In Vitro Comparison of the Efficacies of Natamycin and Silver Nitrate against Ocular Fungi

Yan Xu,1* Guangren Pang,1 Chuanwen Gao,1 Dongqing Zhao,1 Lutan Zhou,1 Shengtao Sun,1 and Bingliang Wang2

Henan Institute of Ophthalmology, Department of Ophthalmology, Henan Provincial People’s Hospital, Zhengzhou, China,1 and Anyang Eye Hospital, Anyang, China2

Received 28 May 2008/Returned for modification 4 September 2008/Accepted 4 January 2009

The in vitro activity of the silver nitrate was assessed in comparison with that of natamycin against 128 corneal Fusarium spp. and 90 corneal Aspergillus isolates. MIC90s of silver nitrate were 2 μg/ml for Fusarium spp. and 1 μg/ml for Aspergillus spp. MIC90s of natamycin were 8 μg/ml for Fusarium spp. and 32 μg/ml for Aspergillus spp. Silver nitrate exhibited potent antifungal activity against ocular fungi in vitro.

The blindness caused by keratomycosis is a serious health problem in the developing world. Keratomycosis has become increasingly prevalent in corneal diseases that are responsible for vision loss in China (17, 24, 26). Clinical studies indicate that keratomycosis constitutes about 46.7% to 61.9% of all cases of suppurative keratitis inpatients, and 35.1% of all patients of infectious keratitis who have undergone penetrating keratoplasty in some regions in China (3, 24, 26). Filamentous fungi, mainly Fusarium spp. or Aspergillus spp., are the most frequently isolated fungi in patients with keratomycosis and the most common ocular pathogenic fungi in China. To date, keratomycosis still remains a therapeutic challenge for the ophthalmologist, as clinical management has been limited due to the relative unavailability of effective antifungal agents. Corneal lesions fail to resolve in many patients who receive antifungal treatment, and some patients get marked loss of vision and eventually perforation of the cornea, ultimately requiring penetrating keratoplasty, or even enucleation or evisceration (15, 17, 19, 24). Therefore, it is very important and urgent to develop new broad-spectrum antifungal agents that are active against a wide variety of ocular fungal pathogens, perhaps available as antifungal eye drops that combat this vision-threatening infection in the ophthalmologic clinic.

The use of metallic silver as an antimicrobial agent has long been recognized (6, 8). Silver nitrate had been used since the 19th century to prevent ophthalmia neonatorum and treat infections and burns (2, 6, 13). Silver compounds possess the activity of silver nitrate was assessed in comparison with that of natamycin against 128 corneal Fusarium spp. and 90 corneal Aspergillus isolates. MIC90s of silver nitrate were 2 μg/ml for Fusarium spp. and 1 μg/ml for Aspergillus spp. MIC90s of natamycin were 8 μg/ml for Fusarium spp. and 32 μg/ml for Aspergillus spp. Silver nitrate exhibited potent antifungal activity against ocular fungi in vitro.

One hundred twenty-eight Fusarium isolates and 90 Aspergillus isolates from patients with keratomycosis from the Henan Institute of Ophthalmology in Zhengzhou, China, were investigated. These isolates were identified based on morphology by standard methods (16, 21–23). Candida parapsilosis ATCC 22019 was used as a quality control.

The antifungal agents tested in this study were silver nitrate (Fourth Air Pharmaceutical Factory, Wuhan, China; minimum purity, 99.8%) and natamycin (Yinxiang Biotechnology Co., Ltd., Zhejiang, China; minimum purity, 95%). Both were dissolved in 100% dimethyl sulfoxide. The stock solutions were prepared at a concentration of 1,600 μg/ml and then were stored at −65°C until tested. All dilutions were made in RPMI 1640 medium (with L-glutamine, without sodium bicarbonate; GIBCO-BRL, Grand Island, NY) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; Serva, Feinbochemica GmbH, Germany) and 10 M NaOH. Final concentrations of both drugs ranged from 0.031 to 16 μg/ml.

A broth microdilution method was used following the Clinical and Laboratory Standards Institute (CLSI) M38-A document (11), which describes a standard method for testing the susceptibility of conidium-forming filamentous fungi that cause invasive fungal infections, including Aspergillus species, Fusarium species, etc., to antifungal agents. Inocula were prepared in accordance with the CLSI M38-A document. The final inoculum was 0.4 × 10⁵ to 5 × 10⁶ CFU/ml.

Following incubation at 35°C for 48 h, the MIC was determined according to the CLSI M38-A document. For both agents tested, the MIC was defined as the lowest drug concentration that prevented any discernible growth. The MIC range, mode, MIC50, and MIC90 were provided for the isolates with the SPSS statistical package (version 13.0). For calculation, high off-scale MICs were converted to the next concentration up.

The MIC range, mode, MIC50, and MIC90 were provided for the isolates with the SPSS statistical package (version 13.0). For calculation, high off-scale MICs were converted to the next concentration up.

The in vitro activities of silver nitrate and natamycin against the Fusarium spp. and Aspergillus spp. are summarized in Table 1. Both the MIC50 and MIC90 of silver nitrate were 2 μg/ml for Fusarium spp. and 1 μg/ml for Aspergillus spp. The MIC50 and MIC90 of natamycin were 4 μg/ml and 8 μg/ml, respectively, for Fusarium spp. and both were 32 μg/ml for Aspergillus spp.

Our results show that the MIC90 of natamycin were 8 μg/ml.
The results of this study suggest that silver nitrate may be superior to natamycin in potency against corneal *Fusarium* and *Aspergillus* isolates in vitro. Particularly, when comparing the MIC\(_{50}\) for both agents, the activity of silver nitrate against *Aspergillus* spp. is 32 times greater than that of natamycin and 4 times higher than that of natamycin when used against *Fusarium* spp. In an earlier study, Wan introduced the application of 3% silver nitrate ophthalmic solution to the eyes of patients with clinical keratomycosis. A higher success rate was achieved with silver nitrate (78%) than without silver nitrate (46%) (20). In a recent study, Zhang reported that in keratomycosis, 92% of 38 patients showed a favorable response to the treatment of (i) subconjunctival injection of 2.5 mg amphotericin B once daily or once on an alternate day and (ii) 0.5% silver nitrate drops given once as an adjuvant on an alternate day. The study showed that silver nitrate may have an adjunctive role in the management of keratomycosis (25). Our result that silver nitrate exhibits potent antifungal activity against corneal *Fusarium* and *Aspergillus* isolates in vitro is in accordance with some good outcomes with silver nitrate for patients with keratomycosis.

Wan and Zhang reported that no significant ocular or systemic adverse effects were observed during the study periods (20, 25). During the 20th century, instillation of silver nitrate eyedrops to newborns had been the best prophylaxis against ophthalmia neonatorum caused by *Neisseria gonorrhoeae* (5, 7, 14). To date, in the developing world and some developed countries, silver nitrate is still being used for prophylaxis in the neonatal period because of its effect against gonococcus (1, 10). Chemical conjunctivitis is much more commonly seen with silver nitrate eye prophylaxis than with other medications, including erythromycin, tetracycline, gentamicin, and 2.5% povidone–iodine ophthalmic solution (1, 5, 10, 14). However silver nitrate eye prophylaxis caused no sustained deleterious effects and even provided some benefit to infants born to women without *Neisseria gonorrhoeae* infection (5, 10).

In conclusion, in vitro susceptibility data suggest that silver nitrate may be effective against corneal *Fusarium* and *Aspergillus* isolates and that a prospective evaluation of efficacy and safety would be required to further develop its clinical applications.

**REFERENCES**


