Evaluation of Polyhexamethylene Biguanide (PHMB) as a Disinfectant for Adenovirus

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Importance: Swimming pools can be a vector for transmission of adenovirus ocular infections. Polyhexamethylene biguanide (PHMB) is a disinfectant used in swimming pools and hot tubs.

Objective: To determine whether PHMB is an effective disinfectant against ocular adenovirus serotypes at a concentration used to disinfect swimming pools and hot tubs.

Design: In vitro laboratory study.

Interventions: The direct disinfecting activity of PHMB was determined in triplicate assays by incubating 9 human adenovirus types (1, 2, 3, 4, 5, 7a, 8, 19, and 37) with PHMB concentrations of 50 and 0 ppm (micrograms per milliliter) for 24 hours at room temperature to simulate swimming pool temperatures or 40°C to simulate hot tub temperatures.

Main Outcome Measures: Plaque assays were performed to determine adenovirus titers after incubation. Titers were log_{10} converted and mean (SD) log_{10} reductions relative to controls were calculated. Virucidal (>99.9%) decreases in mean adenovirus titers after PHMB treatment were determined for each adenovirus type and temperature tested.

Results: At room temperature, 50 ppm of PHMB produced mean reductions in titers less than 1 log_{10} for all adenovirus types tested. At 40°C, 50 ppm of PHMB produced mean reductions in titers less than 1 log_{10} for 2 adenovirus types and greater than 1 but less than 3 log_{10} for 7 of 9 adenovirus types.

Conclusions and Relevance: At a concentration of 50 ppm, PHMB was not virucidal against adenovirus at temperatures consistent with swimming pools or hot tubs. Recreational water maintained and sanitized with PHMB can serve as a vector for the transmission of ocular adenovirus infections.


DENOVIRAL OCULAR INFEC-
TIONS (epidemic keratocon-
junctivitis [EKC], follicu-
lar conjunctivitis, and
pharyngeal conjunctival
fever) are the most commonly occurring
ocular viral infections in the world.1 These
infections are known to occur in community-
and office-based outbreaks.5 Outbreaks of adenoviral ocular infections can be
detrimental to the community because of the significant patient morbidity and the socioeconomic consequences of
time lost from school and work.5 Among the community-based outbreaks, swim-
ing pool water has been reported to be a vector for transmission.2,3 Derrick2 first
showed in 1943 that swimming pools can act as vectors for the transmission of
“swimming bath conjunctivitis.” The description of the patients’ symptoms sug-
gested that these cases were pharyngeal conjunctival fever.3 This observation came
10 years before the first adenovirus was iso-
lated.11 Some of these swimming pool–
related outbreaks have been linked to in-
deficient chlorination of the swimming
pool water.5,6,9

Proper maintenance and sanitization of recrea-
tional water is essential to preventing
disease transmission. Chlorine is the
most common sanitizing agent used in
swimming pools. However, proper chlo-
ринation of swimming pool water has been
associated with eye irritation in some
people.12 Eye irritation, chlorine mainte-
nance, and other factors have lead to the
development of alternate, non–chlorine-

Based agents to disinfect recrea-
tional water, such as swimming pools and hot tubs. One such agent is polyhexamethylene bigu-

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biocide registered for numerous applications by the US Environmental Protection Agency under the Federal Insecticide, Fungicide, and Rodenticide Act and supported under the Biocidal Products Directive in Europe. It is unaffected by sunlight, water temperature, or pH fluctuations, and its stability allows water to be properly maintained for longer periods, generally 7 to 14 days before additional PHMB is required. The agent is also used in ophthalmology as a topical treatment for keratitis and is an active antimicrobial agent in several types of adenovirus.

The use of PHMB for swimming pool and hot tub disinfection prompted us to determine whether PHMB is an effective disinfectant against common ocular serotypes of adenovirus.

METHODS

VIRUSES AND CELLS

Clinical ocular adenovirus isolates of types 1, 2, 3, 4, 5, 7a, 8, and 19 were collected anonymously from patients who sought care because of typical adenoviral ocular disease at The Charles T. Campbell Ophthalmic Microbiology Laboratory at the UPMC Eye Center, University of Pittsburgh, Pittsburgh, Pennsylvania, and frozen at −70°C. The viruses are part of a retrospective clinical collection of adenovirus isolates. The isolates were deidentified and stored for diagnostic test validations. The serotypes of the adenovirus isolates were determined using serum neutralization. No clinical isolates of adenovirus type 37 (Ad37) were recovered, so the ATCC reference strain of Ad37 was used. The types tested represent the most common adenovirus types that cause ocular infections (Ad8, Ad19, and ATCC Ad37) and 1.0 × 10⁶ PFU/mL of multiple clinical ocular isolates of Ad1, Ad2, Ad3, Ad4, Ad5, Ad7a, Ad19, and ATCC Ad37 and 1.0 × 10⁶ PFU/mL of Ad8. Fifty microliters of each test virus was added to duplicate sets of 2-mL screw-capped freezing tubes (Sarstedt) containing 50 ppm of PHMB at a concentration of 50 ppm (micrograms per milliliter), prepared in tissue culture medium. This resulted in a final PHMB concentration of 50 ppm; this concentration was used as according to the manufacturer’s instruction for recreational water disinfection. One set of tubes containing the virus-PHMB mixtures was incubated for 24 hours on a bench top at room temperature to simulate swimming pool conditions. The second set of tubes containing the virus-PHMB mixtures was incubated in a 40°C bench-top water bath for 24 hours to simulate hot tub conditions. After incubation, 500 µL of tissue culture medium containing 20% fetal bovine serum was added to each tube in preparation for the plaque assay.

Determination of Viral Titers (Plaque Assay)

Immediately after incubation and the addition of 500 µL of tissue culture medium, the samples were serially diluted in tissue culture medium containing 10% fetal bovine serum for five 10-fold dilutions and inoculated in duplicate onto A549 cell monolayers in 24-well multiplates. After a 3-hour adsorption period, the wells were filled with tissue culture medium containing 0.5% methylcellulose, except for Ad8, for which the wells were filled with standard tissue culture medium. After 7 to 10 days of incubation, the medium was removed, the cells were fixed and stained with 0.5% gentian violet solution containing formaldehyde, and the number of plaques were counted with a dissecting microscope. The viral titers were calculated and expressed as PFU per milliliter.

Statistical Analysis

Viral titers were log₁₀ converted, and log₁₀ differences in titers from the appropriate negative controls were calculated for each trial. The mean (SD) log₁₀ differences in titers were calculated from the data of the 3 trials. A mean reduction in titer of at least 3 log₁₀ (99.9% reduction) relative to negative control values was considered a virucidal reduction.

Results

The mean log₁₀ reductions from 3 trials of PHMB treatment on adenovirus survival at room temperature and 40°C are presented in the Table. At room temperature, PHMB at a concentration of 50 ppm produced no mean reductions of adenovirus titers greater than 1 log₁₀ for any of the adenovirus serotypes tested. At 40°C, 50 ppm of PHMB produced mean reductions in titers greater than
2 but less than 3 log_{10} for Ad2, Ad3, Ad19, and Ad37. The mean reductions in titers for Ad1, Ad4, and Ad5 were greater than 1 but less than 2 log_{10}. The mean (SD) reduction in titer for the important ocular serotype of Ad7a approached 1 log_{10} (−0.91 [0.26] PFU/mL), whereas 50 ppm of PHMB had no effect on Ad8 at 40°C. No virucidal reductions were demonstrated for PHMB at 50 ppm for either temperature tested.

**COMMENT**

Adenoviruses are hardy viruses that have the ability to survive at least 8 weeks in a desiccated state, 3 to 4 weeks in multidose bottles of topical fluorescein,18 and at least 5 days in liquid transport medium without a significant loss in infectivity after cross-country shipment.29 The hardiness of adenoviruses may contribute to their ability to cause outbreaks of ocular infections. Therefore, to prevent disease transmission, it is of utmost importance to eliminate adenoviruses from potential vectors of transmission, such as contaminated tonometer tips and ophthalmic instruments in ophthalmologists’ offices and recreational water.

Many disinfectants have been tested for their abilities to kill adenoviruses. Rutala and colleagues20 performed an extensive study evaluating the efficacy of a number of disinfectants against Ad8. They determined that 0.55% ortho-phthalaldehyde, 2.4% glutaraldehyde, 2.65% glutaraldehyde, 6000 ppm chlorine, 1900 ppm chloramine, 70% ethanol, 65% ethanol with 0.63% quaternary ammonium compound, 79.6% ethanol with 0.1% quaternary ammonium compound, and 0.2% peracetic acid were effective against Ad8. Other common disinfectants, such as 70% isopropyl alcohol, 3% hydrogen peroxide, 4% chlorhexidine, and 10% povidone-iodine, were ineffective against Ad8.20 These results may not extend to all adenovirus types, however, because disinfectants have a range of efficacy against different adenovirus types.21

To our knowledge, there have been no earlier studies evaluating the efficacy of PHMB as a disinfectant against adenovirus. The results of the current study demonstrated that at room temperature, PHMB treatment at a concentration of 50 ppm produced no reductions of adenovirus titers greater than 1 log_{10} for any of the adenovirus serotypes tested after 24 hours of incubation. We conclude from these data that PHMB is an ineffective disinfectant against adenovirus at room temperature in concentrations used in swimming pools.

At a concentration of 50 ppm, PHMB was more effective when the temperature was raised to 40°C. This concentration produced decreases in adenovirus titers greater than 1 log_{10} for 7 of 9 adenovirus serotypes but did not demonstrate virucidal efficacy (99.9% reduction) against any of the adenovirus serotypes tested. However, 50 ppm of PHMB had little or no effect on Ad7a and Ad8, 2 major causes of ocular adenovirus infections. From these data, we conclude that PHMB may be more effective for some, but not all, adenovirus serotypes when the incubation temperature is raised to 40°C. Several factors may contribute to the increase in PHMB effectiveness at the higher temperature. Pinto et al22 demonstrated that exposure to PHMB of the MS2 bacteriophage—used as a surrogate virus for nonenveloped mammalian viruses, including adenovirus—led to the formation of viral aggregates, which were probably caused by changes in viral surface hydrophobicity. They concluded that the formation of these aggregates reduced the virucidal activity of PHMB. A follow-up study by the same group and using the same virus demonstrated that increasing the incubation temperature from 20°C to 40°C and 50°C decreased the viral aggregates and increased the antiviral activity of PHMB.23 Pinto et al23 speculated that high temperatures may also produce conformational changes in the viral capsid that increase the sensitivity of viral nucleocapsids to PHMB. These factors may have also contributed to an increase in effectiveness of PHMB against adenovirus at 40°C.

Based on the data from the current study, swimming pool water sanitized with PHMB would not effectively kill adenovirus contained in the water. Therefore, these swimming pools are at greater risk to act as vectors for the transmission of adenoviral ocular infections than those properly sanitized with an agent such as chlorine. Hot tub water may be more effectively sanitized with PHMB, because the increased temperature increased the effectiveness of PHMB. However, PHMB was not effective against all the adenovirus serotypes tested, so it may not be the optimal sanitizing agent for hot tub water.

These data are also important because PHMB is used as a disinfectant in several multipurpose contact lens solutions. The concentration of PHMB in these solutions is usually 0.0001% (1 ppm [μg/mL]),13 which is 50 times lower than the concentration tested in the current study. It was previously demonstrated that contact lens solutions containing 0.0001% polyaminopropylbiguanide or 3% hydrogen peroxide solution were ineffective in sterilizing contact lenses infected with Ad8 and Ad19.24 On the basis of the data in the current study, we predict that the PHMB contained in multipurpose contact lens solutions would be ineffective for disinfecting contact lenses contaminated with adenovirus. Because the multipurpose contact lens solutions containing PHMB were not tested in this study, the disinfection efficacy of the formulated solutions against adenovirus remains unknown.

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REFERENCES


