mediators of tryptase release. Previous studies have shown that mast cells play a key role in the regulation of several physiological processes in the body.[14,15] The mast cell is involved in wound healing and bone remodelling. These two processes would be impaired without mast cells.[16,17] Mast cells store pre-formed tryptase that are released when stimulated to affect fibroblast and epithelial cell proliferation, leukocyte recruitment and collagen synthesis in damaged tissues such as tryptase.[18,19] In addition to wound healing, the processes of angiogenesis and lymphangiogenesis are also influenced by mast cell.[20,21] They produce a variety of angiogenic mediators such as histamine, tryptase, matrix metalloproteinases (MMP)-2 and MMP-9, vascular endothelial growth factor A, platelet-derived growth factor and fibroblast growth factor.[22–24] A number of studies have revealed that tryptase performs critical functions in the pathogenesis of inflammation. It regulates expression of fibrinogen (including activation of tissue matrix metalloproteinases), stimulates proliferation of fibroblasts and synthesis of collagen, and promotes eosinophil chemotaxis and IL-8 production.[25] In addition, abnormal vascularisation, the essential aspect of chronic inflammatory responses, is involved in tissue remodelling and carcinogenesis.[26,27] It has been reported that mast cell release of tryptase, basic fibroblast growth factor (bFGF/FGF2) and vascular endothelial growth factor (VEGF) can promote neovascular sprouting and angiogenesis.[28] Thus, tryptase may promote inflammation primarily by control of fibrinogen expression and modulation of tissue vascularisation. In summary, mast cells have the ability to secrete a wide range of enzymes and tryptase is involved in a large number of organismal metabolism, growth and damage repair processes.

Mast cells can stimulate tryptase release by neutrophils, further promoting extracellular matrix degradation and inflammatory processes.[29–31] Furthermore, tryptase-activated neutrophils have traditionally been viewed as a frontline defence against microbial infections, carrying proteases that can destroy invaders but might also cause considerable collateral damage to the host.[32] Once activated, neutrophils release soluble factors and granules into the extracellular environment both to facilitate their translocation, and to promote fixation or killing of pathogens.[33] Neutrophil degranulation has been associated with the pathogenesis of dry eye, as has upregulated release of various soluble inflammatory mediators including MMP-9, lysozyme and tryptase.[34–36] In turn, tryptase induce the secretion of large amounts of lactoferrin and IL-8 from neutrophils, thereby activating inflammatory cells, promoting acute-phase protein synthesis, causing fever and leading to pathological inflammatory damage.[37] Therefore, neutrophils are also essential effector cells for the actions of tryptase.

The mechanisms by which tryptase exert these effects are not well understood. The PARs are members of the wider G-protein coupled receptor (GPCR) class of transmembrane proteins,[38] and to date, four PARs have been identified: PAR1, PAR3 and PAR4—which are all activated by thrombin—and PAR2, which is activated by myeloblastin or tryptase.[39,40] Thus, PAR2 is a major site of tryptase action. In fact, PAR2 is widely distributed in human tissues. Its expression has been detected at the mRNA level in glandular epithelial cells of the stomach, intestine, pancreas and salivary glands, as well as in tracheal epithelial and vascular endothelial cells, kidney, liver, and the peripheral and central nervous system. Importantly, it is also expressed in the conjunctival, uveal and retinal tissues of the eye.[41–44] PAR2 is a crucial element of the inflammatory response by involvement in neutrophil recruitment, increased pain, swelling, and perfusion. PAR2 also presents in the eye to mediate inflammatory responses resulting from the injury. We therefore believe it constitutes an important target in ocular inflammation.

To summarise the above, in tissue injury and disease states, tryptase production and consequent downstream signalling are mainly associated with mast cells and neutrophils via PAR2 activation. This can promote local inflammatory responses and exacerbate tissue injury.

Research in Ophthalmic Disease

Keratoconjunctivitis

In the pathology of keratitis, tryptase can stimulate expression of inflammatory proteins and directly influence extracellular matrix degradation, loss of epithelial barrier function and activation of MMP-9.[45–47] Mast cells degranulate and release tryptase after exposure to allergens such as pollen. Tryptase could activate PAR2 in the conjunctival epithelial cells, in contrast, patients have lower levels of PAR2 in conjunctival epithelial cells.[48,49] In addition, tryptase may in turn activate other cells or toxic products, leading to chronic inflammation and hypersensitivity, particularly in eosinophils.[50] Recent studies have shown that tryptase secreted by mast cells settled at the corneal rim may be involved in maintaining the ocular surface microenvironment under normal conditions in addition to participating in IgE-induced inflammatory responses.[51] These suggest that tryptase plays an important role in the pathogenesis of allergic conjunctivitis.

Allergic Eye Disease

Furthermore, mast cells are essentially and functionally involved in the allergic state. In ocular tissues, these cells are abundant in the uveal and conjunctival bundles, including the choroidal microvasculature in rats, as well as human choroidal tissue.[52,53] Tryptase is a gelatinase with powerful gelatin degrading properties.[54] Trypsin alone or in combination with MMPs can lead to extracellular matrix damage and may therefore be involved in the destruction of the choroidal matrix.[55] The accumulation of oxidised lipids prevents the normal flow of nutrients and metabolites, leading to ageing of the eye and consequently to diseases such as age-related macular degeneration.[56] Tryptase secreted by mast cells is stimulated to be secreted in the choroid and attracts LDL to bind to it, leading to lipid deposition and triggering a chronic inflammatory response.[57]

Uveitis

Uveitis is an ocular autoimmune disease with predominantly vascular leakage and aseptic inflammation. Its pathogenesis is currently unclear, although macrophages apparently have an essential role.[58] As already mentioned, a significant population of mast cells is found in the choroidal and uveal tissue, mostly distributed in close proximity to blood vessels in the endochoroidal vascular layer.[59] In IgG4-related ocular disease, Mikulicz disease causes swelling and fibrosis of its extraocular muscles, trigeminal nerve and optic nerve, and both the number of mast cells and the secretion of tryptase in the orbital tissues of patients are significantly increased in the disease state.[60] The granules of these choroidal mast cells contain both chymotrypsin and tryptase,[61] and degranulation in response to various immune and non-immune stimuli induces release of tryptase (as well as other factors), promoting inflammatory responses in the surrounding tissues.[62] Thus, many mast cells are present in the intrachoroidal vascular layer, which are capable of releasing a range of mediators involved in ocular inflammatory responses, wound healing and host defence. Tryptase production by mast cells may therefore be a significant aspect of the pathogenesis of both allergic and infectious ocular inflammation.

Dry eyeThe tear film in patients with dry eyes is often associated with tear instability, hyperosmolarity and ocular surface inflammation and damage.[63] These pathological changes are associated with dysfunction of the corneal epithelial barrier, which impairs tear film permeability and cellular circulation. Both the conjunctiva and cornea of dry eye patients have significantly more mast cells than normal. Tryptase secreted by mast cells also plays an important role in the pathology of dry eye, promoting the expression and activation of pro-inflammatory cytokines, affecting the degradation of extracellular matrix components and the loss of epithelial barrier function.[64] In addition, tryptase activates corneal PAR2 receptors, which in turn activate MAPK and NF-κB signalling pathways to continue to enhance the local inflammatory response. Furthermore, PAR2 receptors are also associated with neuropathic pain, which may be clinically relevant to the ocular pain felt by some patients with dry eyes.[65]

Fundus Lesions

Mast cell degranulation and tryptase secretion can also mediate the production of monocyte chemotactic protein 1, and promote endothelial cell proliferation and angiogenesis by upregulating expression of downstream factors through an autocrine pathway. For example, tryptase induces VEGF production, whereas this is not the case with TNF-α, histamine

and other chemokines.[66] Tryptase released by choroidal mast cells may therefore make an important contribution to inflammatory and angiogenic processes in the retinal pigment epithelium. Tryptase might also stimulate migration of retinal pigment epithelial cells by regulating ERK1/2 phosphorylation,[67] since it rapidly induces phosphorylation and activation of both kinases in retinal pigment epithelial cells. This suggests that PAR2 is expressed in these cells, can be stimulated by secreted Tryptase, and induces cell migration via ERK1/2 activation. In support of this, PAR2 knockdown in mice produced significant intravitreal neovascularisation, which could be rescued by treatment with a PAR2 agonist peptide, accelerating normal haematologic reconstitution.[43] Thus, retinally expressed PAR2 seemingly regulates oxidative stress-induced retinal inflammation, such that activation of the receptor can inhibit inflammation and promote hemodialysis in ischaemic retinal lesions. Thus, in the context of retinal neuronal disease, activation of PAR2 may promote angiogenesis, help restore local tissue circulation to retinal neurons, and promote healing of lesions. However, the tryptase-activated PAR2 receptor may play opposing roles in different diseases, and the exact mechanisms involved should therefore be explored in further studies.

Mast cells are widely distributed in the eye, but are temporarily absent from the retina and their presence in the vitreous is only detected in pathological states.[68,69] Study has shown that vitreous concentrations of tryptase are significantly higher in patients with idiopathic pre-macular and macular fissures than in patients with proliferative diabetic retinopathy and retinal detachment.[70] Recent studies have identified bursa premacularis as a specific capsule-like structure present in the pre-macular vitreous.[71,72] Mast cells have been found within the bursa premacularis, which may be the source of the mast cells and their secreted tryptase in the vitreous.[73] In the physiological state, they serve to maintain the function of the retinal stem cells, but in the presence of photostimulation they can lead to cellular ageing and increased tryptase secretion leading to the activation of the fibrotic process and the formation of the pre-macular membrane and macular fissures.[70,74] Additionally, In early age-related macular degeneration (AMD) with geographic ("map-like") atrophy, recent work has shown an increase in both the number of choroidal mast cells and their rate of degranulation. The sustained degranulation in these lesions releases tryptase into Bruch's membrane and the choroidal stroma, resulting in retinal pigment epithelium deficiency, reduced electroretinogram amplitudes, and retinal and choroidal thinning.[75] Therefore, endogenous tryptase may contribute significantly to the formation of atrophic fundus lesions such as those found in AMD, and targeting regulation of tryptase regulation might therefore serve as a promising new research direction.

In summary, tryptase secreted by mast cells not only plays an important role in the maintenance of normal ocular function, but also induces inflammatory responses, fibrosis and angiogenesis in pathological situations (Figure 1). Therefore, treatment targeting tryptase and its receptors in pathological situations may have a better therapeutic effect in controlling the inflammatory response, fibrosis formation and angiogenesis.

Therapeutic Targeting of Tryptase

PAR2 Drug Therapy

The primary receptor for endogenous trypsin is PAR2, so the effects of extracellular trypsin secreted from mast cells and neutrophils should be attenuated if inhibition of PAR2 signalling leads to suppression of downstream pro-inflammatory processes. Because mast cells can also act as antigen-presenting cells to activate T cells, inhibition of PAR2 could potentially inhibit acute inflammatory responses mediated by both innate immune cells and trypsin, reducing the recruitment of T cells to traumatised tissues.[76,77] Blocking PAR2 activation may therefore offer therapeutic benefits in a subset of conditions where it has been implicated in inflammatory pathogenesis. In other disease contexts, however, PAR2 activation is beneficial. It has been shown to be important in colitis, arthritis, inflammatory bowel disease and allergic asthma.[78–80] Because of this two-sided nature of PAR2 action, different treatment approaches are needed for different diseases, and the appropriate clinical applications of PAR2 antagonists therefore require further research and evaluation.

Various approaches have been investigated to interfere with PAR2 function; for example, development of small-molecule inhibitors, as well as tethered ligand blockers (including blocking antibodies). Early small-molecule PAR2 antagonists were peptidomimetic drugs based on the tethered ligand sequence that is exposed by trypsin cleavage of the

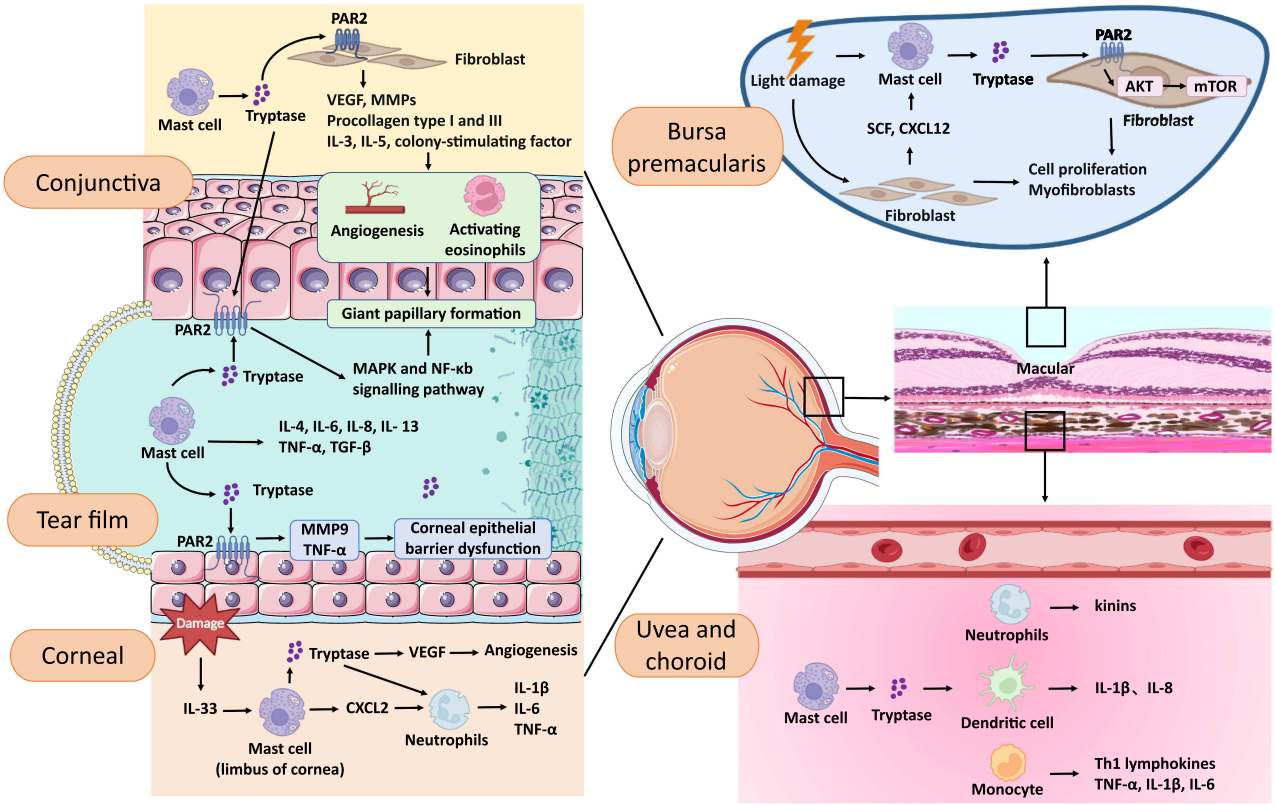

Figure 1 The distribution of tryptase in ocular tissues and their local role following injury and their mechanisms.

receptor. In a mouse model of arthritis, N^1-(3-methylbutyryl)-N^4-(6-aminohexanoyl) piperazine (ENMD-1068) was shown to inhibit PAR2 activation induced by trypsin and to reduce symptoms, but a lack of efficacy ultimately prevented the drug from entering clinical trials.[81] A compound developed later, *N*-[(2*S*)-3-Cyclohexyl-1-[[(2*S*,3*R*)-3-methyl-1-oxo-1-spiro[indene-1,4′-piperidine]-1′-ylpentan-2-yl]amino]-1-oxopropan-2-yl]-1,2-oxazole-5-carboxamide (GB88), is a potent and reversible PAR2 antagonist. It was shown to block receptor activation by endogenous trypsin both in vitro and in vivo, and also to alleviate the inflammatory response in experimental colitis in rats.[82] Interestingly, GB88 selectively inhibits Ca^{2+} release via the PAR2-stimulated signalling pathway without affecting the activation of MAP kinases.[83] This suggests that the compound might inhibit coupling of PAR2 to one or more of its partner G proteins, but not all of them. Thus, the compound can modulate Ca^{2+} mobilisation suggesting that its pharmacological effects may vary in different disease states.

Studies with PEGylated ligand sequences found that function-blocking antibodies targeting the PAR2 tethered ligand sequence can inhibit trypsin-mediated inflammatory responses in vitro, and reduce joint inflammation mediated by PAR2 in vivo, although no clinical trials have yet been conducted.[84] Among currently developed sequences based on the tethered ligand of PAR2, some display antagonism and exert anti-inflammatory effects, whereas others show agonist activity, exerting pro-inflammatory effects.[85] These observations may be related to the function of PAR2 itself, and therefore, the therapeutic approach based on antibodies targeting the tethered ligand sequence of PAR2 needs further investigation.

That said, PAR2 receptors are widely distributed in ocular tissues, and topical ocular administration of antagonists—which could include eye dosing, conjunctival sac irrigation, subconjunctival injection, peribulbar injection and retrobulbar injection—would avoid first-pass metabolism compared with systemic administration, allowing the drugs to act directly on the eye and its surrounding tissues. Thus, PAR2-targeted treatment in the eye may have fewer adverse effects and drug complications. Although considerable progress has been made in characterising biased signalling in vitro and

in vivo, and in elucidating PAR function in vivo, only one FDA-approved drug has thus far resulted, which targets the orthosteric binding site of PAR1.[86] Therefore, development of new drugs targeting PAR2 has strong clinical implications. According to existing studies, the small-molecule inhibitor GB88 blocks some PAR2-mediated signalling pathways, but not all of them, and is therefore a useful research tool.[87,88] Small molecule PAR2 ligands with differential effects on downstream pathways may also be sought. However, there is a need for multiple small molecule PAR2 ligands with differential effects on downstream pathways, in order to improve clinical efficacy and reduce the frequency of adverse events. And with the understanding of how small molecules modulate the function of such important receptors by binding to different pockets on and within proteins, there are far-reaching implications for the structure-based drug design of novel therapeutic agents.[89] For example, structure-based drug design might represent an attractive and feasible strategy for the development of new generations of PAR2 inhibitors. These different designs would help develop new PAR2-related drugs that maximise therapeutic efficacy with fewer side effects.

Drug Therapy for Mast Cells

Pharmacological targeting of mast cells is also possible. Ketotifen fumarate is an H_1-histamine antagonist and serotonin release inhibitor with three major known modes of action: inhibition of histamine receptors, prevention of eosinophil accumulation and stabilisation of mast cells.[90,91] Although the latter effect is not well understood, it may relate to the capacity of the drug to counteract plasma membrane deformation during degranulation of mast cells.[92] Furthermore, topical ketotifen fumarate has been shown to reach the posterior ocular tissues, inhibit the cytosolic processes of mast cells, prevent mast cell degranulation in rats, and protect the retinal pigment epithelium. Topical spot-on delivery of ketotifen fumarate allows the drug to reach the retinal pigment epithelium, choroid and sclera; and although it is also detected in plasma after this route of administration, this is at levels significantly lower than in the ocular tissues.[93] This suggests that ketotifen fumarate eye drops may partially enter the systemic circulation, but at small and relatively safe doses. Additional research has revealed that from the conjunctival and corneal surfaces, drugs can enter the eye via the transvitreal, uveoscleral and periocular pathways.[94] However, the amount of drug that penetrates from these surfaces into the eye is dependent on the nature of the drug and its formulation, the anatomical features of the eye, and differences between species. Whether ketotifen fumarate can be used in this way therefore requires further experimental and clinical validation.

Drug Therapy for Neutrophils

Therapeutic targeting of neutrophil trypsin release is also now available. A recently developed serine protease inhibitor, UAMC-00050, inhibits trypsin secretion by neutrophils, in turn reducing activation of PAR2, attenuating downstream inflammatory responses, and preventing tissue damage. In addition, UAMC-00050 reduces the antigen-presenting function of centrophils and inhibits T-cell aggregation.[95] In dry eye studies, the inhibitor demonstrated therapeutic potential by significantly reducing levels of two cytokines, TNF-α and IL-1α, in tear fluid;[44] however, it is not easy to preserve, and development of a stabilising carrier formulation is therefore needed for further preclinical and clinical investigations.

Nonetheless, treatments targeting endogenously produced trypsin can intervene not only in terms of its secretion (from mast cells and neutrophils), but also through modulation of the trypsin receptor (PAR2). Both aspects are effective in controlling the inflammatory response and tissue oedema caused by trypsin, and although there are currently few effective therapeutic agents, the available data do offer theoretical support for future development of clinical treatments acting via this mechanism.

Therapeutic Use of Trypsin

Trypsin and Its Clinical Applications

In the human body, various enzymes are essential for processes involving protein hydrolysis in cells and tissues—including angiogenesis, along with tissue morphogenesis and repair—through which pain, swelling of tissues, and healing time are reduced.[96] Thus, enzyme therapy is sometimes used, with oral administration being the most common

route for systemic diseases. Ingested enzymes have been shown to protect against oxidative stress and affect cell signalling and immune function.[97] Thus, enzyme therapy appears to dampen the physiological overreaction of the body to disease and injury, allowing it to more effectively utilise its inherent repair mechanisms to restore damaged structures.

The use of protein hydrolytic enzyme preparations dates back to 1876, when pineapple protease (bromelain) was first extracted and applied therapeutically.[98] Since then, protein hydrolases have not only been used as anti-inflammatory and anti-oedema agents, but have also been found to possess fibrinolytic, immunomodulatory and analgesic properties.[99] When clinical and experimental studies demonstrated that orally administered exogenous protein hydrolases displayed high bioavailability without loss of functional activity, use of these enzymes in the clinical greatly increased. They were mainly used to promote tissue repair, and exogenous protein hydrolases thus emerged as a useful therapeutic strategy for treatment of acute injuries.

Trypsin is one of the most important exogenous protein hydrolases, and it entered clinical use in the 1960s.[100] When taken orally, it is absorbed through the intestinal tract and acts throughout the body.[101] Trypsin is an endopeptidase which selectively hydrolyses peptide chains at arginine and lysine residues. It can hydrolyse natural or denatured proteins, fibronectin and mucin into smaller peptides or amino acids.[102] This selective hydrolytic effect of trypsin is mainly related to the structure of the protein. It is said that trypsin only hydrolyses denatured protein, which can be produced under the influence of external physical or chemical factors. Once denatured, the structure of substrate proteins will become looser, exposing the specific hydrolysis sites and allowing trypsin to break them down into shorter amino acid chains.[103] Additionally, when used therapeutically, trypsin can promote the penetration of chemotherapeutic drugs and antibiotics into the target lesion, and also has an auxiliary effect on the absorption of drugs.[104] Clinical use of trypsin has additionally been shown to help prevent inflammation and haematoma formation, as well as displaying antioxidant properties by reducing formation of free radicals, thereby helping maintain redox homeostasis over the long term.[105] One study demonstrated that oral trypsin use can modulate the dental implant microenvironment by increasing fibronectin-induced angiogenesis, nerve protrusion extension and migration activity; and also identified a potential role in promoting pulp regeneration in older teeth.[106] Trypsin treatment in burn patients reduces tissue oedema, inflammation and oxidative stress, thereby reducing tissue destruction and accelerating the repair process.[107] Relatedly, trypsin can be used in post-surgical debridement to directly clean the wound and promote healing of surgical incisions. Furthermore, systemic application of trypsin in patients with ankle sprains was shown to reduce joint oedema and soft tissue damage, and improve the rate of recovery.[108]

In summary, trypsin is used as a complementary protein hydrolase for the treatment of various injuries and inflammatory diseases.[109] It accelerates wound purification in tissues and promotes granulation tissue regeneration. Due to the protective effect of naturally occurring trypsin inhibitors in vivo, it causes no (or minimal) damage to normal tissues.

Mechanism of Action of Exogenous Trypsin

At the onset of inflammation and tissue injury, α1-antitrypsin and α2-macroglobulin rise dramatically, leading to fibrinolytic enzyme shutdown to maintain the tissue inflammatory response, which can cause oedema and delayed repair. Trypsin application can target the early stages of inflammation to exert fibrinolytic effects, and its anti-inflammatory properties might also be due to the fact it can reduce the effects of pro-inflammatory cytokines such as IL-1, TNF-α and IFN-γ in affected tissues.[110] Compared to fibrin, α1-antitrypsin has a stronger affinity for trypsin, so treatment with exogenous trypsin restarts the fibrinolytic process in inflamed and injured tissues: the fibrin barrier is broken, promoting absorption of fibrin exudate. This helps restore the local microcirculation, clear inflammatory oedema, and promote tissue repair.[111] Trypsin not only acts directly to reduce the level of reactive oxygen species (ROS) and oxidative stress, but also to enhance the levels of both non-enzymatic and enzymatic antioxidants in the body. This further boosts its efficacy as an anti-inflammatory and antioxidant agent, attenuating inflammatory responses and promoting faster healing.[112] Thus, exogenous trypsin reduces tissue destruction and inflammatory oedema in patients with acute injury, thereby promoting rapid healing.

In addition, trypsin may also play a role in promoting wound healing by regulating the differentiation of fibroblasts. After injury has occurred, monocytes are recruited by chemokines to the vicinity of the wound and to the site of tissue damage.[113] Once in the injured tissue, monocytes differentiate into fibroblast-like cells called fibroblasts.[114] Fibroblasts are CD45+ cells that express collagen and contribute to the formation of scar tissue, a key component of wound healing and fibrotic disease,[115] and the development of the body's connective tissue response to foreign or invasive objects.[116] Increased fibroblast differentiation in the wound healing environment enhances wound healing and decreased fibroblast differentiation results in slow wound healing.[117] Further studies have shown that the effect of trypsin on fibroblast differentiation is not mediated by PAR as cells treated with trypsin in protein-free medium do not increase fibroblast formation, nor does chymotrypsin replace trypsin to enhance fibroblast differentiation. Thus trypsin enhances the differentiation of monocytes into fibroblasts in culture, and albumin is necessary for this enhancement to occur. This suggests that topical trypsin and trypsin-treated serum promote wound healing through trypsin fragments that promote fibrous cell differentiation.[118] And it has been shown that difficulties in wound recovery are associated not only with infection, age and diabetes, but also with reduced albumin concentrations in the wounded area.[119,120] Therefore a mixture of trypsin and albumin may have better efficacy in wound healing.

Trypsin also acts on macrophages. Exogenous application of trypsin may therefore have anti-infective properties, potentially by inducing enhanced phagocytic activity of macrophages, thus enhancing phagocytosis under infectious conditions.[121] Furthermore, trypsin acts directly on macrophages to polarise them towards the M2a phenotype and promote wound healing.[122] Notably, postoperative use of trypsin can counter the ongoing loss of albumin and prealbumin following surgery,[123] meaning that it could help prevent potentially fatal complications such as postoperative shock.

In summary, trypsin achieves its anti-inflammatory, anti-oedema, wound healing and anti-infective effects through both direct and indirect modes of action. Directly, trypsin acts on the lesion area to break down denatured proteins, as well as displaying anti-fibrin exudation, anti-inflammatory, blood breakdown and anti-infective properties. Indirectly, trypsin augments the phagocytic activity of macrophages to exert additional anti-infective effects (Figure 2).

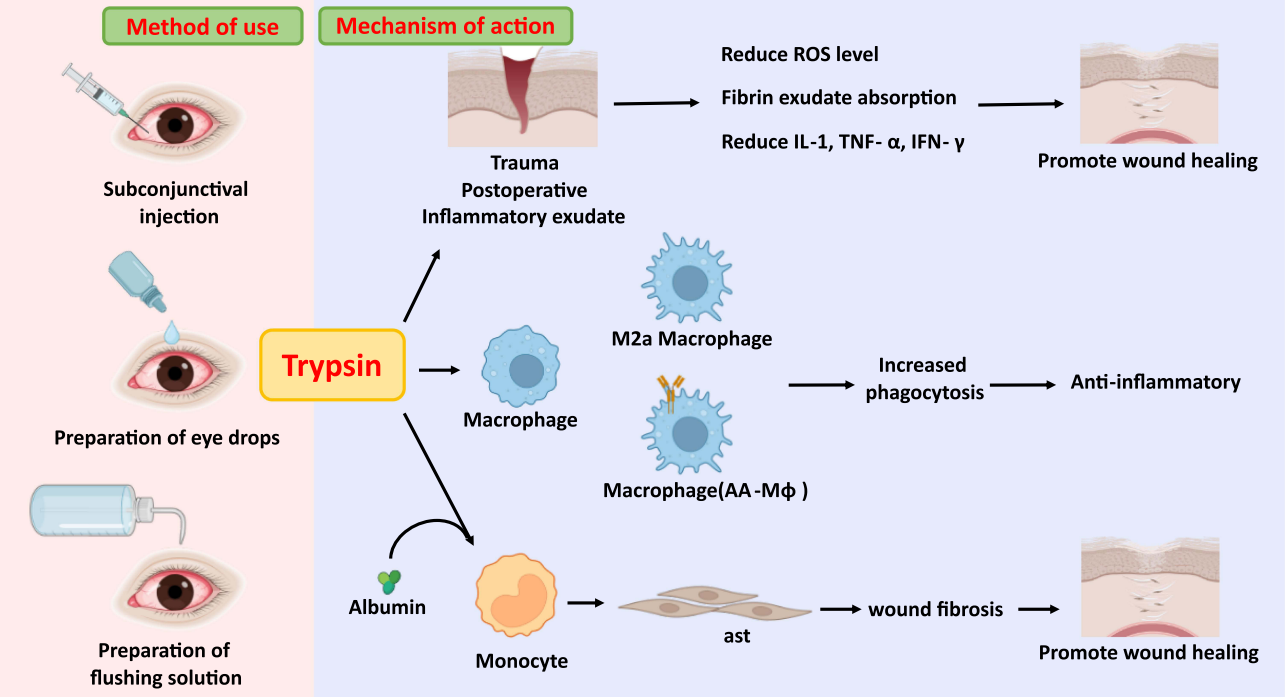

Figure 2 Patterns of exogenous trypsin use in the eye and its anti-inflammatory and pro-repair effects on tissue damage and their mechanisms.

Exogenous Trypsin in Ophthalmic Diseases

Although trypsin has been widely used in the treatment of systemic conditions, it is less commonly employed for ophthalmic diseases. The main uses of trypsin for injection—a lyophilised powder with potential for treatment of ophthalmic diseases—are currently to prepare rinsing solutions for ocular surface irrigation, to configure eye drops for spotting, and for subconjunctival injection.[124] We believe that trypsin for injection can inhibit tissue oedema, improve tissue permeability and exert anti-inflammatory effects in the treatment of a range of ocular inflammatory diseases, or in post-surgical contexts. In support of this, previous work has shown that trypsin performed significantly better than control during treatment of conjunctivitis. The manifestation of conjunctival congestion ceased, various symptoms disappeared (including photophobia, foreign body sensation and lacrimation), and there was no recurrence within one month.[125] Furthermore, for lacrimal tract diseases, trypsin for injection can break down necrotic tissue and pus in the nasolacrimal duct, and denature protein-related secretions.[126] Use of trypsin for injection following ocular trauma or ocular surgery can reduce tissue oedema and pain, clean the wound to help prevent infection, break down abnormal protein exudates and promote absorption of accumulated blood.

As mentioned above, the main treatment modalities with trypsin for injection are currently eye spotting, irrigation and subconjunctival injection. Eye spotting is performed using antibiotic drops or artificial tear drops containing 250 units/mL trypsin. Irrigation also uses 250 units/mL, in saline solution. Subconjunctival injection is carried out with ≤5000 units/mL trypsin in sterile water.[124] Currently, there are no studies on trypsin for injection beyond these uses—ie, for intraocular and postocular applications—but if it proves safe and stable, it may play an important auxiliary role in treating intraocular and postocular inflammatory conditions in the future. Applying trypsin for injection in ophthalmic diseases or after surgical procedures could dissolve blood clots, fibrin exudates and necrotic tissues; improve tissue permeability; inhibit edema and inflammatory reactions; and promote the rapid diffusion and dissolution of locally applied drug formulations.

Although normal tissues should be largely unaffected by injection of trypsin, allergic reactions could occur, given that it is a protein-based drug. The drug itself is recommended for intramuscular injection only in patients who have taken a skin scratch test before use. Therefore, in clinical applications of trypsin for injection, all patients should have the skin scratch test first to help ensure the safety of the drug. The injection route may increase the patient's pain, but lidocaine could be used in the formulation to manage any painful reaction. Topical application of trypsin can also result in sclerosis, meaning that careful monitoring is required during clinical application in ophthalmic diseases. Attention should also be paid to the possibility of other local adverse drug reactions when trypsin is applied opthalmologically, such as elevated intraocular pressure, corneal oedema, and conjunctival congestion and oedema.

Conclusion

In summary, endogenously produced tryptase regulates the inflammatory response in the body and exogenously intervened trypsin, which can have a therapeutic effect on wounds or damaged wounds. In ocular diseases, endogenously secreted tryptase is mainly derived from mast cells, and can activate PAR2, thereby contributing to the inflammatory response. Intervention with mast cells, PAR2 receptors and their downstream binding small molecules can modulate the anti-inflammatory effects of tryptase. Conversely, exogenous interventions with trypsin mainly produce anti-inflammatory, anti-oedema, and abnormal protein breakdown effects, as well as displaying anti-infective properties through direct action and regulation of macrophage function. The safety and efficacy of injectable trypsin as a marketed drug have already been demonstrated in ophthalmic diseases, providing a basis for its investigation in treatment of a wider range of ophthalmic conditions. Nevertheless, further research is needed on the clinical applications of trypsin in ophthalmology, to accumulate the relevant clinical data, analyse adverse drug reactions and complications, and refine the therapeutic role of trypsin in each disease.

Data Sharing Statement

The literature used and cited in this study is available in peer-reviewed journals and is publicly accessible.

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Disclosure

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