·Basic Research·

Time-dependent matrix metalloproteinases and tissue inhibitor of metalloproteinases expression change in *fusarium solani* keratitis

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Abstract

• **AIM: To investigate matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) expression during the progress of *fusarium solani* (*F. solani*) keratitis in a rat model.**

• **METHODS: A rat model of *F.solani* keratitis was produced using corneal scarification and a hand-made contact lens. MMPs and TIMPs expressiond were explored in this rat model of *F.solani* keratitis using real-time polymerase chain reaction (PCR) and DIF. GM6001 (400 μmol/mL) was used to treat infected corneas. The keratitis duration, amount and area of corneal neovascularization (CNV) were evaluated.**

• **RESULTS: MMP-3 expression was 66.3 times higher in infected corneas compared to normal corneas. MMP-8, -9, and -13 expressions were significantly upregulated in the mid-period of the infection, with infected- to-normal ratios of 4.03, 39.86, and 5.94, respectively. MMP-2 and -7 expressions increased in the late period, with the infected-to-normal ratios of 5.94 and 16.22, respectively. TIMP-1 expression was upregulated in the early period, and it was 43.17 times higher in infected compared to normal corneas, but TIMP-2, -3, and -4 expressions were mildly downregulated or unchanged. The results of DIF were consistent with the result of real-time PCR. GM6001, a MMPs inhibitor, decreased the duration of *F. solani* infection and the amount and area of CNV.**

• **CONCLUSION: MMPs and TIMPs contributed into the progress of *F.solani* keratitis.**

• **KEYWORDS:** fungal keratitis; *fusarium solani*; metalloproteinases; tissue inhibitors of metalloproteinases
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INTRODUCTION

Scarring of the cornea resulting from suppurative keratitis is an important cause of preventable blindness. In some developing countries in the tropics, corneal infections are the second most common cause of blindness, after untreated cataracts [1]. Suppurative corneal ulcers may be caused by bacteria, fungi, and protozoa. However, fungal keratitis has been recently found to play an important role. *Fusarium solani* (*F.solani*) is the most common fungal corneal isolate in China [2]. We have made a experimental model of *F.solani* keratitis in rats in our previous study, which is used in this study[3].

The main pathological features of corneal infections are suppurative inflammation, histopathological changes and changes in a variety of proteolytic enzymes that have a direct connection with corneal tissue degradation in fugal keratitis. Matrix metalloproteinases (MMPs) are a conserved family of zinc-dependent proteinases that act as both regulators and effectors of many normal and pathological processes including developmental tissue remodeling, wound healing, angiogenesis, inflammation, and tumor progression [4]. Overexpression of MMPs has been correlated with pathologic conditions such as corneal ulceration, epithelial ingrowth, keratoconus and other complications[5-7]. MMPs are aslo the main enzymes that degrade the extracellular corneal matrix (ECM) that play a critical rloe in the process of neovascularization [8], and these enzymes have been studied by many scholars [9-11].

Tissue inhibitors of matrix metalloproteinases (TIMPs) are also important in fugal keratitis research [12]. The altered balance of MMPs and TIMPs triggered by inflammatory cytokines enhance matrix degradation of the corneal

stroma [13-15]. However, the role of MMPs and TIMPs in the progress of fugal keratitis is unclear.

Yuan *et al* [16] investigated the expression of MMPs and TIMPs during the inception and progression of experimental *candida albicans* keratitis, and this is a comprehensive study of MMPS and TIMPs in one kind of fungal keratitis in recent research. Yuan *et al* [16] found transcriptional and translational levels of MMP-8, -9, -13, and TIMP-1 increase during the early stages of *C. albicans* keratitis, confirming findings for MMP-9 and TIMP-1 in other infectious keratitis models and suggesting roles for MMP-8 and -13. We systematically examined the expression patterns of 7 MMPs and 4 TIMPs in murine *F.solani* keratitis.

GM6001 (Iloasmtat, Galardin) MMP inhibitor is a potent inhibitor of collagenase. It is a broad spectrum inhibitor of MMPs for MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 with K_i of 0.4 nmol/L, 0.5 nmol/L, 27 nmol/L, 0.1 nmol/L, 0.2 nmol/L respectively. GM6001 is widely used to inhibit MMPs *in vitro* experiments and in animal models. In this study, GM6001, as a synthetic MMPs inhibitor, was used to treat *F.solani* keratitis in a rat model and to confirm the action of MMPs.

MATERIALS AND METHODS

Fungi *F.solani* strains were purchased from the China General Microbiology Culture Collection Center (CGMCC), species number 3.5840. The *F.solani* was inoculated into potato dextrose agar solid medium for 7d at 24℃, harvested in sterile phosphate buffered saline (PBS), and diluted with sterile saline, to yield an inoculum with 1×10^8 CFU/mL.

Animals Normal adult Wistar rats, each weighing 180 to 220 g [provided by the Department of Animal Science, Fudan University, license number SCXK (Shanghai) 2002-0002]. The animals were provided a standard diet and water *ad libitum* and housed in a temperature (21℃-23℃) and humidity (45%-50%) controlled room under a constant 12h light, 12h dark cycle. The protocols were approved by the Animal Care and Use Committee of Tongji University and are in accordance with the National Institutes of Health "Guide for the care and use of laboratory animals" (NIH publication No. 85-23, revised 1996). All Wistar rats were examined for eye disease using a slit lamp before surgery. Rats were anesthetized intraperitoneally using rodent combination anesthesia, and both corneas of each rat were scarified using a hypodermic needle to create a superficial wound of intersecting marks in a grid pattern [15]. A 5 μL inoculum (1×10^8 CFU/mL) of *F.solani* was applied to the right eye, while sterile PBS dilution buffer was applied to left eye as a mock-infected control. A hand-made contact lens with a curvature of approximately 3.13 mm was made from parafilm M filmand applied to each eye [3]. The eyelids were sutured shut for one day. Rats were sacrificed on 1, 2, 3, 6, 10 and 14d post inoculation (p.i.), and the eyes were enucleated for analysis.

Scoring Rats were scored daily for up to 14d p.i. with the aid of a dissecting microscope and slit lamp [15]. Briefly, a grade of 0 to 4 was assigned to each of 3 criteria: area of corneal opacity, density of corneal opacity, and corneal surface regularity, as previously described[15].

RNA Extraction and Quantitative Real -time Polymerase Chain Reaction Corneas were dissected from freshly enucleated eyes, and from the surrounding conjunctiva, Tenon capsule, and uvea. Randomly grouped five-cornea pools were prepared in triplicate from *F.solani*-infected and mock-infected control animals at days 1, 2, 3, 6, 10 and 14 p.i., and from normal unmanipulated rat corneas. Total RNA was immediately extracted (Triziol, Invitrogene), and dissolved in RNase free water, separated using agarose gel electrophoresis, and the rest was stored at -80℃ until use.

Total RNA, isolated from normal rat corneas, *F.solani*-infected corneas and mock-infected corneas on days 1, 2, 3, 6, 10 and 14 p.i., was quantified spectroscopically at an absorption of 260 nm. The first-strand cDNA was synthesized using 0.5 μg total RNA and a RT regent Kit (SYBR® PrimeScript RT reagent Kit, Takara Biotechnology). Real-time polymerase chain reaction (PCR) was performed using an assay with primers specific for the various MMPs and TIMPs transcripts (Table 1). The threshold cycle (Ct) for each target mRNA was normalized to β-actin mRNA. Normalized Ct results were used to calculate gene expression levels, and the mean results were used to determine the relative fold changes between experimental groups.

Direct Immunofluorescence Corneas from infected eyes and mock-infected eyes on 1, 2, 3, 6, 10, and 14d p.i., and the corneas from the 5 normal rats were fixed in 4% paraformaldehyde, embedded in paraffin, and serially sectioned at a thickness of 4 μm. The sections were deparaffinized, and blocked with 10% normal donkey serum in PBS for 1h to decrease nonspecific binding. The following primary antibodies were diluted 1:100, applied to the blocked sections, and incubated overnight at 4℃: MMP-2, -9 (AB19016, AB19167, Millipore), MMP-3, -7, -8, and TIMP-1, -2, -3, -4 (sc-6839, sc-26680, sc-8848, sc-5538, Santa Cruz Biotechnology).

GM6001 Treatment With an initial concentration of 2.5 mmol/mL, GM6001 (Chemicon), a specfic MMPs inhibitor, was diluted into a concentration of 400 μg mol/mL using PBS buffer, and was kept at pH 6.5 to 7.5, stored in small quantities at 4℃. Rats (n =20) were inoculated with *F.solani*, and randomly divided into a GM6001 group (400 μmol/mL GM6001) or a saline group (0.9% normal saline). At 2d p.i., rats were given eye drops (25 μL, 5 times per day between 8:00 and 16:00). All experimental eyes were coated with erythromycin eye ointment every night to prevent bacterial infection, and an tropicamide drop was administered to avoid closure of the pupillary membrane.

The duration of infection, and the amount and area of corneal neovascularization (CNV) were used to evaluate the

Table 1 MMPs and TIMPs primers

Gene	Genbank no.	Primer	Product
MMP-2	NM_031054.2	FW: 5'-GTAAAGTATGGGAACGCTGATGGC-3'; RV: 5'-CTTCTCAAAGTTGTACGTGGTGGA-3'	135 bp
MMP-3	NM_133523.2	FW: 5'-GTTCCTTGGGCTGAAGATGAC-3'; RV: 5'-TGGAAAGGTACTGAAGCCACC-3'	106 bp
MMP-7	NM_012864.2	FW: 5'-GCAGACATCATAATTGGCTTCG-3'; RV: 5'-AGTCCTCACCATCCGTCCAG-3'	157 bp
MMP-8	NM_022221.1	FW: 5'-ACTGGGCTCTAAGTGCCTATGAC-3'; RV: 5'-ATCTCCAGCATTGGTTGTTTACG-3'	147 bp
MMP-9	NM_031055.1	FW: 5'-GGGCTTAGATCATTCTTCAGTG-3'; RV: 5'-GCCTTGGGTCAGGTTTAGAG-3'	137 bp
MMP-10	NM_133514.1	FW: 5'-AGACAGGCACTTCTGGCGTAG-3'; RV: 5'-TCTTTGGGTAACCTGCTTGGA-3'	194 bp
MMP-13	NM_133530.1	FW: 5'-CAAGCAGCTCCAAAGGCTACAAC-3'; RV: 5'-GGAAACATCAGGGCTCCAGGGTC-3'	103 bp
TIMP-1	NM_053819.1	FW: 5'-GCCTCTGGCATCCTCTTGTTG-3'; RV: 5'-CATAACGCTGGTATAAGGTGGTC-3'	163 bp
TIMP-2	NM_021989.2	FW: 5'-ACACGCTTAGCATCACCCAGAA-3'; RV: 5'-CAGTCCATCCAGAGGCACTCAT-3'	130 bp
TIMP-3	NM_012886.2	FW: 5'-GCCGTTTATGGAGTTGATTTGG-3'; RV: 5'-AGTGCGGTCTCATTCTTTCTGG-3'	130 bp
TIMP-4	NM_001109393.1	FW: 5'-CAGTATGTCTACACGCCATTTGA-3'; RV: 5'-TCTGGTGGTAGTGATGATTCAGG-3'	193 bp
β-actin	NM-031144	FW: 5'-CGTAAAGACCTCTATGCCAACA-3'; RV: 5'-TAGGAGCCAGGGCAGTAATC-3'	100 bp

therapeutic efficacy of GM6001. Corneas from the experimental eye were placed into paraffin, and dyed using hematoxylin-eosin (HE) stain. Three random slices of each specimen were assessed, and six random high-power field (HPF; ×400) areas from each slice were assessed. The amount of CNVs within the full field, excluding the pipe diameter, was calculated. The CNV area was calculated using the following equation: area (mm^2)=0.2×π×maximal× vessel length (mm)×time (h) of CNV involving the cornea[17].

Statistical Analysis Data are expressed as the mean ±SD. Score were evaluated for statistical significance using a Kruskal-Wallis 1-way analysis of variance on ranks. Pairwise multiple comparison procedures included Dunn's method and the Tukey test. For kinetic analysis of MMPs and TIMPs transcriptional levels, the mean results were compared using ANOVA with the Holm-Sidak method for pairwise multiple comparison procedures. Corneal lesion scoring, the amount and area of CNV and the CNV group were assessed using a paired-sample t-test. P <0.05 was considered to be statistically significant. Data were analyzed using SPSS 12.0 (SPSS Inc, USA).

RESULTS

Duration of *F.solani* Keratitis All eyes inoculated with *F. solani* developed clinical signs of keratitis. The infection was present for 14d and the infected corneas were observed using a slit lamp. There was no inflammation in eyes from mock-infected controls. Histopathologic evaluation of infected eyes revealed partial loss of epithelial integrity, acute inflammatory cells invading the stroma, and PAS staining showed hyphae invading into the mid to deep stroma. Clinical scoring (Figure 1) indicated that the infection was divided into an early period (1d p.i.), a mid-period (2-5d p.i.) and a late period (6-14d p.i.). There were significant differences between control corneas, corneas at 6d p.i., and corneas observed on other days p.i. (P <0.05).

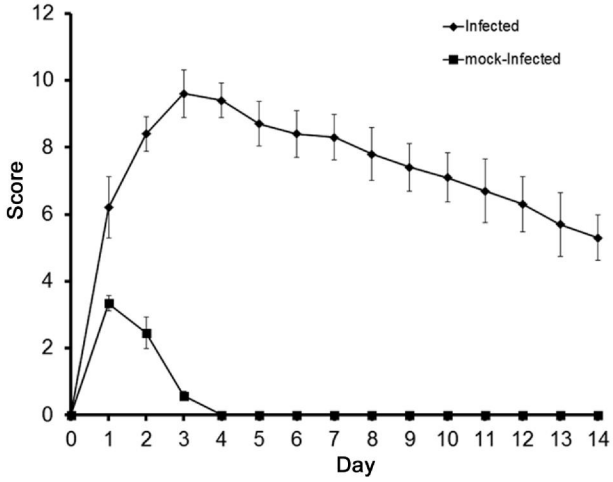

Figure 1 Clinical evaluation of *F.solani* keratitis The disease course was divided into an early period (1d p.i.), a mid-period (2-5d p.i.) and a late period (6-14d p.i.). Each point represents the mean score for each day.

Matrix Metalloproteinases and Tissue Inhibitor of Metalloproteinases Gene Expression MMPs and TIMPs transcript levels in the five *F.solani*-infected cornea pools and in the five mock-infected cornea pools were assessed using real-time PCR, and 7 members of MMPs and 4

Table 2 Gene expression levels in infected and mock-infected corneas $\bar{x} \pm s$

Gene	Group	Pre-infection expression level	Gene expression levels					
			Day 1	Day 2	Day 3	Day 6	Day 10	Day 14
MMP-2	Infected	1	1.83±0.25	2.54±0.55	4.19±0.46	5.94±0.24	11.61±1.75	3.09±0.45
	Mock	1	1.47±0.01	1.22±0.06	0.20±0.05	0.13±0.07	0.98±0.07	1.07±0.08
MMP-3	Infected	1	66.30±2.30	26.61±3.67	17.63±0.12	10.08±0.63	6.73±0.19	14.23±0.39
	Mock	1	14.66±1.12	3.40± 0.33	1.39±0.11	1.00±0.03	1.07±0.01	1.04±0.01
MMP-7	Infected	--	1	3.81±0.03	5.21±0.07	3.66±0.48	8.28±1.37	16.22±0.22
	Mock	--	1	2.01±0.04	0.96±0.07	0.31±0.01	0.20±0.05	0.15±0.04
MMP-8	Infected	--	1	1.72±0.14	4.03±0.03	1.15±0.29	1.09±0.02	0.50±0.06
	Mock	--	1	0.49±0.01	0.41±0.05	0.40±0.05	0.40±0.04	0.39±0.05
MMP-9	Infected	1	22.63±2.65	29.24±0.20	39.86±3.86	20.33±2.93	5.10±0.14	1.28±0.04
	Mock	1	29.58±2.86	11.79±2.66	5.37±0.52	1.27±0.17	1.22±0.05	1.01±0.01
MMP-10	Infected	--	1	0.93±0.09	1.18±0.07	0.16±0.02	0.24±0.02	0.82±0.04
	Mock	--	1	0.58±0.06	0.85±0.01	0.36±0.03	0.28±0.01	0.23±0.05
MMP-13	Infected	--	1	2.95±0.63	5.94±0.74	1.91±0.45	0.06±0.02	0.02±0.01
	Mock	--	1	0.48±0.02	0.26±0.02	0.18±0.01	0.19±0.02	0.18±0.01
TIMP-1	Infected	1	43.17±2.39	14.88±3.25	8.91±0.80	7.56±1.19	3.13±0.47	0.64±0.11
	Mock	1	13.20±1.72	2.81±0.34	1.98±0.02	0.95±0.20	0.74±0.17	0.57±0.02
TIMP-2	Infected	1	0.49±0.03	0.48±0.05	0.62±0.09	1.19±0.11	1.15±0.05	1.38±0.23
	Mock	1	0.54±0.12	0.90±0.18	1.07±0.18	1.24±0.21	1.41±0.17	1.47±0.12
TIMP-3	Infected	1	0.74±0.16	1.25±0.07	0.54±0.10	0.29±0.06	1.01±0.09	0.81±0.15
	Mock	1	0.89±0.09	1.02±0.19	1.02±0.21	1.05±0.07	1.08±0.13	1.12±0.14
TIMP-4	Infected	1	0.11±0.02	0.17±0.01	0.15±0.03	014±0.03	0.61±0.03	0.93±0.01
	Mock	1	0.44±0.02	0.63±0.05	0.83±0.08	0.85±0.03	0.99±0.01	0.99±0.02

Five cornea pools (each with triplicate samples) of normal, mock-infected, or fungal *F.solani*-infected corneas at 1, 2, 3, 6, 10, 14d p.i. were assessed using real-time PCR and reported as the mean Ct number±SD (normalized to β-actin). --: No expression.

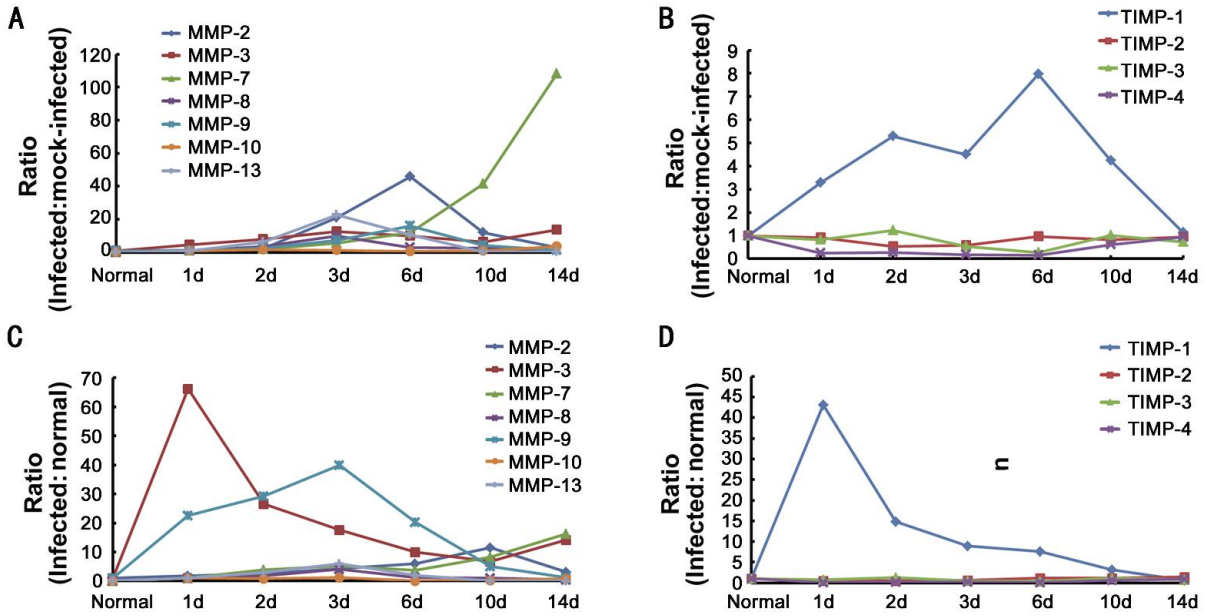

Figure 2 Differential gene expression ratios of MMPs and TIMPs in *F.solani* keratitis compared to control (mock-infected) and normal rat corneas Total MMPs and TIMPs mRNA expression compared to controls and normal corneas was quantified using real-time PCR, with Ct values normalized to β-actin. A: MMPs ratios of infected compared to mock-infected corneas; B: TIMPs ratios of infected compared to mock-infected corneas; C: MMPs ratios of infected compared to normal corneas; D: TIMPs ratios of infected compared to normal corneas.

members of TIMPs were detected. Table 2 and Figure 2 shows the MMPs and TIMPs genes expression in the infected corneas and mock-infected corneas. The gene expression was considered to be normal before infection or mock-infection. MMP-7, -8, -10, -13 were not expressed in the normal cornea. MMP-3 expression was 66.3 times higher in the infected cornea compared to the normal cornea. MMP-8, -9, -13 expression was significantly upregulated in

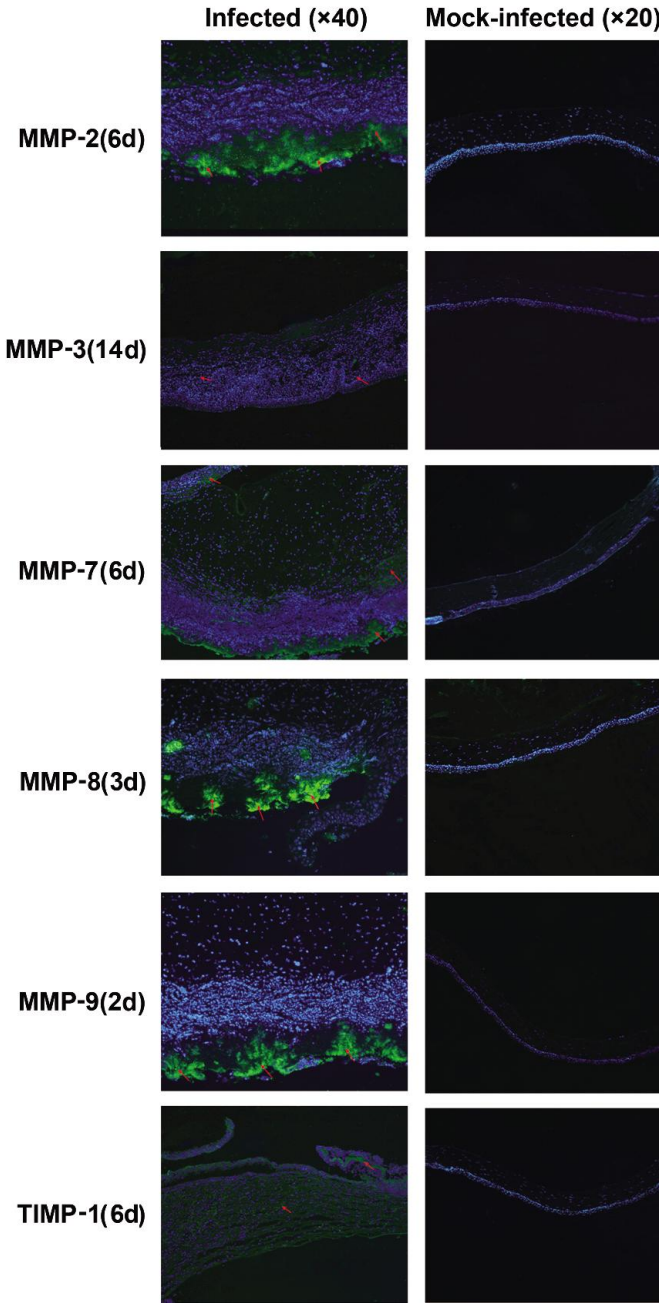

Figure 3 DIF staining of MMP–2, –3, –7, –8, –9 and TIMP–1 in infected and mock–infected corneas DAPI (blue)-nuclei, DIF (green)-MMPs and TIMPs.

the mid-period (infected-to-normal ratios of 4.03, 39.86, and 5.94, respectively). MMP-2, -7 expression was increased in the late period (infected-to-normal ratios of 5.94 and 16.22, respectively). TIMP-1 was upregulated in the early period, and was 43.17 times higher in infected compared to normal corneas, but TIMP-2, -3, -4 expressions were mildly downregulated or unchanged.

Matrix Metalloproteinases and Tissue Inhibitor of Metalloproteinases Protein Expression Protein expression was determined using DIF staining, and these results were consistent with the transcript levels measured using real-time PCR. MMP-8, -9 were expressed during the mid-period, MMP-2, -7 were expressed during the late period, and TIMP-1 was expressed 1d p.i. (Figure 3).

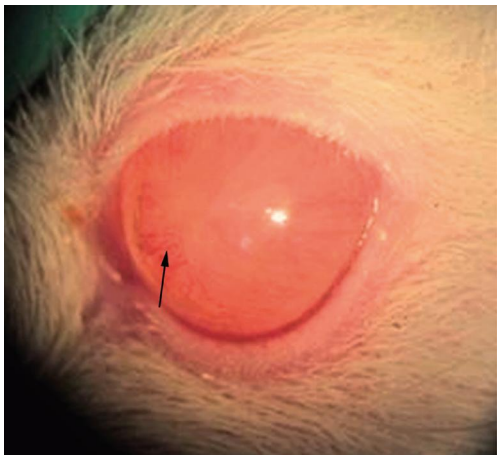

Figure 4 CNV at day 14 p.i.

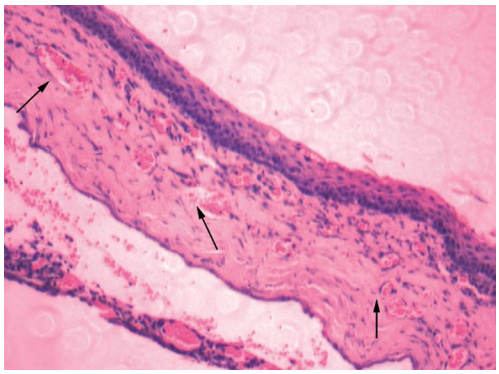

Figure 5 CNV stained using HE (×100).

Table 3 GM6001 efficacy and area of CNV

Groups	Duration of infection (d)	Amount of CNV (×400)	Area of CNV (mm^2)
Saline	14.20±0.92	8.90±1.10	38.01±1.74
GM6001	11.50±0.71	8.10±0.74	32.51±1.51
P	<0.001	0.087	<0.001

Efficacy of GM6001 Treatment GM6001, a MMPs specfic inhibitor, treatment decreased the infection duration to 11.50 ±0.71d compared to 14.20 ±0.92d in rats that received saline drops. The amount and area of CNV were significantly reduced in the infected group that received GM6001 compared to the infected group that received saline (Table 3, Figures 4, 5).

DISCUSSION

Leukocyte-derived MMPs that are involved in fungal keratitis include collagenase (MMP-8), gelatinase (MMP-9), stromeylsin (MMP-10), and elastase (MMP-12), which are released from polymorphonuclear leukocytes (PMNs) or macrophages soon after microbial inoculation [16]. Our study confirms that MMP-8 and MMP-9 expression increased during *F.solani* keratitis. MMP-8 and MMP-9 expression is upregulated in the early-and mid-period of the infection at the RNA level, and DIF showed their expression in the corneal epithelium. Previous studies found that MMP-8, -9 expression was significantly increased in the tear ducts, corneal tissue and serum samples taken from eyes with fungal keratitis [12], the mRNA levels for MMP-9, -10 were

significantly up-regulated in the migrating corneal epithelium [18]. Infiltrating PMNs in the cornea with fungal keratitis contributed to the increased MMP-8 and MMP-9 activities, thereby enhancing tissue destruction and derangement. More expression of MMP-9 is found in mock-infected cornea than *F.solani* infected cornea in 1d, MMP-9 increases immediately after superficial corneal wounding[19] and fungal infection perpetuates its upregulation. MMP-2, -7 play an important role during the corneal repair process, remodeling and angiogenesis. MMP-2 expression was increased during corneal neovascularization and that this neovascularization was mainly localized to the cells infiltrating areas of new vessel formation [20]. In addition, MMP-2 may appear later during the wound-healing process, MMP-2 is important for ECM remodeling in corneal ulcers[21], and MMP2 is important in the development of corneal stromal ulcers [22]. MMP-7 was found to prevent CNV formation, basal epithelial cells express MMP-7 during the migration proliferation phase of corneal wound healing after excimer keratectomy[23]. We found, using real-time PCR, that the expression of MMP-2, -7 is upregulated in the late period of *F.solani* infection, and DIF results are consistent with the real-time PCR findings, which suggests that MMP-2, -7 play a role in the recovery process and ECM remodeling in *F. solani* keratitis.

Stromelysin-1 (MMP-3) degrades proteoglycan core proteins, laminin, fibronectin, elastin, gelatin, and collagen types III, IV, V, VII, and IX [24]. The degradation of the epithelial basement membrane during keratolysis could also be caused by MMP-3 [25]. MMP-3 can be upregulated by IL-1β and TNF-α in corneal epithelial cells [26], and secreted by epithelial cells in the newly formed multi-layer or on the bottom of the deep stromal fibroblasts [27]. We found that MMP-3 expression was upregulated in the early period of *F.solani* keratitis, MMP-3 expression may be an early corneal response to corneal inflammation, MMP-3 increased significantly in 1d both in mock-infected cornear and infected conear. In addition, MMP-3 plays an important role in epithelial basement membrane and extracellular matrix remodeling [28], and this may explain why MMP-3 expression increased in the late period in *F.solani* keratitis.

We found that there was no MMP-13 expression in the normal rat cornea, which is consistent with results presented by Ye *et al* [29]. By digesting collagen and proteoglycan, MMP-13 may be involved in reparative processes of ulcerative keratomycosis [16]. In our study, we found that MMP-13 expression increased in the mid-period, and MMP-13 mRNA was detected in epithelial cells of wounded corneas, but not in normal controls, Zou *et al* [30] found more MMP-13 at day 3 post infection, but less MMP-13, at later time points in experimental Candida albicans keratitis. MMP-13 may play an important role in the gelatinase B-associated proteolytic cascade that allows rapid turnover of the ECM components during corneal wound healing[28]. It's worth mentioning that MMP-13 may activate MMP-9 in the corneal wound [28], in our study, the change trend of MMP-9 was consistent with MMP-13. These results of divergence expression of MMPs family suggested difference function in keratitis.

TIMPs are the major endogenous regulators of MMPs activity in tissues. Adequate TIMPs expression reduces damage to the corneal stroma during infection. TIMP-1 was upregulated during *F.solani* keratitis, and TIMP-1 expression was amplified by fungal infection within 1d to 43.17 times that of the normal cornea, and 3.32 times that of the control (mock-infected) cornea. This indicates that early in the course of the disease, MMPs expression is elevated and TIMP-1 increases to antagonize the elevated MMPs expression. As the disease progresses and healing eventually occurs, TIMP-1 expression begins to decline. Though increased by corneal trauma, the expression of TIMP-1 is amplified by fungal infection within 2d to >5 times that of scarified controls. TIMP-2, -3, -4 expression, on the other hand, was mildly downregulated or unchanged. There may be a network adjustment mechanism between MMPs and TIMPs during *F.solani* keratitis. Corneal homeostasis depends on interactions between MMPs and TIMPs, an important mechanism for the regulation of the MMPs activity is *via* binding to a family of homologous proteins referred to as TIMPs [31], positive correlation was showed between MMP-9 and TIMP-1 in inflammatory cell immunoreactivity in fungal affected Paraffin-embedded equine corneal samples [32], but the mechanism is not known and requires further research. The ratio of MMPs to TIMPs may be important to determine the course of *F.solani* keratitis.

In addition, there were obvious differences in gene expression of MMPs and TIMPs between mock-infected (control) and normal rat corneas, which proved that the expression of MMPs and TIMPs were changed with corneal trauma.

GM6001 is a synthetic MMPs inhibitor that inhibits MMPs activity through recognition of the MMPs substrate recognition site. GM6001 is considered to be the most powerful synthetic MMPs inhibitor. GM6001 (400 μmol/mL) was used to treat *F.solani* keratitis, and we found that GM6001 can shorten the duration of infection and decrease the amount and area of CNV, and these results can confirm the action of MMPs.

In conclusion, this study showed the time-dependent expression of MMPs and TIMPs in a rat *F.solani* keratitis model. We have shown that tissue and inflammatory responses induced upregulation of some MMPs after infection of the cornea with *F.solani* MMP-3, -7, -8, -9 may contribute to ulcerative keratitis and facilitate fungal growth and extension, and MMP-2, -3 and TIMPs may play an important role in corneal healing. A MMPs inhibitor could

be a potential candidate to treat *F.solani* keratitis. This study didn't research molecular mechanism of the changes, which is our further work.

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