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REVIEW

Update on the management of fungal keratitis

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Abstract
Purpose The aim of this article is to introduce the recent advance on the studies of fungal keratitis published over past 5 years.
Methods We performed literature review of articles published on PubMed, Google Scholar, CNKI and Web of Science relevant to the diagnosis, pathogenesis and novel treatment of fungal keratitis.
Results Excessive inflammation can lead to stromal damage and corneal opacification, hence the research on immune mechanism provides many potential therapeutic targets for fungal keratitis. Many researchers discussed the importance of earlier definitive diagnosis and were trying to find rapid and accurate diagnostic methods of pathogens. Develop new drug delivery systems and new routes of administration with better corneal penetration, prolonged ocular residence time, and better mucoadhesive properties is also one of the research hotspots. Additionally, many novel therapeutic agents and methods have been gradually applied in clinical ophthalmology.
Conclusion The diagnosis and treatment of fungal keratitis are still a challenge for ophthalmologist, and many researches provide new methods to conquer these problems.

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Introduction

Fungal keratitis is a severe corneal infection which can cause stromal destruction, perforation, endophthalmitis and corneal scar formation. The high misdiagnosis rate, the lack of effective antifungal agents and the poor therapeutic effect are main cause leading to decrease visual acuity and blindness [1]. The incidence and prevalence of fungal keratitis are affected by geographical location and climate change [2]. Epidemiological studies indicate that incidence rate of fungal keratitis in the developing countries which depends on agriculture is significantly higher than that in the developed countries [3]. Yeast (*Candida*) or filamentous fungi (*Fusarium* and *Aspergillus*) are the most common fungi responsible for fungal keratitis [4]. Filamentous fungi are the main pathogen of fungal keratitis in the tropics and subtropics regions, while yeast is the main pathogen in the temperate areas [5]. A clinical retrospective study confirmed that fusarium is the main cause of fungal keratitis in China, accounting for almost 53.5% of all cases [6].

The risk factors for fungal keratitis include plant trauma, contact lenses, long-term use of steroids, etc. Compared with other infectious keratitis, the therapeutic effect of fungal keratitis is limited due to

individuals have different responsiveness to conventional therapy [7]. At present, the pathogenesis of fungal keratitis remain unclearly and the uniqueness in the physiology and anatomy of ocular tissue results in poor penetration and low retention ability of conventional drug delivery systems [8]. Therefore, how to design effective drugs and methods are still a challenge for ophthalmologists. In this article, we will introduce the recent advance on the studies of fungal keratitis published over past 5 years.

Materials and methods

Collection of English articles relevant to the management of fungal keratitis, a thorough review was performed on the literature published on PubMed, Google Scholar, CNKI and Web of Science. The key words used were fungal keratitis, fungal infection, epidemiology, diagnosis, management, therapy, mechanism, inflammation, corneal ulcer, immune, drug delivery, drug formulation, surgical treatment. These were used alone or in various combination. References of all literature deemed to be relevant were also included.

Immune mechanism

As a common blindness-causing disease, the inflammatory response to pathogens is the main cause of corneal damage and vision loss in fungal keratitis [3]. Recently, researches on the mechanisms of overactive host innate immune inflammatory response in fungal keratitis have become hotspots. In the initial stage of fungal keratitis, pathogenic fungi activate pattern recognition receptors (PRRs) and induce chemokines and cytokines to recruit immune cells to the corneal and promote fungal clearance [9]. As the main infiltrating cells in keratitis, the excessive recruitment of neutrophils associated with the progression of fungal keratitis [10]. Excessive inflammation can lead to stromal damage and corneal opacification [11]. Thus it's crucial to control inflammation in fungal keratitis, and these studies might provide novel targets for its therapeutic strategies.

Xu et al. [12] confirmed that Dectin-1 recruit neutrophils and macrophages to participate anti-fungal immunity through IL-1β, IL-6, CCL2, CXCL1, CXCL2 in early period of innate immune in rat fungal keratitis. And as an important regulator of the host immune and inflammatory response, macrophage migration inhibitory factor (MIF) can inhibit the random migration of macrophages and promote their locally infiltration [3]. Xu et al. found that deficiency of MIF has protective effect on A. fumigatus keratitis. Inhibition of MIF reduced the inflammatory response and the expression of TNF-α and IL-6 in infected rats. Also, many studies have shown that miRNAs modulate ocular infection through its regulation on innate and adaptive immunity, sensory innervation and neuroimmune/neuroinflammation, angiogenesis and neovascularization [13]. Boomiraj et al. [14] predicted several highly dysregulated miRNAs (miR-511-5p, miR-142-3p, miR-155-5p and miR-451a) may be involved in wound healing in infected corneas, moreover, miR-451 may have some therapeutic effects through its over-expression can reduce MIF.

Wedelolactone, triptolide and nerolidol are extracted from plants. Some researchers from China indicated that neutrophil recruitment and IL-1β maturation could be suppressed by wedelolactone in Aspergillus fumigatus keratitis, and zymosan-induced production of IL-6, IL-8 and MCP-1 by human corneal fibroblasts (HCFs) could be inhibited by triptolide [9, 11, 15]. In addition, nerolidol could inhibit the LOX-1/ IL-1β signaling. Combined antifungal medicine with the extractions might reduce lesion severity in fungal keratitis.

Jiang et al. [16] found the expression of indoleamine 2,3-dioxygenase (IDO) in mice cornea was consistent with the severity of A. fumigatus keratitis, and IDO participates in the process of immune regulation by controlling the balance between Th17 and regulatory T (Treg) cell subsets. And IL-17 produced by Th17 cells could suppress CX43 expression through the AKT signaling pathway to inhibit the occurrence and development of fungal keratitis and promote the recovery of corneal damage [17].

Autophagy is a kind of biological phenomena widely existing in eukaryotic cells. It plays an important role in phagocytosis of pathogens, regulation of inflammatory response, reduction of apoptosis and maintenance of internal environment. Li et al. [18] demonstrates that expression of autophagy increased the severity of A. fumigatus keratitis, while autophagy inducer alleviated the severity of keratitis through regulating the recruitment of polymorphonuclear

neutrophilic leukocytes (PMNs), balancing the production of proinflammation and anti-inflammation cytokines.

Furthermore, as a lipid mediator derived from polyunsaturated fatty acids, Maresin1 can relieve corneal inflammation by inhibiting neutrophil recruitment and reducing the expression of CXCL1 and IL-10 [19]. And, Min Yin et al. [20] proved that calcitonin gene-related peptide (CGRP) might have potential therapy effect on A. fumigatus keratitis by regulating the pro-inflammatory and anti-inflammatory mediators.

Novel diagnostic methods

The diagnosis of infectious keratitis is difficult due to its diverse pathogens and clinical manifestations [21]. Unclear diagnosis hinders the further treatment and leads to poor prognosis. Preliminary clinical diagnosis of fungal keratitis were considered according to main predisposing factors such as corneal injury caused by plant matter, contact lens usage and long-term therapy with topical/systemic antibiotics or steroid and typical clinical manifestations include white infiltrates, endothelial plaque, hypopyon and corneal ulcer with a toothpaste-like surface, feathery margins and satellite lesions [22]. Traditional diagnostic methods mainly includes staining and culture of infected corneal tissue [23]. The diagnosis speed and accuracy are greatly limited. The rapid staining method and KOH are superior to others but the positive rate still affected by many factors. Additionally, some morphology-based fungal identification methods such as in vivo confocal microscopy (IVCM) require professional operation and can't always provide enough resolution for identifying fungal species due to the image quality were influenced by the extent of inflammatory cells infiltration [24].

Molecular detections such as polymerase chain reaction (PCR) and DNA sequencing characterized by high sensitivity and specificity. Compared with the traditional culture method, PCR offers several advantages including rapid analysis and the ability to analyse specimens away from the collection site, however, it still has many disadvantages including cross-contamination and false negative results [25]. DNA sequencing is not suitable for clinical practice due to the complicated manipulation and high cost. Hence, it is crucial to explore novel diagnostic methods of pathogens.

Rui Tian et al. [21] found TLR4 is the representative differentially expressed gene (DEG) specific to bacterial keratitis, and SOD2 is the representative DEG specific to fungal keratitis through analyzed DEGs in bacterial and fungal keratitis. Parthiban et al. [2] revealed the down-regulation of zinc $\alpha - 2$ glycoprotein (ZAG) in tear samples of patients with *Aspergillus flavus* keratitis by detecting the proteome of tear samples from patients with different stages of infection while previous study showed the up-regulation of ZAG in tear samples of patients with keratitis induced by Fusarium infection. Furthermore, they found ZAG level could be used as an indicator of infection by *A. flavus* or *F. solani* even in the early stage of the disease. And recently some researches are trying to build intelligent corneal confocal microscopy image analysis and diagnosis system which can diagnose fungal keratitis automatically by using data augmentation and image fusion [26].

Treatment

Local and systematic treatments are generally used in fungal keratitis. Topical administration through eye-drops and intraocular injection (subconjunctival, intracameral, intrastromal or intravitreal) are most commonly used. Systematic treatments can be administered orally on intravenously. The common therapeutic drugs can be classified into three categories, polyene macrolide antibiotic (natamycin (5%), amphotericin B (0.25%)), imidazole anti-fungal drugs (miconazole (0.5%)), pyrimidine anti-fungal drugs (flucytosine (1%)). Combination of drugs has synergic effect in fungistasis, and therapeutic surgery should be considered when patients respond poorly to medical therapy, such as debridement, conjunctival flap or penetrating keratoplasty. Additionally, new methods of treatment are explored and mentioned as follow [27].

Drug formulation

For conventional delivery systems, the pre-corneal drug absorption is extremely limited due to the specificity in anatomy and physiology and protective mechanisms such as blink reflex and lacrimal

secretion and drainage of ocular [28]. Therefore, many researchers are committed to the development of new drug delivery systems and new routes of administration with better corneal penetration, prolonged ocular residence time and better mucoadhesive properties [4].

Vesicular drug delivery systems

Liposome is a kind of subminiature spherical carrier preparation, which is made by encapsulating drugs in the membrane formed by lipid like bimolecular layer. It was prepared to provide superior pharmacokinetics and biodistribution while minimizing toxicity. The excellent biocompatibility, cell membrane like structure and ability to encapsulate both hydrophilic and hydrophobic drugs of liposomes made it an ideal ophthalmic drug delivery system [28].

Zhang et al. [29] showed rapamycin liposome eyedrops might become a promising strategy for the treatment of fungal keratitis by significantly inhibiting the expression of monocyte chemotactic protein-1 (MCP-1). However, as another vesicular system for drug delivery, niosomes was considered that have better stability than liposomes [8]. El-Nabarawi et al. [30] developed dual-purpose natamycin (NAT)-loaded niosomes in ketorolac tromethamine (KT) gels topical ocular drug delivery system to improve NAT ocular bioavailability. And Verma et al. [8] suggested that Nat loaded trimethyl chitosan (TMC) coated mucoadhesive cationic niosomes (Muc-Cat-Nios) is an effective way in treating fungal keratitis via cationic Nios showed greater mucoadhesive potential that extended the release time of drug.

Drug-loaded nano-system

Drug-loaded nano-system has many advantages such as targeting drug delivery, controling release of drug, reducing the toxicity and improving drug loading. Chhonker et al. [31] prepared Amphotericin-B loaded lecithin/chitosan nanoparticles to improve the bioavailability and precorneal residence time of Amphotericin-B. And Younes et al. [32] prepared corneal targeted Sertaconazole nitrate loaded cubosomes which has superior corneal penetration power. Both the NAT solid lipid nanoparticles (NAT-SLNs) prepared by Khames et al. [4] and a thermosensitive hydrogel containing sertaconazole loaded nanostructured lipid carriers developed by Tavakoli et al. [33] have higher antifungal activity and cornea permeation.

Polymeric micelles is also a kind of nanoscopic drug carriers. The small molecule drugs were wrapped by micelles to increase its water solubility and stability. Guo et al. [34] prepared self-assembled poly(ethylene glycol)-block-poly(glycidyl methacrylate) (PEG-b-PGMA) micelles to deliver the natamycin sustainable and reduce administration frequency.

Currently, researches on formulations utilizing newer vesicular delivery system is still on the way of experiment phase, and ophthalmic preparation is not yet commercially available.

Contact lens and microneedle ocular patch (MOP)

In order to carry and release drugs sustainably. Huang et al. [35] fabricated a hydrogel-based hybrid theranostic contact lens loaded with Voriconazole (Vor) which consists of quaternized chitosan (HTCC), silver nanoparticles and graphene oxide (GO). MOP is a minimally invasive corneal delivery device, Roy et al. [36] fabricated amphotericin B (AmB) containing MOP which mimics the curvature of contact lens to enhance corneal retention of drug. It's application significantly increased the corneal distribution of AmB and reduced the Candida albicans load within cornea in rabbit infection model.

Promising therapeutic agents and methods

Fungal infection of cornea often induces the formation of biofilms which can be encased in the protective extracellular matrix [37]. And difficult removal of biofilms leads to poor therapeutic effect of many antifungal drugs. Therefore, the lack of safe and effective anti-fungal agents is an important reason for the difficulty of the treatment of fungal keratitis [38].

As an antimicrobial agents with a mechanism of membrane disrupting, antimicrobial peptides (AMPs) have the potential to be a effective agent for the treatment of fungal keratitis [39]. Wu et al. [40] demonstrated that the β-sheet forming peptides can remove fungi biofilms both in vitro and mouse model safely and effectively. β-sheet forming peptides were as effective as AmB in reducing the number of *C. albicans* and higher concentration of peptides have stronger fungicidal effect. Dogan et al. [41] showed

that n-butyl-2-cyanoacrylate (nB2CA) exhibited in vitro antifungus efficiency against a wide range of yeast and filamentous fungi which are the most common causes of fungal ocular infections.

Umbilical cord mesenchymal stem cells (uMSCs) possess properties of anti-inflammatory and immunomodulatory, anti-apoptotic and antimicrobial [42]. Researches of uMSCs involve many fields of Ophthalmology. Zhou et al. [43] suggests that subconjunctival injections of uMSCs exerts anti-inflammatory and anti-fibrotic effects in fungal keratitis, the corneal opacity, scar formation area and corneal thickness can be reduced by uMSCs administration, accompanying with down-expression of α-SMA, TGFβ1, CTGF and COLI through TGFβ1/Smad2 signaling pathway regulation.

In addition, Behrens-Baumann et al. [44] reported a patient with fungal keratitis refractory to common antifungal therapy but resolved with systemic and topical terbinafine treatment. And some studies indicated that eugenol, dimethyl itaconate (DI), AMPs and matrix metalloproteinases (MMPs) inhibitors might have potential protective effect on fungal keratitis [10, 45, 46].

Inotophoresis is a non-invasive physical technique which is used to increase molecular transport across biological membranes [47]. Gelfuso et al. [47] showed ocular iontophoresis increase the penetrability of the Vor and improves drug efficiency in fungal keratitis. Photodynamic antimicrobial chemotherapy (PACT) impairs the cell wall or cell membrane and DNA in the target organism by reactive oxygen species to result in antimicrobial effect [48]. Sueoka et al. [48] demonstrated that PACT with the chlorin derivative TONS 504 and an LED device inhibits the growth of the filamentous fungi *F. solani* and *A. fumigatus*, and the antifungal effect of PACT on *F. solani* is stronger. Additionally, it has been proved that commonly used ophthalmic agents with nonantifungal effect enhance the in vitro activity of first-line antifungal drugs to exert synergistic action [49].

Photoactivated chromophore for infectious keratitis cross-linking (PACK-CXL)

Collagen cross-linking (CXL) is a photooxidative collagen crosslinking technique which promote the formation of covalent bonds between collagen molecules in the cornea by photochemical activation of riboflavin [50][51]. PACK-CXL has the function of antibacterial and enhancing corneal tissue, but it's efficacy for fungal keratitis is still controversial.

Some researchers showed that adjunctive CXL treatment is effective for promoting ulcers healing while some others believed that PACK-CXL is not helpful for fungal keratitis [52, 53]. Through comparing the therapeutic effects between patients performed CXL combined 5% NAT eye drops and other patients received medical treatment alone. Vajpayee et al. showed that adjunctive CXL treatment did not have any advantage over medical management. In addition Mikropoulos et al. [54] first reported a case of intraoperative PACK-CXL application combined successfully with penetrating keratoplasty for the management of refractory fungal keratitis specifically in a patient with irradiation-related local immunosuppression.

At present, the role of PACK-CXL in fungal keratitis is still unclear. More research is needed to confirm whether it can help in the treatment of fungal keratitis.

Surgical treatment

If corneal infection of fungal keratitis aggravate in spite of adequate drug treatment, surgical treatment should be considered [25].

Through clinical research, Zhong et al. [55] indicated that full-thickness conjunctival flap covering surgery with amniotic membrane transplantation (FCCS + AMT) may be a good choice for the patients with severe fungal keratitis without corneal perforation, and may save the eyeball and provide a greater opportunity for corneal transplantation. Kitazawa et al. [56] reported that antifungal medications usually unable to eradicate the infection in the cases where retrocorneal plaques may penetrate the anterior chamber, however, retrocorneal plaque aspiration may contributes to improve the diagnostic accuracy, reduce secondary inflammation and prevent the development of corneal perforation.

Therapeutic keratoplasty (TKP) is required for those patients who cannot be helped by other therapies [57]. Recurrence of fungal infection is an important cause of corneal grafting failure. Through the retrospective research on 198 eyes underwent therapeutic penetrating keratoplasty (ThPK) for fungal keratitis, Mundra et al. [58] found that the recurrence risk of fungal infection was related to the size of the infiltrate, but not to the fungal specie. And as glucocorticoids

aggravate fungal keratitis through increasing fungal aggressivity and reducing the infiltration of neutrophils, lack of appropriate anti-inflammatory drugs in the postoperative period limited the visual restoration and secondary rehabilitation of patients [59, 58].

Other studies suggested that smaller grafts might reduce the incidence of complications after penetrating keratoplasty, but the recurrence rate increased due to incomplete removal of infection sites [60].

Air-assisted manual therapeutic deep anterior lamellar keratoplasty (TDALK) is a technique that DALK performed by more superficial intrastromal air injection [61]. Compared air-assisted manual TDALK with big-bubble TDALK, Uchio et al. presented that air-assisted manual TDALK can reduce the risk of intraoperative perforation of Descemet's membrane (DM) and provide structural stability and ambulatory vision.

Conclusion

Fungal keratitis is one of the mainly causes of blindness. Lack of rapid diagnosis methods and effective antifungal drugs leading to the poor prognosis. In recent years, more rapid and accurate diagnostic methods, potential therapeutic targets, new pharmaceutical preparations and novel therapeutic methods of fungal keratitis were the research focus of many researches. These studies will provide ophthalmologists with more options for treatment and bring hope to patients suffering from fungal keratitis.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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