In Vitro Activity of Thimerosal against Ocular Pathogenic Fungi

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The in vitro activity of thimerosal versus those of amphotericin B and natamycin was assessed against 244 ocular fungal isolates. The activity of thimerosal against Fusarium spp., Aspergillus spp., and Alternaria alternata was 256 times, 512 times, and 128 times, respectively, greater than that of natamycin and 64 times, 32 times, and 32 times, respectively, greater than that of amphotericin B. Thimerosal’s antifungal activity was significantly superior to those of amphotericin B and natamycin against ocular pathogenic fungi in vitro.

The problem of keratomycosis in developing countries like China is more acute because of its higher incidence and the unavailability of effective antifungals (18, 28, 30). 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 (3, 27, 29). Fusarium spp. and Aspergillus spp. are more commonly associated with keratomycosis, while Candida spp. are rarely implicated as etiological agents of keratomycosis in China (23, 26). Natamycin is the only topical ophthalmic antifungal compound approved in the United States (14). Natamycin is poorly soluble in water. After topical application, natamycin penetrates the cornea and conjunctiva poorly and effective drug levels are not achieved in either the cornea or the aqueous humor (15); it is therefore useful only in the treatment of superficial keratomycosis. Due to the relative unavailability of effective antifungals, keratomycosis fails to resolve in many of the patients who receive antifungal treatment; some patients experience vision loss and eventually corneal perforation, ultimately require penetrating keratoplasty, or even enucleation or evisceration (20, 28). 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.

Thimerosal is a preservative commonly used in ophthalmic solutions, otic drops, topical medicine, and vaccines because of its bactericidal property. However, the efficacy of thimerosal against ocular pathogenic fungi has not been evaluated so far. The present study was performed to determine the antifungal activity of thimerosal versus those of amphotericin B and natamycin against ocular pathogenic fungi in vitro. To our knowledge, this is the first study to determine the antifungal activity of thimerosal against main ocular pathogenic fungi. Results obtained in this study may contribute to the development of new antifungal eye drops.

Two hundred forty-four strains of fungi isolated from patients with keratomycosis from the Henan Eye Institute in Zhengzhou, China, were investigated. These isolates were identified based on morphology by standard methods (22, 23, 25). They included 136 Fusarium isolates, 98 Aspergillus isolates, and 10 Alternaria alternata isolates. Candida parapsilosis ATCC 22019 was used as quality control for each test. Thimerosal (Yili Pharmaceutical Co. Ltd., Beijing, China), amphotericin B (Bristol-Myers Squibb, Princeton, NJ), and natamycin (Yinxiang Biotechnology Co. Ltd., Zhejing, China) were studied. They were all dissolved in 100% dimethyl sulfoxide. The stock solutions were prepared at concentrations of 400 μg/ml for thimerosal and 1,600 μg/ml for amphotericin B and natamycin. Drug dilutions were made in RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid. Final concentrations ranged from 0.0078 to 4 μg/ml for thimerosal and from 0.0313 to 16 μg/ml for amphotericin B and natamycin.

A broth microdilution method was performed by following the Clinical and Laboratory Standards Institute M38-A2 document (13). The final inoculum was 0.4 × 104 to 5 × 104 CFU/ml. Following incubation at 35°C for 48 h, the MIC was determined as the lowest concentration of amphotericin B, natamycin, or thimerosal that prevented any discernible growth.

The MIC range and mode, the MIC for 50% of the strains tested (MIC50), and the MIC for 90% 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.

The in vitro activities of thimerosal, amphotericin B, and natamycin against the isolates are summarized in Tables 1 and 2. When comparing the MIC50 of thimerosal with those of natamycin and amphotericin B, the activity of thimerosal against Fusarium spp. is 256 times greater than that of natamycin.
mycin and 64 times greater than that of amphotericin B, the activity of thimerosal against Aspergillus spp. is 512 times greater than that of natamycin and 32 times greater than that of amphotericin B, and the activity of thimerosal against Alternaria alternata is 128 times greater than that of natamycin and 32 times greater than that of amphotericin B. Therefore, thimerosal’s effect was significantly superior to those of amphotericin B and natamycin against main ocular pathogenic fungi in vitro.

As shown in Tables 1 and 2, thimerosal has activity against different Aspergillus and Fusarium complexes. For each of these genera, this activity remains consistent and does not show significant interspecies variability. On the other hand, natamycin shows various activities against different Aspergillus spp. Most Aspergillus spp. are not susceptible, but Aspergillus fumigatus complex is susceptible to natamycin.

A noteworthy finding is that thimerosal exhibits the greatest activity against Aspergillus spp. in comparison to the effects of all of the antifungals studied in vitro to date. Some studies (10–12) of the in vitro efficacy of traditional and newer antifungals against keratitis and endophthalmitis fungal pathogens show that amphotericin B and voriconazole have the lowest MIC>90 (2 to 4 μg/ml) against Aspergillus spp., closely followed by terbinafine (8 μg/ml), natamycin (16 μg/ml), posaconazole (>8 μg/ml), itraconazole (>16 μg/ml), ketoconazole (>16 μg/ml), caspofungin (>16 μg/ml), 5-flucytosine (>64 μg/ml), and fluconazole (>256 μg/ml). When comparing the MIC>90 of thimerosal with those of other antifungals, the activity of thimerosal against Fusarium spp. is 64 to >8,179 times greater than those of other antifungals. It is very important because Fusarium spp. remain the most frequently isolated ocular fungal pathogens in China, Portugal, Singapore, Australia, and the southern United States (2, 7, 12, 16, 18, 19, 23, 24, 26, 30) and the second most frequently isolated ocular fungal pathogens in India, Nepal, and Saudi Arabia (4, 8, 9, 17).

Successful treatment of otomycosis with thimerosal has been reported by Tisner et al. (21). Recently, our primary work based on clinical trials addressed the suitability of thimerosal for the treatment of keratitis. At the Henan Eye Institute and the Anyang Eye Hospital, 21 patients with filamentous keratomycosis were treated with thimerosal because they were not improving after topical amphotericin B, ketoconazole, and natamycin treatment for 1 to 3 weeks. Twenty of the 21 infections responded well to thimerosal. The keratomycosis healed after topical thimerosal treatment for 14 to 45 days (unpublished data).

Thimerosal is one of the main preservatives used worldwide in topical ophthalmic preparations, at concentrations ranging from 0.004 to 0.01%. Thimerosal has generally been accepted as a safe preservative agent in eye drops, and ocular side effects due to thimerosal are rare. No toxic effects have been observed following topical application of solutions containing thimerosal, even at concentrations 100 times higher than those required for a bactericidal effect (1, 5, 6). The findings from our study indicate that products formulated with thimerosal as both the main drug and a preservative can probably be used to treat keratomycosis successfully. We think that thimerosal has both antifungal and preservative effects without exceeding its value as a preservative and that the benefits of treating keratomycosis outweigh the potential risks for thimerosal.

In conclusion, in this study, thimerosal exhibited potent in vitro activity against main ocular pathogenic fungi and was even more effective than amphotericin B and natamycin. The

### TABLE 1. In vitro susceptibilities of ocular Fusarium isolates to thimerosal, amphotericin B, and natamycin

<table>
<thead>
<tr>
<th>Organism (no. of isolates) and antifungal agent</th>
<th>MIC range (mode)</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
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</thead>
<tbody>
<tr>
<td><strong>Fusarium solani</strong> species complex (82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thimerosal</td>
<td>0.0078–0.0313 (0.0156)</td>
<td>0.0156</td>
<td>0.0313</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.5–16 (1)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Natamycin</td>
<td>4–32 (4)</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>Fusarium moniliforme</strong> species complex (20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thimerosal</td>
<td>0.0156–0.0313 (0.0156)</td>
<td>0.0156</td>
<td>0.0313</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>1–8 (2)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Natamycin</td>
<td>4–8 (4)</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>Fusarium avenaceum</strong> species complex (16)</td>
<td></td>
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<tr>
<td>Thimerosal</td>
<td>0.0156–0.0313 (0.0156)</td>
<td>0.0156</td>
<td>0.0313</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.5–8 (2)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Natamycin</td>
<td>4–32 (8)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><strong>Other Fusarium isolates</strong> (18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thimerosal</td>
<td>0.0078–0.0625 (0.0156)</td>
<td>0.0156</td>
<td>0.0313</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.5–2 (1)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Natamycin</td>
<td>4–8 (4)</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>Fusarium spp.</strong> (136)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thimerosal</td>
<td>0.0078–0.0625 (0.0156)</td>
<td>0.0156</td>
<td>0.0313</td>
</tr>
<tr>
<td>Amphotericin B</td>
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<td>2</td>
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<tr>
<td>Natamycin</td>
<td>4–32 (4)</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Includes 9 strains of Fusarium oxysporum species complex, 5 strains of Fusarium poae species complex, and 4 strains of Fusarium lateritum species complex.

<sup>b</sup> Values are in micrograms per milliliter.
results suggest that thimerosal might play a role as a main drug in the treatment of keratomycosis and should be subjected to a prospective evaluation of efficacy and safety to further develop its clinical applications.

REFERENCES


