In Vitro Activity of nano-silver against Pulmonary Pathogenic Fungi

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Abstract: The in vitro activity of nano-silver versus those of amphotericin B was assessed against 37 plmonary aspergillosis isolates. The activity of nano-silver against Aspergillus spp. is 2 times greater than that of amphotericin B. Nano-silver's antifungalactivity was superior to hose of amphotericin B against plmonary pathogenic fungi in vitro.

[Chao Xu, Chuanwen Gao, Hua Zhang, Jianli Chen. **In Vitro Activity of nano-silver against Pulmonary Pathogenic Fungi.** *Life Sci J* 2013;10(1):3040-3043] (ISSN:1097-8135). http://www.lifesciencesite.com. 373

Keywords*:* nano-silver; Pulmonary aspergillosis; drug susceptibility testing; antifungal

1. Introduction

Pulmonary aspergillosis is a severe disease. Heretofore considered to be an unusual cause of infection, Aspergillus species have emerged as important causes of morbidity and mortality in immunocompromised patients (1-4). Invasive aspergillosis currently constitutes the most common cause of infectious pneumonic mortality in patients undergoing HSCT(hematopoietic stem cell transplantation) and is an important cause of opportunistic respiratory and disseminated infection in other immunocompromised patients(4-11).Many risk factors are associated with Invasive aspergillosis, a serious fungal infection that affects immunocompromised patients, particularly those with hematological malignancies and those who have undergone hematopoietic stem cell or solid organ transplantation.(12) Invasive fungus infections caused by aspergillus spp. occur most frequently in immunocompromised patients. A high infection-associated death rate of up to and over 50% is attributed even today to these fungi. The disease in humans is caused mainly by Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger. Other species, for example, Aspergillus terreus or Aspergillus nidulans are quantitatively less prevalent. (13,14). Amphotericin B, a polyene macrolide, has long been used as the first-line agent for systemic fungal infection because of its broad spectrum. (15)

The silver ion is well known for its broad spectrum Antimicrobial at very low concentrations(16)，And it has been used for centuries in health care delivery due to its antimicrobial and wound healing (anti-inflammatory) properties (17,18). Recently there is an increasing use of silver as an efficacious antibacterial and antifungalagent in wound care products and medical devices (10-22), and the advances in nanotechnology have enabled us to produce pure silver, as nanoparticles, which are more efficient than silver ions (23). Nano-sliver is available as an antimicrobial gel formulation for conventional topical antimicrobial agents, treatment (24). Some studies show that nano-sliver has the antimicrobial activity against bacteria and virus (25-27).Our experiments have demonstrated that Nano sliver exhibited potent antifungal activity against Aspergillus in vitro (28,29). The Aspergillus in our experiments such as Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger are also the most common pathogens of Pulmonary aspergillosis(13).In this study we want to determine the Activity of nano-silver against Pulmonary Pathogenic Fungi.

2. Material and Methods

Thirty-seven strains of Aspergillus isolated were obtained from patients with Pulmonary aspergillosis from the Zhengzhou Central Hospital affiliated to Zhengzhou University, China, were investigated. These isolates were identified based on morphology by standard methods (30-33). Three species were studied, they included 16 Aspergillus fumigatus, 10 Aspergillus flavus, and 8 Aspergillus niger. 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) and amphotericin B (Bristol-Myers Squibb,Princeton, NJ) 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 amphotericin B .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 amphotericin B. 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 (34), 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 $0.4\times10^4$to $5\times10^4$4CFU/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 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 and amphotericin B against the Aspergillus spp. are summarized in Table 1. The MIC50 and MIC90 of nano-sliver were 0.5μg /ml, 0.5μg /ml, 0.25μg /ml, respectively, and were 0.5μg /ml, 1μg /ml, 0. 5μg /ml, respectively, for Aspergillus fumigatus，Aspergillus flavus and Aspergillus niger. The MIC50 and MIC90 of amphotericin B were 1μg /ml, 2μg /ml, 1μg /ml, respectively, and were all 2μg /ml for Aspergillus fumigates, Aspergillus flavus and Aspergillus niger. When comparing the MIC90s of nano-sliver and amphotericin B, the activity of nano-sliver against Aspergillus spp. is 2 times greater than that of amphotericin B. And as shown in Tables 1, nano-sliver has activity against Aspergillus complexes. For each of these genera, this activity remains consistent and does not show significant interspecies variability. Therefore, nano-sliver was effective against main Pulmonary pathogenic fungi in vitro. And it's effect was superior to those of amphotericin B.

Tables 1. In vitro susceptibilities of Pulmonary Aspergillus isolates to Nano-sliver and amphotericin B.

Organism (no. of isolates) and antifungal agent	MIC range (μg /ml)	MIC mode (μg /ml)	MIC50 (μg /ml)	MIC90 (μg /ml)
Aspergillus fumigatus species complex (16)				
Nano-silver	0.25-1	0.5	0.5	0.5
amphotericin B	0.5-4	1	1	2
Aspergillus flavus species complex (10)				
Nano-silver	0.5-1	0.5	0.5	1
amphotericin B	1-32	2	2	2
Aspergillus niger species complex (8)				
Nano-silver	0.125-0.5	0.5	0.25	0.5
amphotericin B	0.25-2	1	1	2
Aspergillus spp.(34)				
Nano-silver	0.125-1	0.5	0.5	1
amphotericin B	0.25-32	1	1	2

4. ***Discussions***

The scientific literature points that Nano-sliver is widely used in medical devices and supplies as a potent antibacterial, antifungal, antiviral, and anti-inflammatory agent. (35,36). Coatings generally comprised of nanoparticles have been used to prevent bacterial infections associated with medical devices, such as wound dressings, catheters, and orthopedic and cardiovascular implants, with different degrees of clinical efficacy (37,38). The findings from our study indicate that nano-sliver is active against main Pulmonary pathogenic fungi. The results suggest that a prospective evaluation of efficacy and safety to develop the nano-sliver's clinical applications such as Fiberoptic bronchoscopy.

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2/25/2013