

Changes in *Staphylococcus aureus* density and lesion severity after topical application of povidone-iodine in cases of atopic dermatitis

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Received 19 November 1996; received in revised form 31 January 1997; accepted 10 February 1997

Abstract

A case-control study was performed to examine the efficacy of 10% povidone-iodine solution applied to atopic dermatitis patients. The density of *Staphylococcus aureus* on the eczematous lesions and the lesion severity before and after the topical application of povidone-iodine were compared. We found a 10–100-fold decrease in the density of *S. aureus* after povidone-iodine treatment in patients colonizing *S. aureus* at an initial density of > 1000 CFU/10 cm². Erythema and exudation also decreased after povidone-iodine treatment in patients colonizing *S. aureus* at an initial density of > 1000 CFU/10 cm². The 10% povidone-iodine solution disinfected *S. aureus* cells when added immediately after the cells were mixed in human plasma; however, 10% povidone-iodine solution only reduced the density of *S. aureus* cells by 10–100-fold when *S. aureus* cells were harvested after a 24 h incubation in human plasma. *Staphylococcus aureus* cells harvested after 24 h incubation in human plasma were often surrounded by fibrin bundles and cells circumscribed by fibrin bundles could not be disinfected with 10% povidone-iodine solution. We suggest that *S. aureus* cells may produce biofilm-like structures in atopic dermatitis patients and that these structures may help *S. aureus* cells resist the 10% povidone-iodine treatment. © 1997 Elsevier Science Ireland Ltd.

Keywords: *Staphylococcus aureus*; Povidone-iodine; Atopic dermatitis; Case-control study

1. Introduction

Eczematous skin lesions of atopic dermatitis (AD) are usually densely colonized with *Staphylo-*

coccus aureus [1–4] and a correlation between the severity of the eczematous lesions and the density of the *S. aureus* colonization has been demonstrated [2,4]. The presence of *S. aureus* at a density greater than 1×10^6 colony-forming units (CFU)/cm² may aggravate the underlying dermatitis, but does not indicate the development of

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a secondary infection. An *S. aureus* density greater than 1×10^7 CFU/cm² may suggest a secondary infection [5,6]. Following antimicrobial treatment, *S. aureus* colonies on skin surfaces readily change from methicillin-sensitive *S. aureus* (MSSA) to methicillin-resistant *S. aureus* (MRSA) [4]. We performed a case-control study of 10% povidone-iodine (PVP-I) solution application on AD patients and determined the usefulness of PVP-I treatment. We also examined the in vitro activity of PVP-I in the presence of human plasma.

2. Materials and methods

2.1. Patients

Patients who fulfilled the criteria of the Japanese Dermatological Association [7] for AD were enrolled in this study. Informed consent was obtained from all patients prior to treatment and from parents of patients less than 20 years old. Pregnant or lactating women and infants less than 6 months old were excluded. Patients with an apparent secondary infection were also excluded from this study. A total of 150 out-patients with AD (73 males and 77 females) were assigned alternately to the PVP-I group or to the non-PVP-I group. The PVP-I group consisted of 75 patients (sex: 36 males and 39 females; age: 18.9 ± 10.9 (mean $\pm$ S.D.) years) and the non-PVP-I group consisted of 75 patients (sex: 37 males and 38 females; age: 18.3 ± 11.4 (mean $\pm$ S.D.) years). Culture samples were taken from the face or neck ($n = 31$), trunk ($n = 7$), and extremities ($n = 37$) of patients in the PVP-I group and from the face or neck ($n = 39$), trunk ($n = 14$), and extremities ($n = 22$) of patients in the non-PVP-I group.

2.2. PVP-I treatment

Povidone-iodine (Meiji Seika Co., Tokyo, Japan), known for its strong antistaphylococcal activity, was applied topically to an area less than 100 cm² once daily for 14 days; each application was followed 2 min later by cleansing with soap. No other oral or topical antimicrobial agents,

antiseptics, or oral corticosteroids were used. Before the start of treatment, we confirmed that the patients had no history of thyroid dysfunction and did not have hypersensitivity to iodine. In the non-PVP-I group, one area less than 100 cm² was selected for observation and cleansed once daily with soap for 14 days in a similar way as in the PVP-I group. No oral or topical antimicrobial agents, antiseptics, or oral corticosteroids were used. We recommended all patients to take a bath or a shower in a similar pattern during the study. Sea bathing, sun bathing, swimming in a pool, bathing in hot spring, and the use of acidic water were not allowed in both groups throughout the study. Topical corticosteroids were not applied to the area where bacteriological stamp sampling was performed, although 28 patients in the PVP-I group and 25 patients in the non-PVP-I group received treatment with hydrocortisone butyrate or clobetasone butyrate ointment. The PVP-I treatment was discontinued in 11 cases: 5 cases of an exacerbated condition, 2 cases of herpes simplex complication, and 4 cases of non-compliance. Five patients discontinued the PVP-I treatment due to exacerbation at 1, 2 ($n = 2$), 5, and 10 days of treatment. All of these patients complained of irritation; the degree of erythema was slightly increased, but vesiculopapules were not observed on the erythemas. One patient from each group was excluded for receiving oral antibiotics during the test period. Eight patients in the PVP-I group and 10 patients in the non-PVP-I group were excluded for not visiting on the appointed days. Thus, the numbers of patients remaining in the study at the time of completion were 55 in the PVP-I group and 64 in the non-PVP-I group.

2.3. Bacteriological examination

Two samples were taken with a Food Stamp 'Nissui' (10 cm²) (Nissui Pharmaceutical Co., Tokyo, Japan) [4] for *S. aureus* on the same lesional area of 10 cm² for 5 s, once at the beginning of the study and once again 14 days later. Before the sampling, the AD lesions were classified as red or light/dark red erythema, with or without exudation. Itching was graded as follows: 3, marked sleep disturbance due to itching;

2, considerable itching, relieved by scratching; 1, slight itching; 0, no itching. We determined CFU after the stamp plates had been cultured for 48 h at 37°C. There were no statistical differences between the two groups in terms of age, sex, lesion site, exclusion, and the density of *S. aureus* on the test lesions at the beginning of the study.

Colonies from the stamp sampling reacting positive to egg yolk were regarded as *S. aureus* colonies. One colony was randomly selected from each culture plate and inoculated onto Mueller Hinton medium (Difco, Detroit, MI, USA). After incubation at 24 h at 37°C, each colony was tested by Staphaurex® rapid latex test kit for the identification of *S. aureus* (Murex Diagnostics Ltd., Dardford, England) and positive strains were regarded as *S. aureus*. The minimal inhibitory concentration (MIC) of each colony isolated from the PVP-I group against oxacillin (MPIPC) was measured using the microdilution method.

2.4. In vitro examination of the bactericidal activities of PVP-I

The in vitro bactericidal activity of PVP-I against *S. aureus* strain no. 1 isolated from an AD lesion was determined by adding 2.5 ml of 0.001% (10 µg/ml), 0.01% (100 µg/ml), 0.1%, 1%, or 10% PVP-I solution to 2.5 ml of sterile saline solution containing *S. aureus* cells at 3.2×10^8 CFU and 2.5 ml of 0.1%, 1%, or 10% PVP-I solution to 2.5 ml of human plasma containing *S. aureus* cells at 3.2×10^8 CFU. Human plasma was taken from a healthy volunteer. The in vitro bactericidal activities of PVP-I against *S. aureus* strains no. 2–5 isolated from AD lesions were determined by adding 2.5 ml of 10% PVP-I solution to 2.5 ml of human plasma containing *S. aureus* cells at 4.4 – 8.4×10^7 CFU. Strain no. 1 was tested immediately after it was mixed in sterile saline solution or human plasma, but strains no. 2–5 were tested after they were incubated in human plasma for 24 h. After 2 min of incubation with sterile saline solution or human plasma, 1 ml of the mixture was collected and mixed with an equal amount of 5% sodium thiosulfate (Wako Pure Chemicals Co., Osaka, Japan) to inactivate PVP-I [8]. A 0.1-ml aliquot of the final mixture was diluted

with saline solution using the 10-fold dilution method. An aliquot of each bacterial suspension was cultured on Mueller Hinton medium plates for 24 h at 37°C ($n = 3$). The CFU were counted and converted to viable cell counts for each of the original mixtures. The coagulase types of *S. aureus* strains consisted of types III (strains no. 1, 3), type V (strain no. 5), and type VII (strains no. 2, 4). Suspensions of *S. aureus* strains no. 1–3, to which 10% PVP-I was added, were centrifuged at $9000 \times g$ for 10 min at 4°C and pellets were embedded in Mueller Hinton medium. The specimens were fixed with 2% glutaraldehyde cacodylate buffer (pH 7.4) for 3 h at 4°C; the specimens were then cut into 1 mm³ pieces. The fixed specimens were then dehydrated with graduated ethanol solution and were embedded in Epon 812. Ultrathin sections were cut with a Porter Blum MT-2B ultramicrotome (SOR VALL, CT, USA) and stained with 11.1% (w/v) uranyl acetate and 2.66% (w/v) lead citrate. The stained sections were examined with a JEM-100 SX electron microscope (JEOL, Akishima, Japan).

2.5. Formation of the membranous structures of 40 *S. aureus* strains isolated from atopic dermatitis lesions

Forty *S. aureus* strains isolated from skin lesions of AD were used. Twenty strains were coagulase type III and 20 strains were coagulase type VII. These 40 strains were grown separately in 8 ml of tryptic soy broth (Nissui Pharmaceutical Co.) at 37°C for 24 h without shaking. Following incubation, the bacterial cells were harvested by centrifugation at $9000 \times g$ for 10 min at 4°C. The bacterial cells were resuspended in sterile saline solution and centrifuged as described above. Resuspension in sterile saline solution and centrifugation were repeated three times. Inoculum suspension was prepared by resuspending the washed bacteria in 0.6 ml of human plasma with 0.9 cm² of a plastic tissue-culture coverslip (Celldesk®) (Sumitomo Bakelite Co., Tokyo, Japan) in 1-ml polypropylene microtubes (IUCHI BIO Systems, Tokyo, Japan). The final suspension in each microtube contained 6.6×10^7 – 3.9×10^8 CFU. After 72 h, materials around the plastic

Table 1

Comparison of the density of *S. aureus* at lesional sites with and without 14 days of PVP-I treatment. The data represent 119 cases: 55 in the PVP-I group and 64 in the non-PVP-I group

PVP-I group				Non-PVP-I group			
Density at the beginning in this study (no. of cases)		Density 14 days later (no. of cases)		Density at the beginning in this study (no. of cases)		Density 14 days later (no. of cases)	
> 1000	(26)	> 1000	(3)	> 1000	(21)	> 1000	(16)
		100–1000	(12)			100–1000	(4)
		< 100	(11)			< 100	(1)
				* [			
100–1000	(14)	> 1000	(3)			> 1000	(2)
		100–1000	(6)	100–1000	(19)	100–1000	(10)
		< 100	(5)			< 100	(7)
		100–1000	(7)			100–1000	(4)
< 100	(15)	< 100	(8)	< 100	(24)	< 100	(20)

* $P < 0.05$.

>1000, 100–1000, <100: CFU/10 cm².

matrix were fixed in 10% formalin solution and embedded in paraffin. Specimens for light microscopy were stained with hematoxylin and eosin (HE) stain and periodic acid-Schiff (PAS) stain.

2.6. Statistical methods

Data obtained in this study were analyzed using the Mann-Whitney *U*-test and chi-square test (Yeat's correction).

3. Results

3.1. Comparison of the density of *S. aureus* at lesional sites before and after 14 days of PVP-I treatment

By the method used, colonies at a density in excess of 1000 CFU/10 cm² were impossible to count; therefore, we used 1000 CFU/10 cm² as a criterion for inclusion in this test. The density of *S. aureus* decreased after PVP-I treatment ($P < 0.05$) when *S. aureus* density was >1000 CFU/10 cm² at the beginning of the study (Mann-Whitney *U*-test; Table 1). The density of *S. aureus* did not

decrease after PVP-I treatment when *S. aureus* density was ≤ 1000 CFU/10 cm² at the beginning of the study (Table 1).

3.2. Comparison of the clinical symptoms at lesional sites with and without 14 days of PVP-I treatment

In cases of *S. aureus* density >1000 CFU/10 cm² at the beginning of the study, the decrease in the degree of erythema was greater in the PVP-I group than in the non-PVP-I group ($P < 0.05$; χ^2 test; Table 2), and the exudation decreased more in the PVP-I group than in the non-PVP-I group ($P < 0.01$; χ^2 test; Table 2). The clinical symptoms did not decrease in the PVP-I group in cases of *S. aureus* density ≤ 1000 CFU/10 cm² at the beginning of this study (Table 3).

3.3. Numbers of MRSA before and after 14 days of PVP-I treatment

At oxacillin MIC ≥ 4 μ g/ml, 9 strains were MRSA before treatment, and 9 were MRSA after treatment.

Table 2

Comparison of the clinical symptoms at lesional sites with and without 14 days of PVP-I treatment. The data represent 47 cases, 26 in the PVP-I group and 21 in the non-PVP-I group, each colonizing *S. aureus* at a density >1000 CFU/10 cm² at the beginning of the study

PVP-I group				Non-PVP-I group			
At the beginning in this study (no. of cases)		14 days later (no. of cases)		At the beginning in this study (no. of cases)		14 days later (no. of cases)	
Degree of erythema							
Red	(24)	Red	(10)	Red	(21)	Red	(16)
		Light/dark red	(14)			Light/dark red	(5)
Light/dark red	(2)	Light/dark red	(2)				
Exudation							
+	(15)	+	(1)	+	(13)	+	(9)
		—	(14)			—	(4)
—	(11)	—	(11)	—	(8)	—	(8)
Degree of itching							
2–3	(25)	2–3	(10)	2–3	(19)	2–3	(13)
		0–1	(15)			0–1	(6)
0–1	(1)	0–1	(1)	0–1	(2)	0–1	(2)

* $P < 0.05$; ** $P < 0.01$

3.4. Bactericidal activities of PVP-I solution against *S. aureus*

As shown in Table 4, the bactericidal activities of PVP-I solution against *S. aureus* strain no. 1 were observed at a concentration of $\geq 0.1\%$ in sterile saline solution and at a concentration of 10% in human plasma. The 10% PVP-I solution reduced the cell density of *S. aureus* strains no. 2–5 by 10–100-fold compared to the density of the untreated control.

3.5. Electron microscopy

Cells from *S. aureus* strain no. 1 were not circumscribed by fibrin bundles and were often ruptured. Intact cells from *S. aureus* no. 2–3 were surrounded by fibrin bundles (Fig. 1A and B). In contrast, cells from *S. aureus* strains no. 2–3 which were not circumscribed by fibrin bundles, were often ruptured (Fig. 1B and C).

3.6. Formation of membranous structures in *S. aureus* strains

The formation of membranous structures (thickness from the Celldesk (HE) > 1 mm; PAS-positive) was 3/20 (coagulase type III) and 12/20 (coagulase type VII).

4. Discussion

PVP-I frequently causes irritant contact dermatitis, but an allergic contact reaction to this antiseptic is rare [9]. A patch test with 10% PVP-I solution produced erythema in normal controls [10] but a patch test with 0.5% PVP-I solution was negative in normal controls [9], suggesting that high concentrations of PVP-I may be irritant to the skin. Five patients who had not used PVP-I solution before the study discontinued the treatment due to exacerbation of their condition. Their symptoms of exacerbation disappeared within a

Table 3

Comparison of the clinical symptoms at lesional site with and without 14 days of PVP-I treatment. The data represent 72 cases, 29 in the PVP-I group and 43 in the non-PVP-I group, each colonizing *S. aureus* at a density ≤ 1000 CFU/10 cm² at the beginning of the study

PVP-I group				Non-PVP-I group			
At the beginning in this study (no. of cases)		14 days later (no. of cases)		At the beginning in this study (no. of cases)		14 days later (no. of cases)	
Degree of erythema							
Red	(24)	Red	(20)	Red	(28)	Red	(19)
		Light/dark red	(4)			Light/dark red	(9)
		Red	(1)			Red	(2)
Light/dark red	(5)	Light/dark red	(4)	Light/dark red	(15)	Light/dark red	(13)
Exudation							
+	(7)	+	(5)	+	(14)	+	(12)
		—	(2)			—	(2)
—	(22)	—	(22)	—	(29)	—	(29)
Degree of itching							
2–3	(22)	2–3	(17)	2–3	(24)	2–3	(18)
		0–1	(5)			0–1	(6)
0–1	(7)	2–3	(1)	0–1	(19)	2–3	(2)
		0–1	(6)			0–1	(17)

few days of discontinuing PVP-I treatment. Although a patch test with PVP-I was not done, we suspect that the exacerbation by PVP-I in this study may be due to irritation.

Transient congenital hypothyroidism has been observed in pregnant and in lactating subjects when PVP-I is applied frequently [11–13]. Iodine goitre and hypothyroidism were induced in infants by the cutaneous absorption of iodine [11–

13]. One of the dangers of PVP-I topical therapy in thermal injuries is the absorption of the therapeutic agent with subsequent metabolic and toxic complications [14]. Two patients, one 30 years old with a 75% burn, the second 72 years old with a 35% burn, were treated with PVP-I. In both patients, severe metabolic acidosis developed. Pietsch and Meakins [14] suggested that iodophors should not be used topically for burns greater

Table 4

Bactericidal activities of PVP-I solution against *S. aureus* strains isolated from atopic dermatitis patients

<i>S. aureus</i> strain no.	PVP-I (10%)+ plasma (CFU/ml)	PVP-I (1%)+ plasma (CFU/ml)	PVP-I (10, 1, 0.1%)+ saline solution (CFU/ml)	PVP-I (0.01%)+ saline solution (CFU/ml)	Control (CFU/ml)
1	0	2.5×10^3	0	8.6×10^3	3.2×10^8
2	4.6×10^6	N.D.	N.D.	N.D.	3.6×10^7
3	7.4×10^5	N.D.	N.D.	N.D.	6.3×10^7
4	6.3×10^6	N.D.	N.D.	N.D.	2.3×10^7
5	3.0×10^6	N.D.	N.D.	N.D.	5.5×10^7

Strain no. 1 was tested immediately after PVP-I was mixed in sterile saline solution or human plasma. Strains no. 2–5 were tested after *S. aureus* cells of each strain were incubated in human plasma for 24 h.

N.D., not done.

than 20% of the body surface or in the presence of renal failure. We suggest that a 10% PVP-I solution should be used because of its strong bacteri-

dal activities in human plasma; however, the area to which the solution is topically applied must be cleansed with soap within a few minutes of application to reduce irritation and the absorption of PVP-I. Pregnant or lactating women and newborn infants should be excluded from PVP-I treatment. This study indicates that 10% PVP-I solution should be used in AD patients colonizing *S. aureus* at a density > 1000 CFU/10 cm² within a limited area for the shortest possible duration.

Colony counts of $2.2 \times 10^5 \pm 0.44 \times 10^5$ (mean $\pm$ S.D.) CFU/cm² obtained by the scrub technique corresponded to 1000 CFU/10 cm² colony counts obtained by the contact-plate technique [4] using the method of the croton oil-inflamed mouse model [8]. Because the contact-plate technique is a fast and easy procedure, this technique is used to examine the density of *S. aureus* on AD lesions. Our previous study noted a decrease of erythema in 28 of 39 cases and a decrease of exudation in 28 of 32 cases; both effects were associated with a decrease in colony density following antimicrobial (cefdinir) treatment [4]. The PVP-I treatment reduced the colony density of *S. aureus* on the lesions that contained > 1000 CFU/10 cm² at the beginning of the treatment. Consequently, the degree of erythema and the exudation decreased in the PVP-I group. These results suggest that *S. aureus* on AD lesions at a density > 1000 CFU/10 cm² may exacerbate erythema and exudation.

MRSA strains were isolated from AD patients following antimicrobial (cefdinir) treatment in 13 of 37 cases of hospitalized patients and in 7 of 59 cases of out-patients [4]. In contrast, the transition from MSSA to MRSA was not observed in PVP-I treatment. In this case, PVP-I treatment may have some advantage over treatment with antibiotics.

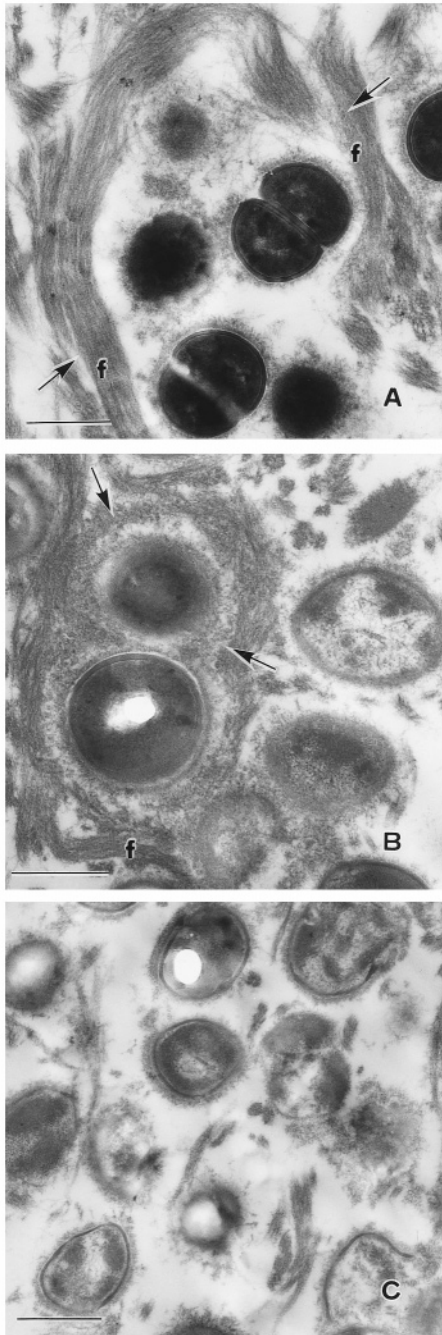


Fig. 1.

Fig. 1. Electron microscopy. The 10% PVP-I solution was added to *S. aureus* cells harvested after 24 h incubation in human plasma. After 2 min, 1 ml of the mixture was collected and mixed with an equal amount of 5% sodium thiosulfate. (A) Intact *S. aureus* cells were surrounded by fibrin bundles (arrow); f, fibrin. Bar, 0.5 μ m. (B) Intact *S. aureus* cells were surrounded by fibrin bundles (arrow). In contrast *S. aureus* cells not circumscribed by fibrin bundles were often ruptured. f, fibrin. Bar, 0.5 μ m. (C) *Staphylococcus aureus* cells not circumscribed by fibrin bundles were often ruptured. Bar, 0.5 μ m.

The formation of membranous structures by *S. aureus* strains on a Celldesk after a 72-h incubation in human plasma was observed in our previous study: in 8 of 20 strains isolated from impetigo (coagulase type I), in 10 of 20 strains isolated from impetigo (coagulase type V), and in 2 of 20 strains isolated from furuncle (coagulase type IV) [15]. Because the membranous structure surrounding the *S. aureus* cells was PAS-positive, we suggest it is a biofilm. The frequency of the formation of this biofilm was more prominent in *S. aureus* strains isolated from impetigo (coagulase types I, V) and AD (coagulase type VII) than in *S. aureus* strains isolated from furuncle (coagulase type IV) ($P < 0.05$, Fisher's exact probability method). Abundant fibrins surrounding *S. aureus* cells are the first step for biofilm production by *S. aureus* cells harvested in human plasma [15]. *Staphylococcus aureus* cells harvested after 24 h incubation in human plasma were often surrounded by fibrin bundles and cells circumscribed by fibrin bundles could not be disinfected with 10% PVP-I solution. *Staphylococcus aureus* cells were observed to be clustered and scattered between corneocytes in AD patients, and an intense labeling for fibronectin, fibrinogen, IgG, and IgE was found on the surfaces of *S. aureus* cells and in the epidermal intercellular spaces [16]. These results suggest that *S. aureus* cells may produce biofilm in AD patients and that the formation of biofilm-like structures may help *S. aureus* cells resist the 10% PVP-I treatment given to AD patients. Further study is necessary to clarify the specific mechanisms of *S. aureus* involvement in AD.

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