Chlorhexidine is a better antiseptic than povidone iodine and sodium hypochlorite because of its substantive effect

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Key Words:
Anti-infecting agents
Chlorine compounds
Iodine compounds
Biguanides

Background: The present study compared both the antiseptic efficacy of sodium hypochlorite against that of chlorhexidine gluconate in isopropyl alcohol and the substantive effect of chlorhexidine, povidone iodine, and sodium hypochlorite.

Methods: This was a 2-step study that included volunteers. In step 1, 4 skin areas were tested for bacteria in colony-forming units (CFU): 2 were controls to determine baseline bacteria or the effect of scrubbing, and 2 were treated with 10% hypochlorite or 2% chlorhexidine in isopropyl alcohol. Every subject was tested 4 times. The second step tested the substantive effect of 10% povidone-iodine and the aforementioned antiseptics.

Results: For the first step, 30 volunteers were studied, resulting in 120 determinations for each control and antiseptic. No differences between chlorhexidine gluconate (median 115 CFU/cm²) and sodium hypochlorite (median 115 CFU/cm²) were found. Both antiseptics were significantly different from rubbing control (317 CFU/cm²) and basal control (606 CFU/cm²). Only chlorhexidine showed a substantive effect.

Conclusion: We consider that chlorhexidine gluconate in isopropyl alcohol, sodium hypochlorite, and povidone-iodine is equally effective for procedures that do not require a long action. However, chlorhexidine is desirable for procedures such as catheter insertion, skin preparation for surgery, or hand-washing prior to surgery.

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Antisepsis are one of the most powerful weapons in infection control. Their clinical impact has been proved since the nineteenth century, when Ignaz Semmelweis introduced hand hygiene with a sodium hypochlorite solution, leading to an impressive reduction in morbidity and mortality related to childbed fever.1 In Mexico, every year, 450,000 new cases of hospital-acquired infections are reported, with 32 deaths per 100,000 habitants and associated costs of 1.5 billion US dollars.2 Nearly one-third of these infections could be prevented with asepsis and antisepsis protocols as well as hand hygiene.3,5

Nowadays, available antisepsics are limited because many of them have been removed from the clinical practice because of their toxic effects or infection outbreaks from intrinsic and extrinsic contamination.6,7 Antisepsics more commonly used for health care are alcohol, chlorhexidine, and iodine compounds. Alcohol has resisted the test of time, having only rarely been associated with contamination; it has an extended spectrum of activity and rapid action, although it is volatile and flammable, requiring sealed containers to keep the ideal concentration. Povidone-iodine has considerable spectrum and has been used for decades, with only few problems of contamination with gram-negative bacilli and allergic reactions8,9; it is still the standard of use in many institutions through the world. In a previous study, we reported that the antisepsis properties of sodium hypochlorite are not inferior to those of povidone-iodine.9

Chlorhexidine is currently recommended for skin preparation before surgery and insertion of intravascular devices5,10,11; nevertheless, chlorhexidine is an expensive substance, which limits its availability and distribution, especially in developing countries.9 Chlorhexidine has an inherent substantive effect, which is the ability of only a few antisepsics to remain linked in its active form to

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Conflicts of interest: None to report.
certain biologic surfaces such as the stratum corneum of the skin. Substantivity is an important characteristic that could explain the reported superiority of chlorhexidine against other antiseptics. Nevertheless, substantivity has been poorly studied because almost every study design tested indirect methods such as bacterial counts within specific periods after the antiseptics application. Because bacterial counts may represent only a delay in population recovery, a new method that could overcome this concern is needed.

The present study was conducted to compare the antiseptic efficacy of 10% sodium hypochlorite against that of 2% chlorhexidine gluconate in 70% isopropyl alcohol in healthy volunteers. Additionally, we conducted tests to prove an eventual substantive effect of chlorhexidine, povidone iodine, and sodium hypochlorite. With the results of the present and those of a previous study, we expected to gather information to conclude whether chlorhexidine has an immediate antiseptic effect and substantivity similar to that of sodium hypochlorite and povidone-iodine.

MATERIAL AND METHODS

Experimental design

The study was designed in 2 steps to answer 2 main research questions. The first step compared the antiseptic efficacy of sodium hypochlorite and chlorhexidine. The second step tested the substantive effect of sodium hypochlorite, chlorhexidine, and povidone-iodine.

The first step was a phase 3 nonrandomized efficacy study in 2 arms of human volunteers, blinded to the outcome assessor. Healthy adult volunteers with no history of skin allergies or atopy were included between April and December 2011. The main outcome measure was the bacterial counts in cultures of skin treated with chlorhexidine or with sodium hypochlorite. The secondary outcomes were the presence of allergy or skin reaction. The second step evaluated the substantive effect of chlorhexidine, sodium hypochlorite, and povidone-iodine. The protocol was reviewed and approved by the investigation board of the University of Guanajuato, Department of Medicine and Nutrition, and registered in clinicaltrials.gov (NCT01321125). Signed informed consent was obtained from each participant. The sponsor had no involvement in the design or conductions of the investigation.

Study products

Two products were used to test the main outcome: a standard agent, 2% wt/vol chlorhexidine gluconate in 70% vol/vol isopropyl alcohol (ChloraPrep, Enturia, TX), and 10% wt/vol sodium hypochlorite of electrolytic production (Except; Pisa SA de CV, Guadalajara, Mexico). To test the substantive effect, the same products were used, in addition to povidone-iodine 10% wt/vol (Isodine; Boehringer-Ingelheim Promeco SA de CV, Mexico City, Mexico).

First step

Preparatory phase

For stabilization of the skin microbiota, all volunteers used neutral soap and shampoo without antiseptics over a period of 2 weeks, being advised to avoid swimming in pools. After that phase, every subject was assessed to check that he or she had at least 100 aerobic bacteria per square centimeter of the forearm skin, which was be determined before entering the study. Volunteers were instructed to not take a shower 24 hours prior to the experiment.

Methods of intervention

For the primary measurement, 4 areas of approximately 25 cm² each were selected from the forearms. Two areas were designated as controls; the first one, the basal control, was used to determine the baseline bacterial count; the second one was the rubbing control; a cotton swab impregnated with sterile saline solution was rubbed to test the influence of the rubbing itself into bacterial counts. The other 2 areas were rubbed with chlorhexidine or sodium hypochlorite. Rubbing control or antiseptics were rubbed with circular movements toward the periphery, covering the area of study; solutions were left on the skin for 60 seconds before culturing, allowing them to dry. To conclude the trial, every volunteer had to be examined 4 times, each one separated by at least 15 days, alternating the areas in every subsequent test; therefore, every area was used for both controls, and for each antiseptic. All the volunteers were instructed to keep using the neutral soap and hair shampoo without antiseptics during the entire follow-up period.

Microbiologic methods and neutralizer

 Cultures were performed by the same trained technologist, following the quantitative technique described by Williamson and Kliger. Briefly, a scrub cup of 5 cm² of internal area was pressed over the skin zone to be tested. With the use of a pipette, the technologist added 3 mL of broth (Neutralizing broth D/E; DIFCO, Mexico City, Mexico) containing a neutralizing agent for halogens (0.1% sodium thiosulphate) and chlorhexidine (L-α-lecithin) and a detergent agent (1% solution Tween-80) as washing solution. A sterile rubber policeman (a hand-held flexible natural-rubber scraper attached to a glass rod) was used to rub the skin for 2 minutes. After this, 3 mL of new washing solution was added, and the abrasive scrub was repeated. These 2 washes were gathered together, and 50 μL of this volume was dropped on a plate containing neutralizing agar (Neutralizing agar D/E; DIFCO), which has the same characteristics of the neutralizing broth. The solution was distributed across the surface using a sterile plastic spreader. The plates were incubated at 35°C ± 2°C for 24 ± 4 hours in ambient atmosphere. After incubation, the outcome assessor counted the colonies to determine the colony-forming units (CFU) per square centimeter (CFU/cm²) of skin.

Second step

Method for testing the substantive effect

To test the substantive effect, 3 fingers were selected and then washed; the first finger was swabbed with chlorhexidine, the second one with povidone-iodine, and the third one with sodium hypochlorite. The antiseptics were left to dry for 60 seconds, followed by a second wash with distillate water, to remove any antiseptic excess. Finally, each finger was covered with a sterile dressing. After 2 hours, the dressing was removed, and the finger-tips were tested, placing them delicately for 30 seconds on a Mueller-Hinton agar (BD/BBL; Mexico City, Mexico). The plates were swabbed with a 0.5 McFarland solution of Escherichia coli ATCC 25922. Finally, the plates were incubated at 35°C ± 2°C for 24 ± 4 hours in ambient atmosphere. After incubation, the outcome assessor searched for inhibition zones.

Statistical analysis

To test significant differences in non-normal distribution data, we used a range test (Kruskal-Wallis) with 3 degrees of freedom, corrected for ties. A post hoc test of Kruskal-Wallis for multiple comparisons of z values was used to determine which arm was different. The α level for significance was established at 5%. For the first step, a minimal sample of 16 volunteers was calculated to find a difference of 100 CFU/cm², with a power of 80, and bilateral error.
of 5%. To test the substantive effect, we performed an initial pilot study in 3 volunteers, and we found that only chlorhexidine had such an effect; therefore, for step 2, we tested only 10 volunteers.

RESULTS

We enrolled 31 healthy volunteers, 18 women and 13 men, for testing between April and December 2011. One was withdrawn from the study because she did not tolerate the sampling technique, showing demographic lesions in all the tested areas. The median age of the remaining women was 23 years (range, 18–31 years) and, for men, 22 years (range, 19–26 years). Every enrolled subject was tested on 4 separated occasions, thus completing the first phase of the study; we obtained 120 determinations for each intervention, for a global of 480 determinations. The medians and interquartile ranges of every intervention are shown in the Table 1.

Table 1

<table>
<thead>
<tr>
<th>Agent or control</th>
<th>n</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Median</th>
<th>Range</th>
<th>Interquartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Sodium hypochlorite</td>
<td>120</td>
<td>140</td>
<td>154</td>
<td>115</td>
<td>0-692</td>
<td>0-173</td>
</tr>
<tr>
<td>2% Chlorhexidine gluconate/70% isopropyl alcohol</td>
<td>120</td>
<td>135</td>
<td>104</td>
<td>115</td>
<td>0-404</td>
<td>58-173</td>
</tr>
<tr>
<td>Basal control</td>
<td>120</td>
<td>881</td>
<td>646</td>
<td>606</td>
<td>173-2,423</td>
<td>404-1,111</td>
</tr>
<tr>
<td>Rubbing control</td>
<td>120</td>
<td>139</td>
<td>327</td>
<td>317</td>
<td>58-1,615</td>
<td>159-533</td>
</tr>
</tbody>
</table>

After the analysis, the data did not show a normal distribution, so a Kruskal-Wallis test for non-normally distrusted data was used, in which we found a significant difference between groups ($\chi^2_H = 60.24, P < .01$). Subsequently, we performed a Dunn test for multiple comparisons of the mean ranks (post hoc test of Kruskal-Wallis for multiple comparison on z values difference were shown if z values <2.6383).

This test showed that there were no differences between chlorhexidine gluconate (median, 115 CFU/cm$^2$) and sodium hypochlorite (median, 115 CFU/cm$^2$). Both antiseptics were significantly different from the 2 controls. Of note, rubbing control reduced almost 50% the median of growth (317 CFU/cm$^2$) when compared against that of the basal control (606 CFU/cm$^2$). No subject developed skin reactions related to the tested antiseptics (Fig 1).

In the substantive effect test, chlorhexidine inhibited the bacterial growth on every agar zone in contact with the treated skin; neither sodium hypochlorite nor povidone-iodine showed such an effect. An agar plate from this test can be seen in the Figure 2.

DISCUSSION

This trial could not find a difference in the immediate antiseptic effect after 1 minute of exposure to sodium hypochlorite and chlorhexidine; in a similar study, our group could not find a difference in the activity of sodium hypochlorite and povidone-iodine.9 These findings may suggest a wider application of sodium hypochlorite; nevertheless, the substantive effect seems to be a determinant factor in the recommendation of chlorhexidine for invasive procedures, at least for those with a prolonged intervention or involving the insertion of catheters.5,11

On the other hand, we found that only chlorhexidine has substantivity. The substantive effect is the linkage of an antiseptic to the biologic surfaces because of electrochemical forces. Chlorhexidine links itself to the stratum corneum of the skin, allowing the tissue to act like a reservoir of the antiseptic thus perpetuating the inhibition of bacterial growth.18,25 Agents with substantive effect such as chlorhexidine have a major persistent effect as the inhibition in bacterial growth is potentiated. This is an important finding because the skin is a reservoir of potential pathogens.26-28

To our knowledge, only few reports have discussed substantivity,2-14,16,18,25 which lacks a standardized test to prove it. Almost every previous study has measured indirect outcomes such as bacterial counts as a surrogate of substantivity when a prolonged persistent activity is demonstrated. Substantivity can be mistakenly considered as a synonymous of persistent activity, which is defined from the persistently low bacterial counts in culture,1 whereas substantivity means the actual effect of the antiseptic even against freshly introduced organisms. A novel method that overcomes this concern was required to demonstrate the importance of substantivity beyond any doubt. In this trial, we introduced an easy and novel method to test the substantive effect in a safer way for the volunteers because it avoids the interaction with bacteria that was required in previous studies.12-14,16,18,25,29
Chlorhexidine is the current recommendation for skin preparation before performing invasive procedures, but its daily use in some institutions is limited because it is unavailable or expensive. Nevertheless, its substantivity makes it desirable for prolonged procedures, such as the installation of intravascular devices or surgical procedures. Alcohol is an effective and inexpensive antiseptic, with a wide spectrum and quick action, but it is volatile and therefore inflammable. Iodophors are a frequent selection in hospitals because historically they have been considered very efficient; however, they can be inactivated by blood or serum proteins, are associated with skin reactions, and can cause potential damage on newborns. Sodium hypochlorite has been in use for hundreds of years; it is inexpensive, nonirritating, and nontoxic, and it has, to the best of our knowledge, never been associated with contamination-related outbreaks or pseudo-outbreaks. Recently, it has been widely used to care for the exit sites of hemodialysis and peritoneal dialysis catheters as well as for dentistry procedures. Chlorhexidine is generally used in alcohol dilution, and its substantivity makes it desirable for prolonged procedures, the power is small to detect reactions present per thousands of CFUs of skin area, which could eventually be important for specifying the antimicrobial effect of a 2% triclosan-detergent preparation on the skin. In real life, it is inexpensive, nonirritating, and nontoxic, and it is used to care for the exit sites of hemodialysis and peritoneal dialysis catheters as well as for dentistry procedures. It is reasonable to propose its use for other procedures where a substantive effect is not required such as venipunctures or wound care.

The present study has a few limitations. First, it was not designed to show differences under 100 CFUs of skin area, which could eventually be important for specific organisms. Second, although none of the volunteers suffered from adverse skin reactions, the power is small to detect reactions present per thousands of users, which could be of relevance. Finally, as it happens for other studies, chlorhexidine was tested in suspension with alcohol, making it difficult to discriminate the relative contribution of the 2 substances to the antiseptic effect. However, these limitations do not affect the main findings of the study because, in real life, chlorhexidine is generally used in alcohol dilution, and its substantive effect is clearly an effect of the former.

In conclusion, with the results of this and a previous study, we consider that 2% chlorhexidine gluconate in 70% isopropyl alcohol, 10% sodium hypochlorite, and 10% povidone-iodine are equally effective for procedures that do not require a long action, such as venipuncture for laboratory test. However, chlorhexidine is a better selection than sodium hypochlorite and povidone-iodine for skin preparation when a substantive effect is desired for procedures such as catheter insertion, skin preparation for surgery, or handwashing before surgery.

References