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Research Article

Time-Kill Comparison of Povidone Iodine to Hypochlorous Acid against Endophthalmitis Isolates of Staphylococci

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Abstract

Purpose: We compared the antibacterial activity of Povidone Iodine (PI) and Hypochlorous Acid (HA) against endophthalmitis isolates of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* using time-kill studies.

Methods: Time-kill studies of PI, HA (0.008%), and HA (0.01%, Avenova), were conducted in duplicate against Methicillin-Susceptible *Staphylococcus aureus* (MSSA), Methicillin-Resistant *Staphylococcus aureus* (MRSA), and Coagulase Negative *Staphylococcus* (CNS) at time points 1, 2, 10, and 30 minutes. Each antiseptic was inoculated to a final bacterial concentration of 10^6 CFU/mL. The main outcome measure was based on a bactericidal decrease in colony counts units (CFU) ($3 \log_{10}$ or 99.9% decrease).

Results: At 1 minute, HA (0.008% and 0.01%) were more bactericidal than PI for decreasing the CFU of MRSA, MSSA, and CNS ($p=0.045$, Fisher's Exact Test). At 2 minutes, the bactericidal effect was equivalent for PI and HA (0.008% and 0.01%) against the three Staphylococcal groups. The bactericidal effects of HA 0.008% and HA 0.01% were equivalent at all time points against all three Staphylococcal groups.

Conclusions: Povidone iodine is the current gold standard for endophthalmitis prophylaxis. This *in vitro* study supports a 2-minute contact time between Staphylococci and PI for a bactericidal effect. The contact time between HA (0.008% and 0.01%) and Staphylococci for a bactericidal effect appears to be reduced to 1 minute.

Keywords: Endophthalmitis; Hypochlorous Acid; *Staphylococcus aureus* **Introduction**

Abbreviations

PI	:	Povidone Iodine
HA	:	Hypochlorous Acid
MRSA	:	Methicillin Resistant <i>Staphylococcus aureus</i>
MSSA	:	Methicillin Susceptible <i>Staphylococcus aureus</i>
CNS	:	Coagulase Negative <i>Staphylococcus</i>
CFU	:	Colony Forming Unit

Antiseptics are used to sterilize the eye before ocular surgery. Endophthalmitis is a very worrisome, but uncommon post-operative complication of intraocular surgery and it is generally believed that *S. epidermidis* is the most common pathogen associated with endophthalmitis [1]. Povidone Iodine (PI) is now the gold standard for reducing the risk of post-operative endophthalmitis, and it has been shown to reduce colony counts on the ocular surface [2].

In recent years, products such as SteriLid have been shown to decrease bacterial load in *in vitro* studies, and have been shown to be comparable to PI [3]. In 2014 the FDA approved I-Lid Cleanser (Nova Bay, Emeryville, CA), which was recently rebranded

as Avenova, as an approved treatment for blepharitis. Its active ingredient is 0.01% Hypochlorous Acid (HA), and it is unclear how effective the product may be at reducing bacterial load of ocular adnexa. It has been shown that using sodium hypochlorite decreases bacterial load on a variety of ophthalmic lenses [4]. However, the concentration used was over 50 times as powerful as Avenova, so the question remains about the products efficacy at decreasing bacterial load.

We hypothesized that there was no difference in the antibacterial activity between PI, HA 0.008%, and HA 0.01%, and that all products would have a fast kill time. The hypothesis was tested using time-kill studies against bacteria isolated from endophthalmitis cases at time points that included 1 and 2 minutes as well as 10 and 30 minutes. Differences between PI, HA 0.008%, and HA 0.01% were based on 90% and 99.9% decreases in bacterial load at each time point.

Materials and Methods

In vitro time-kill studies of PI 5%, HA 0.008% (supplied by Pete Adamson, Institute of Ophthalmology, University College, London, HPA-Scientific, Port Louis, Mauritius), and HA 0.01% were conducted at 22°C in duplicate experiments against bacteria that include: Coagulase Negative *Staphylococcus* (CNS), Methicillin-Susceptible *Staphylococcus aureus* (MSSA), and Methicillin-Resistant *Staphylococcus aureus* (MRSA), at time points 1, 2, 10, and 30 minutes. The bacterial isolates were from de-identified endophthalmitis isolates collected for antibiotic validation testing at the Charles T. Campbell Ophthalmic Microbiology Laboratory at the University of Pittsburgh Medical Center, Pittsburgh, PA.

The inoculum was prepared from an overnight growth of bacteria grown on trypticase soy agar supplemented with 5% sheep blood (TSA, BBL, Sparks, MD). Colonies were picked from the TSA and suspended in trypticase soy broth to about a 1.0 McFarland Standard that represents approximately 108cfu/mL. The initial inoculum, noted as time 0, was confirmed with

standard colony counts. Four 3-mL glass test tubes were used for time points 1, 2, 10, and 30 minutes, with 1 mL of either PI, HA (0.008%), or Avenova (0.01%). Tubes were placed on a vortex mixer to ensure antiseptic and bacteria contact. At time points 1, 2, 10, and 30 minutes 0.1 mL of each representative were removed for standard colony count determination on TSA. The calculations for the final colony counts for each time point were performed. The final colony counts were calculated based upon the volume of inoculums and antiseptic. Outcome measures were based on 90% and 99.9% decreases in colony counts.

The comparative outcome measures were based on 90% and 99.9% decreases in bacterial colony counts compared to the colony counts at time 0. A 90% reduction is a 1-log decrease in colony counts, whereas a 99.9% reduction is a 3-log reduction that is denoted as a bactericidal kill. The 90% and 99.9% reductions were noted at each time point for all bacterial groups. The number of bacterial groups that were reduced by these percentages at each time point were compared between PI, HA 0.008%, and HA 0.01% using Fisher’s Exact (FE) randomization analysis with significance set at P<0.05.

Results and Discussion

(Tables 1-3) present the colony counts at each time point for each bacteria group and the reduction in colony counts compared to time 0 for PI, HA 0.008%, and HA 0.01%. (Tables 4-6) through 6 detail the statistical comparison between PI, HA 0.008%, and HA 0.01% for reaching either 99% or 99.9% bacterial reduction at each timepoint. At 1 minute, HA (0.008% and 0.01%) were more bactericidal than PI for decreasing the CFU of MRSA, MSSA, and CNS (p=0.045, Fisher’s Exact Test). At 2 minutes, the bactericidal effect was equivalent for PI and HA (0.008% and 0.01%) against the three Staphylococcal groups. The bactericidal effects of HA 0.008% and HA 0.01% were equivalent at all time points against all three Staphylococcal groups and statistically better than PI (p=0.002).

Table 1: Time-Kill Data for Povidone Iodine, Hypochlorous Acid 0.008%, and Hypochlorous Acid 0.01% Against Common Endophthalmitis Bacterial Isolates.

	Time 0	1 min	2 min	10 min	30 min
MRSA (E625)					
PI	1.4 x 10 ⁸	2.9 x 10 ⁶	0	0	0
% reduction		99	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
MRSA (E635)					

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PI	1.9×10^8	1.6×10^6	1.0×10^6	0	0
% reduction		99	99	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
MRSA (E716)					
PI	1.3×10^8	1.6×10^6	0	0	0
% reduction		99	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
MRSA (E699)					
PI	2.3×10^8	3.1×10^6	0	0	0
% reduction		99	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
MRSA (E765)					
PI	4.4×10^8	0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
Abbreviations: [MRSA: Methicillin Resistant Staphylococcus aureus, PI: Povidone Iodine, HA: Hypochlorous Acid].					

Table 2: Time-Kill Data for Povidone Iodine, Hypochlorous Acid 0.008%, and Hypochlorous Acid 0.01%, Against Common Endophthalmitis Bacterial Isolates.

	Time 0	1 min	2 min	10 min	30 min
MSSA (E628)					
PI	4.0×10^8	7.0×10^6	0	0	0
% reduction		99	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
MSSA (E772)					
PI	8.7×10^8	0	0	0	0

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% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
MSSA (E721)					
PI	5.0×10^8	1.4×10^6	4.0×10^5	0	0
% reduction		>99	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
MSSA (E736)					
PI	3.9×10^8	1.5×10^6	4.0×10^5	0	0
% reduction		>99	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
MSSA (E749)					
PI	6.6×10^8	4.7×10^6	1.0×10^6	0	0
% reduction		>99	>99	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9

Abbreviations: [MSSA: Methicillin Susceptible Staphylococcus aureus, PI: Povidone Iodine, HA: Hypochlorous Acid].

Table 3: Time-Kill Data for Povidone Iodine, Hypochlorous Acid 0.008%, and Hypochlorous Acid 0.01% Against Common Endophthalmitis Bacterial Isolates.

	Time 0	1 min	2 min	10 min	30 min
CNS (E759)					
PI	7.0×10^7	0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
CNS (E751)					
PI	1.0×10^8	0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9

HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
CNS (E742)					
PI	2.3 x 10 ⁸	0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
CNS (E762)					
PI	7.0 x 10 ⁷	0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
CNS (E771)					
PI	1.0 x 10 ⁸	0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.008%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
HA 0.01%		0	0	0	0
% reduction		>99.9	>99.9	>99.9	>99.9
Abbreviations: [CNS; Coagulase negative Staphylococci, PI: Povidone Iodine, HA: Hypochlorous Acid].					

Table 4: Statistical Comparison of Povidone Iodine, Hypochlorous Acid 0.008%, and Hypochlorous Acid 0.01% for bactericidal effect among the five isolates of MRSA at the four-time points in the study.

	1 min	2 min	10 min	30 min
PI	1/5	4/5	5/5	5/5
HA 0.008%	5/5	5/5	5/5	5/5
HA 0.01%	5/5	5/5	5/5	5/5
P Value ^a	P=0.45	P=1	P=1	P=1

Abbreviations: [PI: Povidone Iodine, HA: Hypochlorous Acid]. ^aP Values result from a Fisher's Exact test at each time point comparing PI, HA 0.008%, and HA 0.01% for bactericidal reduction, which is greater than a 3-log decrease from inoculum (time zero).

Table 5: Statistical Comparison of Povidone Iodine, Hypochlorous Acid 0.008%, and Hypochlorous Acid 0.01% for bactericidal effect among the five isolates of MSSA at the four time points in the study.

	1 min	2 min	10 min	30 min
PI	1/5	4/5	5/5	5/5
HA 0.008%	5/5	5/5	5/5	5/5

HA 0.01%	5/5	5/5	5/5	5/5
P Value ^a	P=0.45	P=1	P=1	P=1

Abbreviations: [PI: Povidone Iodine, HA: Hypochlorous Acid].

^aP Values result from a Fisher’s Exact test at each time point comparing PI, HA 0.008%, and HA 0.01% for bactericidal reduction, which is greater than a 3-log decrease from inoculum (time zero).

Table 6: Statistical Comparison of Povidone Iodine, Hypochlorous Acid 0.008%, and Hypochlorous Acid 0.01% for bactericidal effect among the five isolates of CNS at the four-time points in the study.

	1 min	2 min	10 min	30 min
PI	5/5	5/5	5/5	5/5
HA 0.008%	5/5	5/5	5/5	5/5
HA 0.01%	5/5	5/5	5/5	5/5
P Value ^a	P=1	P=1	P=1	P=1

Abbreviations: [PI: Povidone Iodine, HA: Hypochlorous Acid].

^aP Values result from a Fisher’s Exact test at each time point comparing PI, HA 0.008%, and HA 0.01% for bactericidal reduction, which is greater than a 3-log decrease from inoculum (time zero).

Bacterial endophthalmitis is a devastating complication of intraocular surgery. Bacterial pathogens from the surrounding ocular adnexa such as the eyelids and lashes are often to blame, therefore it is imperative to have effective preoperative prophylaxis against these pathogens to prevent infection. In this study we compared PI to HA 0.008% and HA 0.01% against 15 different bacterial isolates from endophthalmitis samples. PI was bactericidal (decreasing bacterial load by greater than 99.9%) in only 1 of 5 MRSA isolates at one minute, by 2 minutes this increased to 4 of 5 MRSA isolates. PI was also only bactericidal in only 1 of 5 MSSA isolates at 1 minute, and increasing to 5 of 5 isolates at 2 minutes. PI was bactericidal for all 5 CNS isolates at 1 minute. In contrast HA in both 0.008% and 0.01% were bactericidal in 5 of 5 isolates of MRSA, MSSA, and CNS at one minute and thereafter.

Previous studies have shown the *in vitro* bactericidal effects of PI in regard to a variety of endophthalmitis isolates [3]. Recently, *in vivo* studies of cultures taken of the ocular surface at the end of cataract surgery after prophylaxis of the ocular surface and adnexa with PI grew bacterial isolates in 30% of eyes sampled [5]. Furthermore, recent *in vitro* studies have shown the bactericidal effect of dilute hypochlorous acid solutions on MRSA isolates [6,7]. However, this study is the first to show bactericidal effect of hypochlorous acid on endophthalmitis isolates. In addition, dilute hypochlorous acid has been shown not to be cytotoxic in *in vitro* cell viability assays, unlike PI which had a high index of cytotoxicity [7]. Hypochlorous acid in concentrations of 0.008% and 0.01% were bactericidal in all bacterial isolates at 1 minute and beyond, which was statistically significant to PI at 1 minute for MRSA and MSSA isolates.

Conclusion

We conclude that PI, the current gold standard for preoperative endophthalmitis prophylaxis is an effective antiseptic for decreasing bacterial load native to ocular adnexa, at times of 2 minutes after application or greater. Hypochlorous acid solutions of 0.008% and 0.01% are more effective at reducing bacterial load than PI at 1 minute after initial application. It may be noted that HI 0.008% may be more potent since the lower concentration was as effective as HI 0.01%.

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Conflict of Interest

The authors have no “Conflict of Interests” to disclose for the completion of this study as determined by the Office of Research, University of Pittsburgh, Pittsburgh, PA, USA.

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