

Irritancy of antiseptics tested by repeated open exposures on the human skin, evaluated by non-invasive methods

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The aim of the study was to test the irritancy of 6 antiseptics in an open exposure model. The following agents were tested in their normal use concentrations using open exposures, 2× daily for 4 days in 20 subjects: chlorhexidine 4% (CH), chlorhexidine 0.5% in ethanol 70% (CE), ethanol 70% (ET), iodine 1% in ethanol 70% (IE), povidone-iodine 10% (PI) and sodium hypochlorite 0.25% (SH). Responses were evaluated by visual scoring, subjective irritancy scoring, stratum corneum hydration (Corneometer), transepidermal water loss and laser Doppler flowmetry. Exposure to SH had to be discontinued after 4 applications because of severe subjective irritation. The same held true for IE (7 applications), whereas the other agents were exposed 8×. All evaluation methods showed SH to be significantly more irritating than IE, which was in turn more irritating than CH, CE, ET and PI. Thus, it can be concluded that CH, CE, ET and PI were non-irritating in this open exposure model.

Key words: antiseptic; hydration; irritancy; laser Doppler flow; non-invasive methods; transepidermal water loss. © Munksgaard, 1997.

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Traditionally, antiseptics are used on the skin to prevent the spread of micro-organisms and to prevent (post-operative) infection. Furthermore, these types of agents might be useful in reducing the count of micro-organisms in skin diseases that are known to be complicated by a high load of micro-organisms, such as atopic dermatitis.

A surgical hand scrub agent should significantly reduce micro-organisms on intact skin, have a broad spectrum action, be fast acting and/or have a residual effect, and be non-irritating (1). There is a vast amount of literature dealing with the microbicidal action of various types of antiseptics but literature on the irritant potency of antiseptics is sparse. It is a known fact that antiseptics can cause chronic irritant contact dermatitis (2). During their long history, some antiseptics with good microbicidal actions have been discarded because of their irritant potency on the skin, such as iodine tincture (3). Another example of an antiseptic with known irritant properties is sodium hypochlorite, which is used as a wound cleanser (4). However, to our knowledge, the only study in which the irritant potency of different types of antiseptics was investigated on human skin in a comparative way is the

study of Paulson (1). It is necessary to be informed about the exact irritant potency of antiseptics in cases where they are used frequently, particularly when this concerns susceptible skin like that of atopic dermatitis patients.

The aim of this study was to assess the irritancy of antiseptics tested by repeated open exposures on the human skin, in order to establish which antiseptic had the lowest irritant potency. Evaluation was performed by means of visual scoring, subjective irritancy scoring and by the following non-invasive methods: skin hydration by means of the Corneometer, transepidermal water loss (TEWL) and laser Doppler flowmetry (LDF). Different types of antiseptic preparations were tested: chlorhexidine, ethanol, iodine and sodium hypochlorite. These agents were selected based on their documented bactericidal action on *Staphylococcus aureus* (1, 3-5).

Materials and Methods

Subjects

20 subjects (11 female and 9 male) entered the study. Their age ranged from 19 to 29 years (mean, 23 years). None had manifest skin disease. A his-

tory of atopic dermatitis was present in 3 subjects, and a history of mucosal atopy was present in 4 subjects. 1 person had skin type I, and the others had skin types II and III. None of the persons was on medication. The study was performed during the winter months of 1995. The study protocol was approved by the hospital ethics committee. All subjects gave their informed consent.

Exposure

Table 1 summarizes the antiseptics studied. All antiseptic solutions were prepared according to the British Pharmacopoeia 1993. The antiseptics were tested in their normal use concentrations using open exposures of 30 min duration, 2× daily on 4 consecutive days. The minimal time interval between these 2 applications was 3 h. A plastic strip was used with holes of 20 mm diameter, in which 0.8 ml of each solution was applied. The strip was fixed to the volar side of the right forearm with nonadhesive bandages. The distance between the most distal exposure site and the wrist joint was kept at 60 mm. In each subject, this most distal site of the left forearm was exposed to distilled water. The most distal site of the right forearm was not exposed. The antiseptic solutions were exposed to the remaining sites in a sequence that changed cyclically from one subject to the next. After their removal, the skin was cleaned with running water and gently dried with a cotton towel.

Evaluation

On each day, the sites were graded visually for erythema, scaling and fissures, according to Frosch & Kligman (6). Total scores for these signs were recorded. Furthermore, a subjective score for the degree of burning or stinging sensation during and/or after exposure to each agent was recorded, according to the following scoring system: negative (0); weak (1+); moderate (2+); strong (3+). When a subject experienced moderate or strong burning/stinging to a particular solution, further exposure to that solution was stopped. The number of ful-

filled exposures to each solution was registered. On days 1 and 5, the following non-invasive evaluation methods were carried out:

- Firstly, stratum corneum hydration was determined by means of a Corneometer CM 820 PC (Courage & Khazaka, Cologne, Germany) (arbitrary units). The measuring principle of this instrument is described elsewhere (7).
- Subsequently, transepidermal water loss (TEWL) (g/m²h) measurements were performed using a Tewameter TM210 (Courage & Khazaka, Cologne, Germany). The measurements were performed in an air-conditioned room at a temperature of 18–21°C and a relative humidity of 35–50%, in which the subjects acclimatized for 20 min in a comfortable armchair with the forearm uncovered, following the guidelines of the standardization group of the European Society of Contact Dermatitis (8).
- Finally, cutaneous blood flow was measured by laser Doppler flowmetry (Periflux Pf2, Perimed, Stockholm, Sweden) (arbitrary units) following the guidelines of the standardization group of the European Society of Contact Dermatitis (9). The instrument was adjusted to a bandwidth of 4 kHz, gain 10 and time constant 3 s.

Statistical methods

Mean values of the non-invasive evaluation methods were compared by means of *t*-tests, corrected by Bonferroni's modification, i.e., in individual pairwise comparisons (21 pairs) differences were considered statistically significant for a $p \leq 0.0024$ ($=0.05/21$). *t*-tests were used for these variables since they fitted the normal distribution. The values of the visual scores were compared by Wilcoxon's signed rank test, corrected similarly to the *t*-test.

Results

Baseline values for hydration were not different, whereas for TEWL and LDF, the most distal sites had (not significantly) higher values than the other sites (data not shown). The most distal site of the left forearm was exposed to water.

In the majority of subjects, exposure to SH had to be discontinued after 4 applications because of subjective irritation sensation. IE exposure had to be stopped after an average of 7 applications, whereas the other agents were exposed 8 times in all subjects without clear irritation sensations (data not shown).

In 5 subjects, an erythematous reaction could already be discerned after 1 exposure to SH. From day 3 until day 5 the mean score for erythema re-

Table 1. Characteristics of the antiseptics tested

Antiseptic	Abbreviation	Concentration	Solution
chlorhexidine gluconate	CE	0.5	ethanol 70%
chlorhexidine gluconate	CH	4	water
ethanol	ET	70	water
iodine	IE	1	ethanol 70%
povidone-iodine ¹	PI	10	water
sodium hypochlorite	SH	0.25	water

¹ Polyvinyl pyrrolidone-iodine complex.

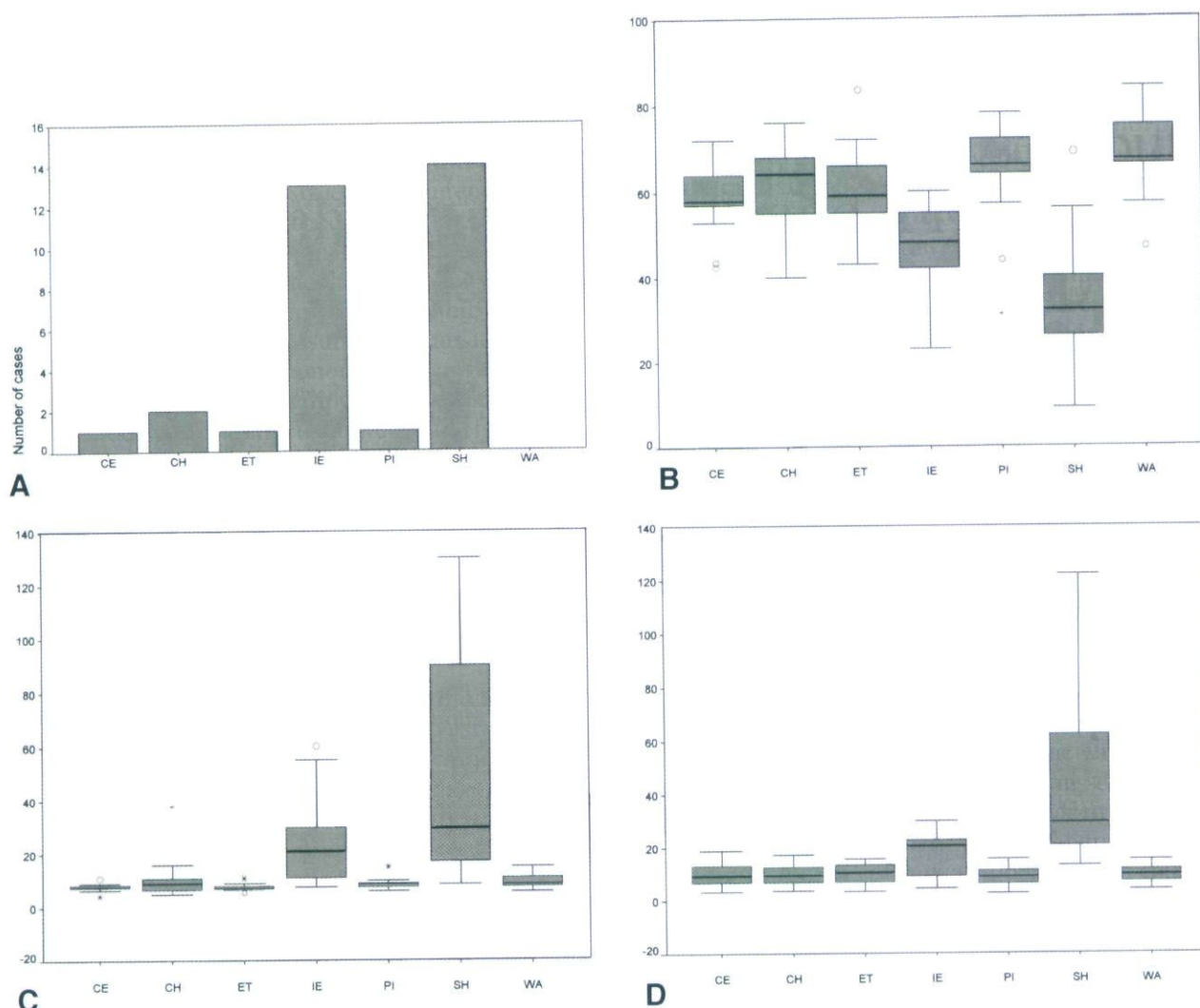


Fig. 1. (A) Bar diagram of the number of subjects having a total visual score ≥ 1 on day 5, after repeated open exposures to: CE, chlorhexidine in ethanol; CH chlorhexidine in water; ET ethanol; IE, iodine in ethanol; PI, povidone-iodine; SH, sodium hypochlorite; WA, water. (B-D) Box plots of responses on day 5. (B) shows the hydration values as determined by the Corneometer (arbitrary units); (C) shows the transepidermal water loss (TEWL) (g/m²h); (D) shows the laser Doppler flow values (arbitrary units). The box plots according to Tukey (19) are defined as follows: the lower boundary of the box is the 25th percentile and the upper boundary is the 75th percentile. The horizontal line inside the box represents the median. Cases with values that are more than 3 box-lengths from the upper or lower edge of the box are designated with empty circles, and cases with values that are within 1.5 and 3 box-lengths from the edges are designated with an asterisk. The largest and smallest observed values that are less than 1.5 box lengths from the edges are shown by lines from the lower and upper box edges.

mained constant, whereas an increasing score for scaling and fissuring was noted. To a lesser degree, the same held true for IE. With the exception of some cases, CE, CH, ET and PI did not induce visible skin changes (Fig. 1A). On day 5, the ranking order of mean values for visual scores was as follows: SH $\geq$ IE $>$ CH $\geq$ CE = ET = PI $\geq$ WA (' $</>$ ', significant difference; ' $\leq/\geq$ ', no significant difference; ' $=$ ', equal). The mean values and box plots of visual scores and non-invasive methods are shown in Table 2 and Fig. 1B-D. For hydration values, the following ranking order was found: SH $<$ IE $<$ CE $\leq$ ET $\leq$ CH $\leq$ PI $\leq$ WA. Using TEWL,

the order was as follows: SH $>$ IE $>$ ET $\geq$ CE $\geq$ CH $\geq$ WA $\geq$ PI. For laser Doppler flowmetry, the ranking order was slightly different, namely: SH $\geq$ IE $\geq$ CH $\geq$ WA $\geq$ PI $\geq$ CE $\geq$ ET.

Discussion

Clinically, sodium hypochlorite (SH) and iodine tincture (IE) were significantly more irritating than chlorhexidine solutions (CH, CE), ethanol (ET) and povidone-iodine (PI). In a study by Paulson, different surgical scrub products were compared (brushes impregnated with chlorhexidine, povi-

Table 2. Mean values and standard deviations of visual score, hydration, transepidermal water loss and laser Doppler flowmetry at the sites exposed to antiseptics and water, on day 5

Antiseptic	Evaluation method							
	visual scoring		hydration		TEWL		LDF	
	mean	SD	mean	SD	mean	SD	mean	SD
CE	0.1	0.2	58.7	7.7	9.8	4.1	7.8	1.5
CH	0.2	0.5	61.3	10.5	9.7	3.8	11.3	8.3
ET	0.1	0.2	59.8	10.0	10.1	3.7	7.6	1.4
IE	1.7	1.8	47.4	11.0	16.7	8.1	24.9	17.3
PI	0.1	0.2	64.0	11.1	8.9	3.6	8.7	2.1
SH	3.3	2.8	34.9	17.0	47.0	37.0	52.1	44.9
WA	0	0	69.2	9.3	9.3	3.2	9.3	2.9

done-iodine, parachlorometaxyleneol and an alcohol-detergent mixture) using a 1× 2-min scrub of the hands and forearms (1). The alcohol-detergent mixture was the only product that induced dryness, erythema and burning sensations (1). This might have been due to the detergent.

Using hydration assessment by means of the Corneometer, significant differences between SH, IE and CE were noted, SH having the lowest value. Differences between CE and the other agents were not significant. To our knowledge, there is no literature on the effects of antiseptics evaluated by the Corneometer. This instrument has been used to monitor skin changes after a 24-h patch test (10) and after repetitive washing (11) with detergents. Hydration values were decreased after both types of exposure to detergents (10, 11), associated with scaling and fissuring, i.e., the late repair phase (10). The low hydration values at the sites exposed to SH and IE in the current study were also associated with scaling.

The irritant potency of SH could be discriminated from that of IE and from the less irritant antiseptics by means of TEWL. This method can determine the barrier function of the stratum corneum (12). TEWL has been used extensively in studies on detergents (10, 11, 13) and other types of irritants (13), though not on antiseptics. An exception is a repeated open exposure study on alcohols by Timmer et al. (14). In that study no TEWL increase was observed. In the current study it was demonstrated that SH and to a lesser extent IE induced an irritant reaction which was associated with barrier function impairment.

Finally, by means of laser Doppler flowmetry SH and IE were also indicated as the most irritant antiseptics tested in this study. Laser Doppler flowmetry has been used to study the blood flow in the superficial dermal microcirculation, in order to assess skin irritation (9). Dykes & Marks (15) have investigated the irritancy of 10% PI solutions using a single short-time occlusive test model with exposure durations varying from 1 to 8 h. The

LDF values and erythema scores were highly dependent on the duration of exposure. In contrast to those findings, PI has been shown to possess a very low irritant potency in the current study.

In this study, 4 evaluation methods have been used in order to determine the irritant potency of various antiseptics. These evaluation methods are supposed to assess different aspects of the cutaneous irritant reaction. Nevertheless, the ranking order in irritancy of the antiseptics was similar in each evaluation method. SH had a high irritant potency, followed by IE with an intermediate irritancy, in its turn followed by a group of antiseptics with low irritancy. This equal ranking order determined by the different evaluation methods may be explained by the fact that the mechanism of irritation of SH and IE includes several aspects of irritation, i.e., barrier function impairment, erythema and scaling. The concomitant presence of scaling may be attributed to the fact that the evaluation was performed several days after the peak of the irritation reaction. The shift from erythema in the acute irritation phase towards scaling and/or fissuring in the subacute/chronic phase is a well-known phenomenon (10, 16). SH is an antiseptic that is used as a wound cleanser to remove bacteria and other contaminants from wounds (3). SH acts through protein denaturation (2). Concentrations in the range of 0.1–0.5% showed marked epidermal hyperplasia in an *in vivo* animal model (17). No deleterious effects were observed in an *in vitro* toxicity assay when a concentration of 0.025% was used (4). This low concentration of SH was shown to exhibit sufficient bactericidal properties (4).

The irritant nature of IE found in the current study confirms the irritant reputation of this agent (2, 3). The mode of action of iodine is probably the binding of nucleotides with subsequent protein synthesis impairment (2). The irritant action of iodine solutions is dependent on the amount of free iodine (2). Therefore, the irritant action of IE, as opposed to non-irritating PI, is caused by the high concentration of free iodine in IE tested in

the current study (10,000 mg/l=1%). The concentration of free iodine in the 10% PI solution tested in our study, is 1.8 mg/l (18). The low irritancy of PI found in our study agrees with the common observation that PI is well tolerated (3, 15). Apparently, the discrepancy between the study of Dykes & Marks (15) and the current study may be due to the difference in exposure technique. PI is a iodophor in which iodine is chemically bound to polyvinyl pyrrolidone (2). The amount of free iodine increases from 1.8 mg/l to 24 mg/l when the concentration of PI is decreased from 10^6 mg/l (=10%) to 70 mg/l (18). This paradoxical phenomenon is possibly the cause of the discrepancy between the occlusive exposure of longer duration (15) and the current short-time open test, namely by a shift of free iodine molecules from the polyvinyl pyrrolidone molecule in the occlusive test. In pilot studies for the current investigation, PI exhibited erythematous reactions when tested by means of occlusion. Repeated open exposure mimics most conditions met with in practice. In the case that antiseptics are used as washes, a wash test would better reflect these conditions. A disadvantage of the (elbow) wash test is the fact that only 2 agents can be compared in the same volunteer (16). Apart from the mechanical act of washing, no basic differences exist between the repeated open exposure test and the wash test. Therefore, repeated open exposure is an appropriate technique for testing these types of agents.

Chlorhexidine induces coagulation of bacterial cytoplasm and destruction of its membrane (2). It has a very low toxicity, even when used on the intact skin of newborns (3). The same holds true for ethanol, which derives its antibacterial activity by way of protein denaturation (2). The current investigation corroborates the clinical observations regarding chlorhexidine and ethanol.

In conclusion, we have demonstrated a highly irritant influence of sodium hypochlorite and iodine tincture as opposed to a low irritancy of chlorhexidine, ethanol and povidon-iodine, by means of a repeated open exposure test using normal use concentrations. Thus, the last-mentioned antiseptics may probably be used without risk on a regular basis in dermatoses that are complicated by high counts of micro-organisms.

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