In Vitro Activity of nano-silver against Ocular Pathogenic Fungi

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Abstract: The in vitro activity of nano-silver versus those of fluconazole and natamycin was assessed against 264 ocular fungal isolates. The activity of nano-silver against Fusarium spp., Aspergillus spp., and Alternariaalternata was 8 times, 32 times, and 2 times, respectively, greater than that of natamycin and 512times, 256 times, and 4 times, respectively, greater than that of fluconazole. Nano-silver's antifungalactivity was significantly superior to those of natamycin and fluconazole against ocular pathogenic fungi in vitro.
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1. Introduction

The problem of keratomycosis has become increasingly prevalent in corneal diseases that are responsible for vision loss in the developing world like china(1,2,3).Clinical studies indicate that keratomycosis constitutes about 46.7% to 61.9% of all cases of suppurative keratitis inpatients. 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, only fluconazole and natamycin are commercially available for ocular use in China. Fluconazole has high bioavailability against Candida spp., but Fusarium spp. And Aspergillus spp. are resistant to it (4,5,6). Natamycin is the only topical ophthalmic antifungal compound approved in the United States (7), but it penetrates the cornea and conjunctiva poorly and effective drug levels are not achieved in either the cornea or the aqueous humor (8) after topical application because it is poorly soluble in water. It is therefore useful only in the treatment of superficial keratomycosis. Due to the relative unavailability of effective antifungals, corneal lesions fail to resolve in many patients who receive antifungal treatment, some patients get vision loss and eventually perforation of the cornea, ultimately require penetrating keratoplasty, or even enucleation or evisceration (2,9). Therefore, it is very important and urgent to explore broad-spectrum antifungals to effectively suppress a wide variety of ocular fungal pathogens and to develop new antifungal eye drops to combat this vision-threatening infection.

Since ancient times, the silver ion has been known to be effective against a broad range of microorganisms. Rencently there is an increasing use of silver as an efficacious antibacterial and antifungal agent in wound care products and medical devices (10,11,12,13) including dental work, catheters, and the healing of burn wounds (14,15,16). Additionally, $AgNO_3$, as eye drops, have been utilized to prevent gonococcal ophthalmicneonatorum in newborns by pediatricians for centuries(17), and our experiment has demonstrated that Silver nitrate exhibited potent antifungal activity against ocular fungi in vitro. 18. Recent advances in nanotechnology have enabled us to produce pure silver, as nanoparticles, which are more efficient than silver ions(19).

2. Material and Methods

Two hundred sixty-four strains of fungi isolated from patients with keratomycosis from the Zhengzhou second hospital and the Henan Eye Institute in Zhengzhou, China, were investigated. These isolates were identified based on morphology by standard methods (20,21,22,23). They included 144 Fusarium isolates, 110 Aspergillus isolates, and 10 Alternaria alternata isolates. Candida parapsilosis ATCC 22019 was used as quality control for each test.

The antifungal agents tested in this study were nano-sliver (Nanux, korea; 2000ppm), natamycin (Yinxiang Biotechnology Co., Ltd., Zhejiang, China; minimum purity, 95%) and fluconazole (Pfizer. American, minimum purity, 100%) They were all dissolved in 100% dimethyl sulfoxide. The stock solutions were prepared at concentrations of 800μg/ml for nano-sliver, 1,600 μg/ml for natamycin and 25600 μg/ml for fluconazole. Drug dilutions were made in RPMI 1640 (with L-glutamine, without sodium bicarbonate; GIBCO-BRL, Grand Island, NY) medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; Serva, Feinbochemica GmbH, Germany). Final concentrations ranged from 0.0313 to 16μg/ml for

nano-sliver, from 0.0625 to 32μg/ml for natamycin and from1 to 512μg/ml for fluconazole. Then they were stored at -65°C until tested.

A broth microdilution method was performed following the Clinical and Laboratory Standards Institute (CLSI) M38-A document (24), 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 0.4×10^4 to 5×10^4CFU/ml. Following incubation at 35°C for 48 h, the MIC was determined according to the CLSI M38-A document. For nano-sliver and natamycin the MIC was defined as the lowest drug concentration that prevented any discernible growth, and the MIC was defined as the lowest drug concentration that prevented 75% or more growth for fluconazole.

The MIC range and mode, the MIC50(MIC for 50% of the strains tested), and the MIC90(MIC for 90% of the strains tested) were provided for the isolates with the SPSS statistical package (version 13.0). For calculation, any high off-scale MIC was converted to the next higher concentration.

3. Results

The in vitro activities of nano-sliver, natamycin and fluconazole against the Fusarium spp. and Aspergillus spp. are summarized in Table 1 and Table 2. Both the MIC50 and MIC90 of nano-sliver were both 1μg /ml for Fusarium spp. and 0.5μg /ml and 1μg /ml, respectively for Aspergillus spp. The MIC50 and MIC90 of natamycin were 4μg /ml and 8μg /ml, respectively, for Fusarium spp. and were both 32μg /ml for Aspergillus spp. The MIC50 and MIC90 of fluconazole were both 512μg /ml for Fusarium spp. and were 128μg /ml and 256μg /ml, respectively, for Aspergillus spp.

When comparing the MIC90s of nano-sliver, natamycin and fluconazole, the activity of nano-sliver against Fusarium spp. is 8 times greater than that of natamycin and 512 times greater than that of fluconazole, the activity of nano-sliver against Aspergillus spp. is 32 times greater than that of natamycin and 256 times greater than that of fluconazole, and the activity of nano-sliver against Alternaria alternata is 2 times greater than that of natamycin and 4 times greater than that of fluconazole. And as shown in Tables 1 and 2, nano-sliver has activity against Fusarium and Aspergillus complexes. For each of these genera, this activity remains consistent and does not show significant interspecies variability. Therefore, nano-sliver's effect was significantly superior to those of natamycin and fluconazole against main ocular pathogenic fungi in vitro.

Table 1. *In vitro* susceptibilities of ocular *Fusarium* isolates to Nano-sliver, natamycin and fluconazole.

Organism (no. of isolates) and antifungal agent	MIC (μg /ml) range (mode)	MIC50 (μg /ml)	MIC90 (μg /ml)
Fusarium solani species complex (85)			
Nano-silver	0.25-2(1)	1	1
natamycin	4-32(4)	4	8
fluconazole	16-512(512)	512	512
Fusarium moniliforme species complex (23)			
Nano-silver	0.5-2(1)	1	2
natamycin	4-8(4)	4	8
fluconazole	32-512(512)	256	512
Fusarium avenaceum species complex (18)			
Nano-silver	0.5-2(1)	1	2
natamycin	4-32(8)	8	8
fluconazole	64-512(512)	512	512
Other Fusarium isolates (18) [1]			
Nano-silver	0.5-2(0.5)	0.5	1
natamycin	4-8(4)	4	8
fluconazole	256-512(512)	512	512
Fusarium spp. (144)			
Nano-silver	0.25-2(1)	1	1
natamycin	4-32(4)	4	8
fluconazole	16-512(454.79)	512	512

[1] Includes 9 strains of Fusarium oxysporum species complex, 5 strains of Fusarium poae species complex, and 4 strains of Fusarium lateritium species complex.

Table 2. *In vitro* susceptibilities of ocular *Aspergillus* and *Alternaria alternata* isolates to Nano-sliver, natamycin and fluconazole.

Organism (no. of isolates) and antifungal agent	MIC (μg /ml) range (mode)	MIC50 (μg /ml)	MIC90 (μg /ml)
Aspergillus flavus species complex (54)			
Nano-silver	0.5-1(0.5)	0.5	1
natamycin	8-2(32)	32	32
fluconazole	64-512(128)	128	256
Aspergillus fumigatus species complex (14)			
Nano-silver	0.25-1(0.5)	0.5	0.5
natamycin	4-32(4)	4	4
fluconazole	64-256(128)	128	256
Aspergillus oryzae species complex (15)			
Nano-silver	0.5-1(0.5)	0.5	0.5
natamycin	4-32(32)	32	32
fluconazole	64-128(128)	128	128
Aspergillus versicolor species complex (13)			
Nano-silver	0.125-0.5(0.25)	0.25	0.5
natamycin	4-32(32)	8	32
fluconazole	32-256(128)	64	128
Other Aspergillus isolates (14)[2]			
Nano-silver	0.0625-0.5(0.25)	0.25	0.5
natamycin	0.25-32(16)	8	32
fluconazole	32-128(64)	64	128
Aspergillus spp. (110)			
Nano-silver	0.0625-1(0.5)	0.5	1
natamycin	0.25-32(32)	32	32
fluconazole	32-512(128)	128	256
Alternaria alternata (10)			
Nano-silver	0.25-1(0.5)	0.5	1
natamycin	2-8(4)	4	4
fluconazole	8-128(64)	16	128

[2] Includes 8 strains of Aspergillus niger species complex, 2 strains of Aspergillus candidus, 2 strains of Aspergillus nidulans, 1 strain of Aspergillus ochraceus, and 1 strainof Aspergillus wentii.

4. Discussions

Nano-sliver has been developed as a potent antibacterial, antifungal, antiviral, and anti-inflammatory agent. (25,26,27). Compared with other metals, silver nanoparticles show higher toxicity to microorganisms while exhibiting lower toxicity to mammalian cells (28). To date, the most promising applications have been shown in the medical fields, such as infection for wound and burn (29,30). Nano-sliver is available as an antimicrobial gel formulation for conventional topical antimicrobial agents, treatment (31). Some studies show that nano-sliver have the antimicrobial activity against bacteria and virus (32,33,34). The findings from our study indicate that nano-sliver is active against ocular fungal.

In conclusion, in this study, nano-sliver exhibited potent in vitro activity against main ocular pathogenic fungi and was even more effective than natamycin and fluconazole. The results suggest that nano-sliver might to be a effective drug in the treatment of keratomycosis and that a prospective evaluation of efficacy and safety to further develop its clinical applications.

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