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Increased Bactericidal Activity of Dilute Preparations of Povidone-Iodine Solutions

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Recent confirmation of intrinsic bacterial contamination of 10% povidone-iodine solution has raised questions regarding the bactericidal mechanism of iodophors and the possibility for survival of vegetative bacterial cells in iodophor solutions. In this laboratory investigation, five different species were exposed to various dilutions of three commercial preparations of 10% povidone-iodine solution; survival was assessed after exposure for time periods varying between 0 and 8 min. All brands of povidone-iodine solution tested demonstrated more rapid killing of *Staphylococcus aureus* and *Mycobacterium chelonei* at dilutions of 1:2, 1:4, 1:10, 1:50, and 1:100 than did the stock solutions. *S. aureus* survived a 2-min exposure to full-strength povidone-iodine solution but did not survive a 15-s exposure to a 1:100 dilution of the iodophor. Both stock and dilute preparations of 10% povidone-iodine solution demonstrated rapid bactericidal action against *Klebsiella pneumoniae*, *Pseudomonas cepacia*, and *Streptococcus mitis*.

Iodine has been recognized as a valuable antiseptic for more than a century. However, iodine burns, caused mainly by a 7% tincture, gave iodine a bad reputation (5). In the 1950s, iodophors, loose complexes of iodine and a carrier, were formulated to eliminate the side effects of iodine while retaining iodine's bactericidal activity (H. A. Shelanski, U.S. patent 2,739,922, March 1956). Iodophors have now been used in clinical practice for more than 20 years; povidone-iodine, the common name for iodine in complex with the nonsurfactant carrier polyvinylpyrrolidone, is currently the most widely used iodophor (5). These preparations enjoy widespread popularity today, attributable to their lack of skin irritation and their absence of odor and staining. More important, only a few investigators have questioned their efficacy (7, 9). However, recent confirmation of intrinsic contamination of a 10% povidone-iodine solution with *Pseudomonas cepacia* startled many microbiologists and chemists considered experts in the antiseptic and disinfectant field (1, 2). Although mechanical protection of *P. cepacia* by organic or inorganic material is now considered the most likely explanation for the finding, debate over the mechanism of the solution's bactericidal activity has continued.

In the work reported here, we studied the bactericidal activity of povidone-iodine by challenging different species of bacteria to various concentrations of povidone-iodine solution. The results have major implications both theoretically, in further defining the possible bactericidal mechanisms of povidone-iodine preparations, and practically, in the use of povidone-iodine by patient-care providers.

MATERIALS AND METHODS

Povidone-iodine solutions used with challenge studies. Three 10% povidone-iodine solutions were used: Pharmadine, lot X80299 (Sherwood Pharmaceutical Co., Mahwah, N.J.); Betadine, lot A09 (Purdue Frederick, Norwalk, Conn.); and Povidine, lot 01509 (National Pharmaceutical Manufacturing Co., Baltimore, Md.). Ten milliliters of full-strength povidone-iodine and 1:2, 1:4, 1:10, 1:50, 1:100, 1:1,000, and 1:10,000 dilutions of each povidone-iodine solution were separately added to 16 by 125-mm screw-cap tubes. The diluent was sterile deionized water. The pH of each test dilution and stock solution was measured.

Challenge bacteria and inoculum size. Five different bacterial species were used in these povidone-iodine challenge studies. One strain each of *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (a clinical isolate), *P. cepacia* (isolated from Pharmadine, lot X80299), and *Streptococcus mitis* (isolated from a human throat) was grown separately in 5 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) for 18 to 24 h at 37°C; a *Mycobacterium chelonei* strain (M3, isolated from a porcine heart valve remnant) was grown in 7 ml of 7H9 broth (Difco) for 72 to 96 h at 22°C. All tubes of broth were centrifuged for 20 min at 2,000 × *g* in a Sorvall (Ivan Sorvall Inc., Newtown, Conn.) angle centrifuge, the liquid phase was discarded, and the pellet was suspended in 5 ml of 0.0003 M phosphate-buffered water (PBW) of pH 7.2. Each bacterial suspension was thoroughly mixed; 1 ml was removed and added to a 9-ml PBW dilution blank. These 1:10 dilutions then became the stock inocula; 1 ml of the 1:10 dilution was

TABLE 1. Effect of concentration of povidone-iodine solutions and length of exposure on the survival of *S. aureus*

Dilutions	pH	No. of organisms recovered/0.1ml[a] after contact time[b]				
		15 s	30 s	1 min	2 min	4 min
Pharmadine						
Stock, 10%	3.2	222	20	1	0	0
1:2	3.3	98	3	0	0	0
1:4	3.5	67	0	0	0	0
1:10	3.7	0	0	0	0	0
1:50	4.0	0	0	0	0	0
1:100	4.8	0	0	0	0	0
1:1,000	5.9	340	38	1	0	0
1:10,000	6.4	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
Betadine						
Stock, 10%	3.8	$>10^3$	508	125	6	0
1:2	3.7	443	58	1	0	0
1:4	3.8	160	1	0	0	0
1:10	3.9	0	0	0	0	0
1:50	4.1	0	0	0	0	0
1:100	4.7	0	0	0	0	0
1:1,000	5.7	43	2	0	0	0
1:10,000	6.7	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
Providine						
Stock, 10%	3.6	$>10^3$	192	38	1	0
1:2	3.5	340	42	0	0	0
1:4	3.6	26	0	0	0	0
1:10	3.8	0	0	0	0	0
1:50	4.0	0	0	0	0	0
1:100	4.6	0	0	0	0	0
1:1,000	5.9	7	1	0	0	0
1:10,000	6.2	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$

[a] Represents 0.1 ml of a 1:10 dilution (1 ml of test povidone-iodine and 9 ml of 0.0003 M containing 0.5% sodium thiosulfate).

[b] 100% survival = 1.7×10^4 organisms/0.1 ml.

added to each of the three brands of povidone-iodine at each of the test concentrations (stock and seven dilutions).

To determine the number of organisms per milliliter of inoculum, 1 ml of each stock inoculum was diluted (10^{-1} to 10^{-6}) in 9-ml PBW dilution blanks. In duplicate, 0.1 ml was removed from the 10^{-3} to 10^{-6} dilution tubes and separately plated on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with 5% sheep erythrocytes (TSAB); each portion was spread over the agar surface with sterile bent-glass rods, and TSAB plates were incubated as previously described for broth cultures. Calculated inocula per milliliter were as follows: *S. aureus*, 1.7×10^7; *K. pneumoniae*, 2.0×10^8; *P. cepacia*, 2.9×10^7; *S. mitis*, 5.6×10^7; and *M. chelonei*, 9.6×10^6.

Sampling and determination of bactericidal effect of povidone-iodine solutions. Samples were removed from povidone-iodine solutions at 15 and 30 s and at 1, 2, 4, and 8 min for subsequent determination of surviving organisms. At each sampling time, 1.0 ml of the povidone-iodine suspension to be tested was removed and added to a 9-ml PBW dilution blank containing 0.5% sodium thiosulfate (to neutralize residual iodine). The 0.5% sodium thiosulfate solution was previously tested and found not to be inhibitory to any of the challenge organisms. Each tube was thoroughly mixed; 0.1 ml was then removed in duplicate and added to the surface of TSAB plates. Test portions were evenly spread over the agar surface with individual sterile bent-glass rods. TSAB plates inoculated with povidone-iodine containing *S. aureus*, *K. pneumoniae*, *P. cepacia*, and *S. mitis* were incubated at 37°C for 24 to 48 h; TSAB plates to which *M. chelonei* was inoculated were incubated at 22°C for 5 days. After incubation, colonies present on TSAB plates were enumerated and recorded per 0.1 ml.

Determination of available iodine. Available iodine concentrations of stock povidone-iodine solutions and three test dilutions (1:2, 1:4, and 1:10) were performed by thiosulfate titration at a commercial laboratory (MacMillan Research, Marietta, Ga.).

RESULTS

All brands of povidone-iodine solution demonstrated more rapid kill of *S. aureus* and *M. chelonei* at dilutions of 1:2, 1:4, 1:10, 1:50, and 1:100 than did the stock solutions (Tables 1 and 2). In most instances, the 1:1,000 dilution killed *S. aureus* and *M. chelonei* as rapidly as did the full-strength solution. Figures 1 and 2 show the effects of povidone-iodine in various concentra-

TABLE 2. Effect of concentration of povidone-iodine solutions and length of exposure on the survival of *M. chelonei*

Dilution	No. of organisms recovered/0.1 ml[a] after contact time[b]					
	15 s	30 s	1 min	2 min	4 min	8 min
Pharmadine						
Stock, 10%	$>10^3$	$>10^3$	$>10^3$	$>10^3$	160	0
1:2	$>10^3$	$>10^3$	$>10^3$	390	0	0
1:4	$>10^3$	$>10^3$	$>10^3$	0	0	0
1:10	$>10^3$	466	0	0	0	0
1:50	$>10^3$	10	0	0	0	0
1:100	$>10^3$	4	0	0	0	0
1:1,000	$>10^3$	$>10^3$	$>10^3$	2	0	0
1:10,000	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
Betadine						
Stock, 10%	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	0
1:2	$>10^3$	$>10^3$	$>10^3$	$>10^3$	15	0
1:4	$>10^3$	$>10^3$	$>10^3$	78	10	0
1:10	$>10^3$	$>10^3$	$>10^3$	0	0	0
1:50	$>10^3$	125	1	0	0	0
1:100	$>10^3$	16	0	0	0	0
1:1,000	$>10^3$	$>10^3$	$>10^3$	32	1	0
1:10,000	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
Povidine						
Stock, 10%	$>10^3$	$>10^3$	$>10^3$	$>10^3$	150	0
1:2	$>10^3$	$>10^3$	$>10^3$	$>10^3$	0	0
1:4	$>10^3$	$>10^3$	$>10^3$	49	0	0
1:10	$>10^3$	$>10^3$	104	0	0	0
1:50	$>10^3$	32	0	0	0	0
1:100	$>10^3$	0	0	0	0	0
1:1,000	$>10^3$	$>10^3$	184	0	0	0
1:10,000	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$

[a] See Table 1.
[b] 100% survival = 9.6×10^3 organisms/0.1 ml.

tions on the survival of *S. aureus* and *M. chelonei*, respectively.

Both stock solution and dilute preparations of povidone-iodine demonstrated rapid bactericidal action against *K. pneumoniae*, *P. cepacia*, and *S. mitis*. These organisms were not recovered from either the full-strength solution or dilutions as high as 1:100 after a contact time of 15 s, nor were they recovered after exposure for 30 s to a 1:1,000 dilution of each of the povidone-iodine solutions. However, no bactericidal action was demonstrated by a 1:10,000 dilution after exposure of the test organisms for up to 8 min.

The available iodine content of all stock povidone-iodine solutions was approximately 1% or 10,000 μg/ml; it decreased in proportion to the degree of dilution. The pH increased with higher dilutions (Table 1).

DISCUSSION

Previous studies have demonstrated that low concentrations of povidone-iodine are bactericidal after vegetative organisms are exposed to the solution for 24 to 48 h (6, 11). However, few studies have assessed the effect of dilute concentrations of povidone-iodine on the survival of bacteria after brief exposure times. Lacey reported enhanced bactericidal activity in low concentrations (less than 0.1%) of povidone-iodine solutions, but detailed experimental data were not provided (8). Our investigation demonstrated that low concentrations (i.e., 0.1 to 1%) were more rapidly bactericidal than a full-strength (i.e., 10%) solution. Indeed, in our experiments, a 1:100 dilution of 10% povidone-iodine solution killed bacteria at least as quickly as solutions of higher concentration.

The chemistry of povidone-iodine is complex and not well understood. Therefore, the phenomenon of increased bactericidal activity with dilution is difficult to explain. Certainly, this study establishes that the available or thiosulfate-titratable iodine, currently the only routine measurement of iodine content in povidone-iodine products, is not directly related to bactericidal activity (Fig. 1 and 2). One hypothesis is that the concentration of "free" iodine (i.e., the elemental iodine in solution) significantly contributes to the bactericidal activity of povidone-iodine solution (4). Trueman has stated that dilution of povidone-iodine results in weakening of the iodine linkage to the carrier polymer with

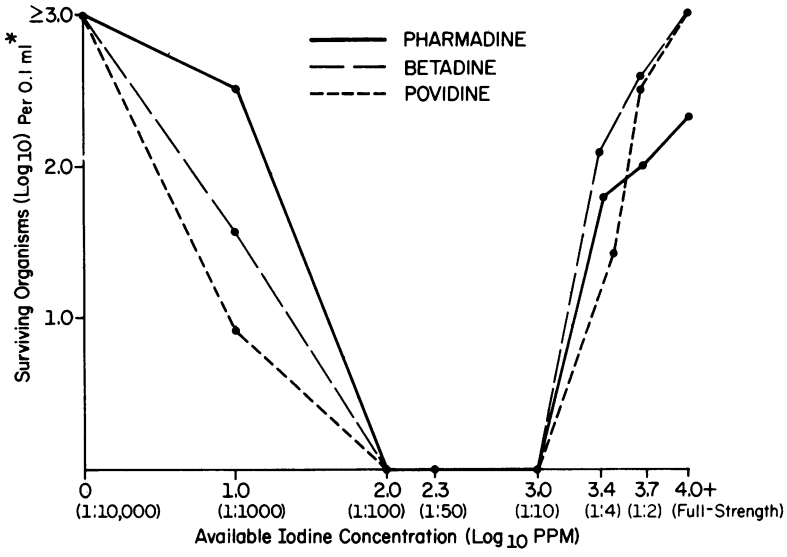

FIG. 1. Survival of *S. aureus* after exposure for 15 s to povidone-iodine solutions of various concentrations. *Maximum survival of 1.7×10^4 cells. +, Full-strength povidone-iodine = 10,000 ppm = 10,000 μg of available iodine per ml. Numbers in parentheses represent the dilution of povidone-iodine.

a concomitant increase in the amount of elemental (free) iodine in solution (14). In the present investigation, we attempted to substantiate this rise in free iodine with dilution. Indeed, with the *n*-heptane extraction assay (M. W. Winicov and W. Schmidt, U.S. patent 3,028,299, April 1962; A. Cantor and M. W. Winicov, U.S. patent 3,028,300, April 1962), levels of free iodine increased with dilution. However, this method of determining free iodine is controversial, and our determinations, particularly those made at higher dilutions (i.e., greater than 1:10) of 10% povidone-iodine solution, were not reproducible. An accurate method for determining free

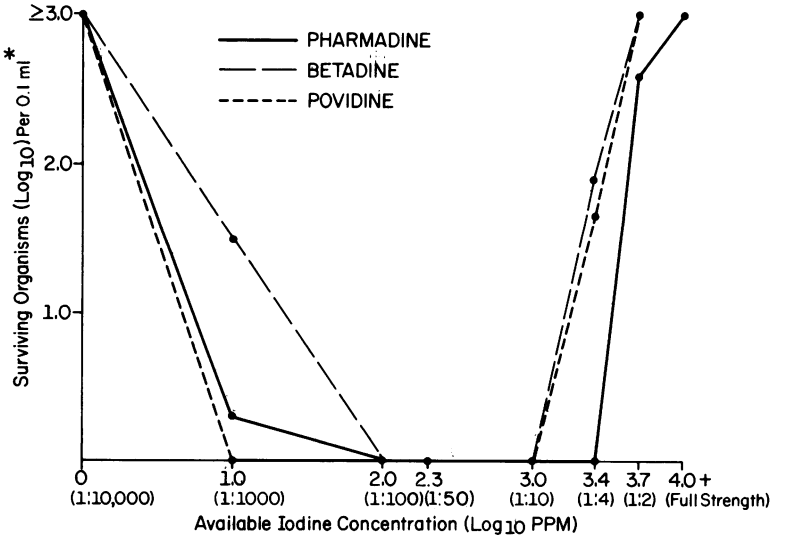

FIG. 2. Survival of *M. chelonei* after exposure for 2 min to povidone-iodine solutions of various concentrations. *Maximum survival of 9.6×10^3 cells. +, Full-strength povidone-iodine = 10,000 ppm = 10,000 μg of available iodine per ml. Numbers in parentheses represent the dilution of povidone-iodine.

iodine levels of povidone-iodine solutions of various concentrations is necessary before this hypothesis may be adequately tested.

The results of our investigation may have important applications to clinical medicine. Manufacturers currently recommend, for antiseptic purposes, that full-strength povidone-iodine solutions be applied for an unspecified period of time. Whether the clinical efficacy of povidone-iodine solutions in preventing infections may be enhanced by diluting stock solutions is not known. It is also unclear whether the increase in free iodine that occurs with dilution will increase the incidence of skin irritation. Furthermore, available iodine is in equilibrium with free iodine; therefore, in the presence of organic debris, the concentration of available iodine may be important as a reservoir for free iodine. In vivo use of dilute povidone-iodine solutions for antiseptic purposes, however, has been reported by several investigators; these uses included irrigation of surgical wounds and preparation for ophthalmic surgery (3, 10, 13). These preparations were described as effective and without serious side effects. Further research is necessary, however, before use of dilute preparations of povidone-iodine may be advocated for use on skin and mucous membranes.

Patient-care providers must distinguish between antiseptics and disinfectants. Ten percent povidone-iodine solution is manufactured for use as an antiseptic (i.e., an antimicrobial agent for use on skin and mucous membranes). However, in recent years, it has become accepted by many as a disinfectant (i.e., an antimicrobial agent for use on inanimate objects). In many institutions, 10% povidone-iodine solution is used liberally for disinfection of blood culture bottle tops, dialysis catheters, bronchoscopes, and other inanimate objects. In our study, a strain of *S. aureus* survived a 2-min exposure to full-strength 10% povidone-iodine solution. This finding raises doubts about categorical recommendations for use of iodophors for disinfection based on levels of available iodine. Manufacturers have recommended dilution of iodophors before use as disinfectants (12). Hospital personnel should be aware that cost effectiveness is not the only reason for this recommendation: this study demonstrated that diluting the stock solution may actually increase its bactericidal activity.

In summary, dilutions of povidone-iodine solutions demonstrated more rapid bactericidal action than did full-strength povidone-iodine solutions. Although 10% povidone-iodine solutions fulfill a useful role in antiseptic practice today, further chemical and microbiological research is warranted. If dilute preparations of povidone-iodine are found to be safe and efficacious, substantial financial savings as well as improved antiseptic care may be realized. In addition, our results suggest that brief exposure of inanimate objects to undiluted solutions of 10% povidone-iodine may be inadequate for disinfection.

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