Enzymatic, Clinical and Histologic Evaluation of Corneal Tissues in Experimental Fungal Keratitis in Rabbits

USHA GOPINATHAN a, T. RAMAKRISHNA b, MARK WILLCOX c, Ch. MOHAN RAO b, D. BALASUBRAMANIAN a, AJAY KULKARNI a, GEETA KASHYAP VEMUGANTI a and GULLAPALLI N. RAO a

aHyderabad Eye Research Foundation, L. V. Prasad Eye Institute, Hyderabad, India, bCentre for Cellular and Molecular Biology, Hyderabad, India and cCooperative Research Centre for Eye Research and Technology, Sydney, Australia

(Received Lund 30 August 2000, accepted in revised form 7 December 2000 and published electronically 20 February 2001)

Mycotic keratitis, being frequently refractive to most of the currently available antifungal therapy, continues to pose a therapeutic challenge to the clinician. In keratitis of infectious etiology stromal dissolution may be brought about by a combination of agent and host factors. An understanding of the source and nature of corneal tissue damage is essential for evolving more effective therapeutic modalities in the treatment of fungal keratitis. In the present study, we have characterized the extracellular proteases produced in vitro by corneal fungal pathogens namely the Aspergillus flavus and Fusarium solani when collagen was provided as the sole nitrogen source. In addition, fungal infected rabbit corneas were investigated for proteolytic activities and nature of inflammatory reaction. Gelatin zymography detected protease bands with molecular mass ranging from 100 to 200 kDa in the culture extracts of A. flavus, and a single major band of molecular mass approximately 200 kDa in the culture extracts of F. solani. A basal proteolytic activity of mass 65 kDa was visualized in all uninfected and infected rabbit corneal extracts. Infected corneas in addition revealed the presence of additional proteolytic species of mass 92 and 200 kDa. The enzyme inhibitory profile suggested that fungal cultures in vitro contained predominantly serine protease activity and to a lesser extent metalloprotease activity. However, fungal infected corneal homogenates showed the presence of metalloproteinase activity alone, the enzymatic activities entirely being sensitive to ethylene diamine tetra acetate (EDTA), a metalloprotease inhibitor. Interestingly, the serine proteolytic activity detected in fungal cultures in vitro was not present in the fungal infected corneas in vivo. However, the possible role of fungal serine proteases in the activation of corneal matrix metalloproteinases (MMPs) cannot be ruled out. Based on the criteria of molecular mass, proteolytic activity in the presence of calcium at neutral pH, and sensitivity to inhibition by a metalloprotease inhibitor, the 65 and 92 kDa gelatinases were identified as MMP 2 and MMP 9, respectively. The expression of 92 and 200 kDa gelatinases correlated positively with the amount of polymorphonuclear cells present in the infected tissues. Activated resident corneal cells or inflammatory cells may largely contribute to the increased proteolytic activities in fungal infected corneas resulting in tissue matrix degradation in fungal keratitis.

Key words: cornea; fungal keratitis; pathogenesis; proteolytic activity; protease inhibitors.

1. Introduction

Worldwide and particularly in the tropics, mycotic keratitis has emerged as a major ophthalmologic problem in recent years. Owing to delayed presentation of the cases to the clinics and limitations in the use of most of the currently available antifungal agents for ophthalmic use, mycotic keratitis often remains refractory to the current antifungal therapy (Foster, 1981; Foster et al., 1981; Johns and O’Day, 1988). Added to these are factors as yet unknown, but contributing to corneal matrix degradation that alters the corneal integrity. Optimizing treatment of fungal keratitis requires meeting several challenges including rapidly reducing the numbers of pathogens and simultaneously controlling the corneal tissue damage.

At the L. V. Prasad Eye Institute, a tertiary eye care center in South India, among the 1977 culture proven cases of infectious keratitis diagnosed between 1991 and 1999, the incidence of fungal keratitis is observed to be 40·6 % (unpublished data). This figure reflects the magnitude of the problem in a tropical country like India. In tropical and sub-tropical regions, filamentous fungi belonging to the genera Fusarium and Aspergillus are the most frequent causes of keratitis (Reddy et al 1972; Liesegang and Forster, 1980; Panda, Mohan and Mukherjee, 1984).

The extracellular bacterial proteases (Brown, Bloomfield and Tam, 1974; Lyerly, Gray and Kreger, 1981; Twining et al., 1993), proteases released by activated polymorphonuclear leukocytes (Steuhl et al.,...
Fungi are known to elaborate extracellular enzymes based on the substrate that they utilize for growth, and extracellularly produced enzymes have been described in certain fungi such as Trichophyton rubrum (Apondaca and McKerrrow, 1989), Coccidoides immittis (Yuan and Cole, 1987), Candida (Borg and Ruchel, 1988) and Aspergillus species (Kothary, Chase and Macmillan 1984; Reichard et al., 1990; Hanzi et al., 1993). Zhu et al. (1990) have also identified extracellular proteases in cultures (in vitro) of a corneal isolate of Aspergillus flavus. In experimental studies using mycelial extracts of the fungus, intracellular fungal proteases have been implicated in corneal matrix degradation in fungal keratitis in rabbits (Burda and Fisher, 1960; Dudley, Chick and Morgantown, 1964). However, the exact source and nature of proteolytic damage occurring to the cornea or the enzymology of collagen degradation following fungal infection has so far not been investigated. It remains to be studied whether fungal or tissue derived products mediate pathogenesis of fungal keratitis. In addition to rendering infected cornea free of fungal elements, inhibition of proteolytic activities of the cornea would be an attractive approach in the management of fungal keratitis. We have therefore attempted to initially characterize the extracellular proteases produced in vitro by corneal isolates of A. flavus and F. solani with a view to understand the types of proteases produced by these fungi when collagen is provided as the sole nitrogen source in the culture medium. Collagen was incorporated into the medium, this being the major protein constituent of the cornea. Secondly, experiments were performed to investigate if fungal or tissue derived factors were responsible for the enzymatic changes occurring to the cornea in experimental fungal keratitis in rabbits.

2. Materials and Methods

Preparation of Fungal Culture Broth

A. flavus and F. solani strains isolated from corneas of patients with fungal keratitis were used for the in vitro and in vivo experiments. Spore suspensions (approximately 1·4 \times 10^4 CFU l^{-1}) were inoculated into one liter of Vogels medium (Kotia, Dhillon and Singh, 1985) in the presence of either insoluble collagen type 1 (Sigma, U.S.A.) or soluble casein (BDH, Poole, U.K.) as the sole source of nitrogen. Casein was incorporated in cultures, this being the substrate for studying the general proteolytic activity. All media were devoid of ammonium salts. Following incubation of media at 35°C for 10 days, the cultures were filtered (Millipore, Bedford, U.S.A.) and tested for enzyme activities.

Analysis of Proteolytic Activity in the Culture Broth

Gelatin (0·15%, w/v) zymography was performed using partially purified samples (ammonium sulphate precipitation) of culture filtrates on a non-reducing 8% sodium dodecyl sulfate polyacrylamide gel (SDS–PAGE) essentially as described earlier (Barletta et al., 1996). To study the inhibitory profiles, the protease inhibitors (Sigma, U.S.A.) used were ethylene diamine tetracetate (EDTA: 10 mM), phenyl methyl sulfonyl fluoride (PMSF: 2 mM), pepstatin (1·0 \mu M) and E64 (2·8 \mu M). Appropriate inhibitor untreated controls and high molecular weight markers (Biolabs, New England, U.S.A.), were also included.

Following the electrophoretic run of samples, lanes were cut out, each of the lanes treated separately with one of the inhibitors (Barletta et al., 1996) and washed with Tris–HCl buffer (pH 7·4) containing Triton x 100 (2·5%) in order to recover the activity of the proteases. The gels were stained using Coomassie blue R 250 and observed for the presence of proteolytic bands which appeared white against a dark blue background.

In vivo Protease Production

All rabbits were treated in accordance with the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic Vision and Research, and the protocol was approved by the Institutional Ethics Committee. Eighteen albino rabbits weighing 2–2·5 kg each were randomly distributed into six groups of three each. For inducing fungal keratitis, spore suspensions of A. flavus (1·25 \times 10^3 CFU per 25 \mu l) and F. solani (2·15 \times 10^3 CFU per 25 \mu l) were prepared according to the method described by O’Day et al. (1991). In order to compare the proteolytic activities between fungal and bacterial infected corneas, bacterial keratitis was induced in one of the groups of rabbits as described earlier (Barletta et al., 1996) using a Pseudomonas aeruginosa strain (2·7 \times 10^7 per 20 \mu l) isolated from a case of human bacterial keratitis. On day zero, the rabbits were anaesthetized using ketamine hydrochloride (O’Day et al., 1991) following which all corneas received the topical anaesthetic, lignocaine hydrochloride (4 %) in their test eye (OD) eyes. Rabbits in groups 1–5 initially received 300 \mu l of the steroid, Depo Medrol (12 mg) subconjunctivally in their right eye (OD). Except for corneas of rabbits in the group 2 that received 20 \mu l of Pseudomonas in the central, midstroma (OD), corneas of rabbits in the remaining groups were injected...
midstromally (OD) with 25 μl of the inoculum (group 1, 0-85% saline; group 3, Aspergillus; group 4, Fusarium; and group 5, killed spores of Fusarium). Rabbits in group 6 did not receive the steroid, but were injected with live Fusarium spores in the right cornea (non-steroid model). The left eye (OS) of rabbits in each group served as the uninoculated control.

The right eyes of all rabbits were examined using a slit lamp biomicroscope on alternate days (from day 1 to day 10) for epithelial defect, size of the infiltrate, hypopyon, vascularization, and endothelial exudates. Rabbits in group 2 were killed on day 4 as the infection progressed rapidly and the remaining rabbits were killed on day 10.

Trehined corneas (12-5 mm) were aseptically excised, washed in saline to remove contaminated blood and each corneal button was divided into three portions. One half of the buttons (right and left) of rabbits in each group was pooled separately and frozen immediately. These pooled samples were processed for enzymatic characterization. The remaining half of each corneal button was divided further into equal halves and one portion each was subjected to microbiologic and histopathologic evaluations, respectively.

Microbiologic and Histopathologic Evaluation

One portion of each corneal button of rabbits in groups 1–6 was inoculated on blood agar plates (BA) and incubated at 35°C for 14 days for determining the viability of the organism. Similarly, the remaining portion of each cornea was formalin-fixed, and paraffin sections subjected to staining by Haematoxylin and Eosin stain (H&E), Gomori Methenamine Silver Nitrate (GMS), Periodic Acid Schiff’s (PAS) and Grams stains.

Enzymatic Characterization

The pooled corneas were extracted with Tris–HCl (pH 7-2) containing 4% SDS. The soluble corneal fractions were matched for OD 280, and subjected to gelatin zymography in the cold (Barletta et al., 1996), with or without inhibitors.

3. Results

In vitro Protease Production and Inhibitor Profile

Gelatin zymography (Fig. 1) revealed proteolytic bands ranging from 100 to 200 kDa in the culture extracts of A. flavus (lanes 1 and 2), and a single major band of molecular mass 200 kDa in culture extracts of F. solani (lanes 3 and 4). The difference in the intensity of proteolytic bands between lanes 1 and 2 of A. flavus and lanes 3 and 4 of F. solani cultures relates to the difference in the type of nitrogen source (casein/collagen) provided in the medium.

There was partial inhibition of proteolytic activities upon treatment of samples with PMSF, a serine protease inhibitor (Fig. 2, lanes 5 and 6), and of others by EDTA, a metalloprotease inhibitor (Fig. 2; lanes 3 and 4). A combination of both PMSF and EDTA inhibited proteases of Aspergillus (Fig. 2) cultures to a greater extent (Fig. 2; lanes 7 and 8). However, some residual proteolytic activity was still observed. A similar pattern of proteolytic activity inhibition was observed with the proteases of Fusarium cultures also (Fig. 3). However, the amount of activity inhibition observed was significantly lesser than that observed for the proteases of Aspergillus cultures. Neither pepstatin nor E64 demonstrated any inhibitory effect on the extracellular proteases of A. flavus and F. solani (data not shown).

In vivo Experiments

Clinical evaluation. None of the eyes (OD) of rabbits in groups 1 (saline-treated) and 5 (killed fungal spores) developed corneal infiltrate. P. aeruginosa-infected corneas [Fig. 4(A)] in group 2 revealed dense yellow–white infiltrates on day 2, the density and depth of which increased rapidly, involving the entire cornea over the next 2 days with copious necropurulent discharge.

Corneas of rabbits in group 3 (Aspergillus keratitis) showed smaller and less dense infiltrates measuring 5-0 mm [Fig. 4(B)] when compared to groups 2 (Pseudomonas keratitis) and 4 (Fusarium keratitis).
Conjunctival congestion with mucoid discharge was noticed in all eyes in this group (group 2). One of the eyes also developed a hypopyon.

Corneas of rabbits in group 4 (Fusarium keratitis) developed a small infiltrate on day 2 which progressed over a period of 10 days [Fig. 4(C)] into a single large dense yellow–white infiltrate (7.0 mm) with mucoid discharge, and conjunctival congestion. Steroid-untreated corneas in group 6 (Fusarium keratitis) developed a small infiltrate on day 2, which progressed to 3 mm by day 5 [Fig. 4(D)]. There was no change in the size or severity of the ulcer over the next 5 days and it appeared almost healed by day 10.

The corneas of rabbits in the groups 2, 3, 4 and 6 showed full thickness infiltrates. An epithelial defect

**Fig. 2.** Characterization of extracellular proteases produced in vitro by *A. flavus*. The culture extracts of *A. flavus* were subjected to gelatin zymography on 8% SDS–PAGE and treated with or without EDTA, and PMSF. Lanes 1 and 2 represent inhibitor untreated samples; lanes 3 and 4, treated with EDTA; 5 and 6, treated with PMSF; 7 and 8, treated with a combination of EDTA and PMSF. Lanes 1, 3, 5 and 7 indicate cultures grown with casein and lanes 2, 4, 6 and 8 indicate cultures grown with collagen.

**Fig. 3.** Characterization of extracellular proteases produced in vitro by *F. solani*. The culture extract of *F. solani* was subjected to gelatin zymography on 8% SDS–PAGE and treated with or without EDTA, and PMSF. Lanes 1 and 2 represent inhibitor untreated samples; lanes 3 and 4, treated with EDTA; 5 and 6, treated with PMSF; 7 and 8, treated with a combination of EDTA and PMSF. Lanes 1, 3, 5 and 7 are cultures grown with casein as the sole source of nitrogen and lanes 2, 4, 6 and 8 indicate cultures grown with collagen as the only nitrogen source.
was present in corneas of all rabbits in the groups 2, 3 and 4 and none of the eyes in the groups 1–6 showed vascularization.

**Microbiologic evaluation.** On culture, corneal buttons from rabbits representing groups 2, 3 and 4 (steroid-models) revealed growth of *P. aeruginosa*, *A. flavus* and *F. solani*, respectively on blood agar plates while corneas from groups 5 (killed fungus) and 6 (non-steroid models) remained culture-negative until day 14 following incubation of the media.

**Histopathologic evaluation.** Corneas in groups 1 (saline inoculated) and 5 (killed fungus) appeared normal. Corneas in group 2 [Fig. 5(A)] with *Pseudomonas* keratitis revealed epithelial ulceration with dense, diffuse inflammatory infiltrates involving two thirds of the stroma. Gram-negative bacilli were detected in Gram-stained corneal sections (data not shown). Corneas infected with *Aspergillus* and *Fusarium* [Fig. 5(B) and (C)] showed polymorphonuclear infiltration throughout the corneal stroma. Corneal sections stained by GMS revealed the presence of fungal filaments in the mid- and deeper stroma (data not shown).

The steroid-untreated group (group 6) however showed only minimal changes. The epithelium was intact with occasional polymorphonuclear cells in the stroma [Fig. 5(D)]. Special stains for visualization of fungus such as GMS and PAS were negative for fungus in corneas of rabbits in the group 6 (non-steroid model).

**Enzymatic characterization of corneal proteolytic activity.** A basal enzymatic activity with an apparent molecular mass of 65 kDa was observed in the uninoculated corneas (OS) and experimental corneas (OD) of rabbits in all groups (Fig. 6). The 65 kDa gelatinase appeared to have minimal proteolytic activity in the uninoculated corneas (Fig. 6, lane 1), and in groups that received saline (Fig. 6, lane 2) and killed fungal suspension (Fig. 6, lane 6), when compared to other groups that showed active infection clinically.

In comparison to corneas infected with *Pseudomonas*, the 65 kDa band expressed more gelatinolytic activity in corneas infected with fungi. The presence of a proteolytic band of approximately 50 kDa was visualized in corneas infected with *Pseudomonas*.

Gelatin zymography enabled the identification of additional gelatinases of molecular mass 58, 92 and 200 kDa in the soluble fractions of rabbit corneas in groups 2 (*Pseudomonas* keratitis, Fig. 6, lane 3), 3 (*Aspergillus* keratitis, Fig. 6, lane 4), and 4 (*Fusarium* keratitis, Fig. 6, lane 5) following infection (steroid-models). Interestingly, the pattern of proteolytic

![Fig. 4. Clinical photographs of rabbit corneas with infectious keratitis. *Pseudomonas* keratitis showing dense large infiltrate involving the entire cornea (A); *Aspergillus* keratitis showing central less dense infiltrate with hyphate edges (B); *Fusarium* keratitis showing central dense infiltrate (C) and non-steroid model of *Fusarium* keratitis showing central small dense infiltrate with well defined margin (D).](image)
Fig. 5. (A–D) Haematoxylin and eosin stained sections of rabbit corneas with infectious keratitis. Pseudomonas keratitis showing dense inflammatory infiltrates in the anterior two thirds of the stroma (arrow) with stromal necrosis [(A) × 250]; Aspergillus keratitis showing stromal abscess (arrow) in mid stroma with diffuse inflammatory infiltrates in all layers [(B) × 250]; Fusarium keratitis showing stromal abscess with inflammatory infiltrates (arrow) in all layers [(C) × 250]; non-steroid model of Fusarium keratitis showing minimal inflammation, few histiocytes and occasional polymorphs with no fungus [(D) × 500].

Fig. 6. Proteolytic activities of rabbit corneas in experimental fungal and bacterial keratitis. Soluble corneal fractions were subjected to gelatin zymography. Note the 65 kDa gelatinase in uninoculated corneal samples (lane 1), vehicle treated corneal samples (lane 2), Pseudomonas keratitis (lane 3), Aspergillus keratitis (lane 4), Fusarium keratitis (lane 5), corneas that received killed Fusarium spores (lane 6). Lanes 3, 4 and 5, in addition reveal gelatinases of molecular mass 58, 92 and 200 kDa.
activities, observed in fungal infected corneal homogenates was quite different from the pattern observed in vitro, in culture extracts of Aspergillus and Fusarium species.

As can be seen in Fig. 7, corneas in group 5 that received killed Fusarium suspension showing the presence of 65 kDa gelatinase alone; lane 2 represents Fusarium infected corneas (steroid-treated) showing gelatinases of molecular mass 58, 92 and 200 kDa and lane 3 represents Fusarium infected corneas (steroid-untreated) showing reduced activity of 58, 92 and 200 kDa gelatinases.

4. Discussion

Fungi are known to elaborate extracellular proteases that are quite specific for the substrates that they utilize for growth (Zhu et al., 1990). The observation made in the current study that fungal corneal pathogens such as A. flavus and F. solani are capable of utilizing collagen in vitro supports the assumption that the ability of a fungus to elicit corneal tissue damage is dependent, at least in part, on the utilization of corneal collagen in vivo by the infecting agent. However, in vitro conditions for fungal growth may not necessarily reflect the actual corneal conditions prevalent in vivo. This prompted us to study the mechanism of pathogenesis of fungal keratitis, which has hitherto, not been investigated.
In this study, the fungal and bacterial corneal ulcers induced in steroid-treated rabbits mimicked human corneal ulcers caused by the respective organisms. It has been the observation of O'Day et al. (1991) that fungal keratitis is difficult to be sustained in rabbits in the absence of steroid treatment. While in the steroid-model we could sustain active corneal infection without fall in clinical severity till day 10, corneal infiltrates of rabbits in the non-steroid model did not show signs of progression of the disease beyond day 5.

The clinical presentation and nature of infiltration are important factors to be considered while investigating corneal tissues for proteolytic activity following infection. Clinically, *Pseudomonas* keratitis took a fulminant course and corneas were excised on day 4. In contrast, fungal keratitis in the steroid-treated rabbits progressed more slowly over a period of 10 days, with a clinical picture characteristic of fungal keratitis. Among the steroid-models of infectious keratitis, stromal necrosis with polymorphonuclear (PMN) infiltration was more evident in *Pseudomonas* keratitis than in corneas infected with fungi. This picture correlated positively with the degree of clinical severity of bacterial and fungal keratitis observed in rabbits, the severity of the ulcers being more pronounced in the former than in the latter group. The absence of fungal elements and the presence of occasional polymorphs in corneal tissues of rabbits in the non-steroid model of *Fusarium* keratitis (as demonstrated by histopathology and microbiological examinations) supported the clinical observation made in this group on day 10 that infiltrates appeared healed indicating no active infection. Interestingly, the expression of 92 and 200 kDa proteases was associated with corneas that showed progressive ulcers and which revealed the presence of dense inflammatory cells.

The gelatinolytic species expressed by normal corneal cells in culture systems have been well characterized (Fini and Girard, 1990). Corneal stromal cells synthesize MMP 2 (of mass 72 kDa) and corneal epithelial cells produce MMP 9 (of mass 92 kDa). The MMP 2 gelatinase is present in a latent proenzyme form in the normal cornea and gets activated by proteolytic cleavage. MMP 2 is extractable from the stroma whereas MMP 9, which is not expressed in a normal cornea, requires induction by some means (Fini and Girard, 1990; Matsubara et al., 1991). The same investigators also observed that the 72 kDa gelatinase of the corneal stroma appears in the region corresponding to a molecular mass of 65 kDa under non-reducing conditions required for zymography. The presence of the 65 kDa gelatinase in both uninfected and infected corneal samples in the current investigation is thus consistent with the observation made by investigators in the studies cited above.

There have been conflicting reports on the expression of the 92 kDa gelatinase in different physiological and pathological conditions (Matsubara et al., 1991; Matsumoto et al., 1993; Twining et al., 1993). A 92 kDa gelatinolytic activity has been demonstrated during corneal wound healing in vivo by Matsubara et al. (1991). This activity was also visualized by the same authors during wound healing in vitro where polymorphonuclear infiltration was precluded. Hence the authors suggested that the 92 kDa protease was not only a product of PMNs but also a product of corneal cells.

According to the hypothesis put forward by Steuhl et al. (1987), corneal damage resulting from activated PMNs was probably more important in the pathogenesis of *Pseudomonas* corneal infection than the bacterial exoproducts. In accordance with this finding is the observation made in keratoconus corneas (non-inflammatory condition), where only the 72 kDa gelatinase (65 kDa gelatinase) was noticed suggesting the association of the 92 kDa enzyme with trauma and inflammation where PMNs come into the picture (Kenney et al., 1994). Human neutrophils are known to synthesize MMP 9, a neutrophil gelatinase, that hydrolyses gelatin and collagen types IV, and V (Hibbs et al., 1985). According to observations made in the current study, the role played by PMNs in extracellular matrix degradation during fungal keratitis deserves attention. The difference in the enzyme activities of fungal-infected corneas between the steroid and the non-steroid models correlated well with the amount of PMN infiltration seen in these two groups. Moreover, in this study none of the eyes that received heat-inactivated fungal spores developed infiltrate clinically or showed inflammatory cells on histopathological examination. In these corneas only the 65 kDa gelatinase could be detected by gelatin zymography indicating that a killed fungus does not elicit inflammatory response and does not express 92 kDa gelatinase.

The expression of a 200 kDa gelatinase is observed to be associated with high amount of 92 kDa gelatinase in rabbit corneas and probably a multimer of MMP 9 (Matsubara et al., 1991). In the current study, the soluble corneal extracts subjected to zymography revealed the presence of 200 kDa gelatinase only in those corneas which also expressed the 92 kDa gelatinase.

It has been indicated (Steuhl et al., 1987) that extracellular bacterial proteases are involved in activating the latent form of tissue gelatinases in *Pseudomonas* keratitis and that these activated forms together with the enzymes released from the infiltrating inflammatory cells lead to uncontrolled activation of MMPs resulting in dissolution of collagen. The relevance of bacterial exoproducts in corneal ulceration has been emphasized by other investigators (Ohman, Burns and Iglewski, 1980; Howe and Iglewski, 1984; Hibbs et al., 1985; Kernacki et al., 1995) as well. In the present study the complete absence of fungal proteases in fungal infected tissue...
seems to indicate that fungal proteolytic enzymes are not directly involved in mediating corneal tissue degradation. However, as demonstrated in our experiment that fungi are capable of elaborating extracellular serine proteases in the presence of collagen in vitro, it could be postulated that minute amounts of serine proteases secreted by the fungus during tissue invasion may play a role in triggering the entire process of activation of corneal cell or inflammatory cell derived proteases although fungal enzymes themselves may escape detection byzymography. In fact, one of the common features of the activation of matrix metalloproteinases is that all of the enzymes characterized so far are partially or completely activated from their precursors by the action of serine proteases such as plasmin and kallikrein (Hibbs et al., 1985; Murphy et al., 1985; Rao et al., 1999).

Our study is the first of its kind to investigate the source and nature of proteolytic activities in rabbit corneas during active fungal keratitis in vivo. This study indicates a common mechanism of matrix turnover during both bacterial and fungal keratitis. This leads us to suggest the use of nontoxic metalloproteinase inhibitors as adjuvants, which may be of great clinical significance in the improved management of fungal and bacterial corneal infections.

Acknowledgements

This research work was supported by grants from the Hyderabad Eye Research Foundation, L.V. Prasad Eye Institute, Hyderabad.

References


