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Efficacy of five alcohol-based skin antiseptics on sebaceous skin used at shorter application times than the current recommendation of 10 minutes

Short title: Shorter application time for sebaceous skin antiseptics.

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Summary

Purpose: Alcohol-based skin antiseptics are recommended with a minimum application time of 10 minutes on skin containing high numbers of sebaceous glands. In clinical practice, a 10 minute application time often is too long. Therefore, we determined the efficacy of skin antiseptics on the forehead and lower back using shorter application times. **Methods:** Five alcoholic solutions were tested in a double-blind trial for their cfu reduction after 3, 4, 5, and 10 minutes on forehead of 20 healthy volunteers and lower back of 10 healthy volunteers and 10 patients against the reference alcohol 70% propan-2-ol, 10 min. **Results:** After an application time of 3 minutes, 3/5 (forehead) and 5/5 preparations (lower back) were at least equally effective as compared to the reference alcohol and an application time of 10 minutes. **Conclusion:** Alcohol-based skin antiseptics do not require 10 min application time. For all of the tested antiseptics a minimum application time of 3 minutes on sebaceous skin can be recommended.

Key words: skin antiseptics; sebaceous skin; application time; alcohol-based antiseptics;

Introduction

Currently, in Germany and Austria all alcohol-based skin antiseptics are recommended with a minimum application time of 10 minutes on skin areas containing high numbers of sebaceous glands.¹ Because currently there is no European Norm available to determine the efficacy of preparations for skin antiseptics, in Germany and Austria the efficacy of a preparation is commonly determined according to the test method of the German Society for Hygiene and Microbiology.² This test method requires 70% v/v iso-propanol during an application time of 10 minutes on skin with a high density of sebaceous glands. This leads to the requirement that the antimicrobial efficacy of any new skin antiseptic must not be significantly above the efficacy of the reference antiseptics with iso-propanol (70%, v/v) for 10 minutes.^{1,3}

In clinical practice, however, a 10 minute application time often is far too long, especially in emergency situations or in settings with high patient volumes, and it makes compliance to the manufacturers' recommendation difficult. As the work leading to the recommendation is 20 years old and it is based on the application of 70% v/v iso-propanol alone, it seems necessary to re-evaluate the recommendation, as new products containing combinations of different alcohols are available now. Recently published data on surgical hand disinfection^{4,5} revealed that for certain propanol-based preparations a shorter application time than the previously recommended 3 minutes is achievable with the same effect on the resident hand flora. The results for surgical hand disinfection support new studies for skin antiseptics, as for particular preparations the application time also might be shorter than 10 minutes. In deed, two skin antiseptics based on 85% ethanol were described recently having an equivalent efficacy on the forehead of healthy volunteers within an application time of only 2.5 minutes.⁶ The efficacy on the bacterial flora of patients using shorter application times and on other anatomic skin sites containing high numbers of sebaceous glands has not been published so far. Therefore, the aim of our study was to determine the efficacy of five alcohol-based skin antiseptics on the bacterial flora of the forehead (only volunteers) and lower back (volunteers and patients).

Methods

Design and preparation of subjects

Each experiment was performed in a double-blind reference-controlled cross-over design.² A minimum of 20 volunteers was recruited per experiment. Only participants with healthy skin on the forehead and the lower back were selected (no injury, eczema or other inflammatory skin disease). Subjects were excluded when they took antibiotics or had previously used a disinfectant or antiseptic solution within the last seven days before any experiment. Bathing and showering was not allowed 24 h prior to an experiment. Hair around the test site was clipped or tied back in order to prevent recontamination of the skin of the forehead during the experiments. **The inclusion and exclusion criteria were applied to both, 20 healthy volunteers in the forehead group and 10 healthy volunteers and 10 patients in the lower back group.** The skin on the forehead and the lower back was divided into five areas of approximately five cm² next to each other. The left area was always chosen to determine the baseline bacterial density. The other test fields were allocated from the left side to right side: 3 min post-value, 4 min post-value, 5 min post-value, 10 min reference treatment post value. A cotton swab was soaked with the tested skin antiseptic and was swabbed strictly over the marked test field. The procedure was repeated as many times as necessary to keep the skin moist with the skin antiseptic for the examined application time.

Products and application

The following preparations were tested: R) propan-2-ol (70%, v/v) as reference alcohol; A) Manorapid synergy, a hand and skin antiseptic based on 57.6% (w/w) ethanol (96%) and 10% (w/w) 1-propanol (Antiseptica GmbH, Pulheim, Germany); B) Skin-Des (Polyalcohol Hautantiseptikum), a skin antiseptic based on 63.1% [w/w] 2-propanol (Antiseptica GmbH, Pulheim, Germany); C) Cutasept F, a skin antiseptic based on 63% (w/w) 2-propanol (Bode Chemie GmbH & Co. KG, Hamburg, Germany); D) VP 365, an alcohol-based antiseptic

containing 85% (w/w) ethanol (Bode Chemie GmbH & Co. KG, Hamburg, Germany); and E) VP 505, an alcohol-based solution containing 65% (w/w) 2-propanol and 0.2% (w/w) mectronium etilsulphate (Bode Chemie GmbH & Co. KG, Hamburg, Germany).

All preparations were colourless and blinded to participants and laboratory staff. All skin antiseptics were tested for application times of 10 minutes, 5 minutes, 4 minutes, and 3 minutes (forehead and lower back). The reference alcohol was always applied for 10 minutes to the skin of the forehead according to the recommended test method.²

Antiseptic phase

One marked skin area was treated with the reference alcohol, three other areas with one of the antiseptic products each. After each skin antiseptis a sample was taken (post-value) immediately after completion of the application (10, 5, 4 or 3 minutes after beginning of the application). Between each application of the product, a resting period of at least one week elapsed in order to allow complete reconstitution of the normal skin flora.

Determination of the pre-values and post-values

Sampling and cultivation were done according to the test method of the German Society for Hygiene and Microbiology.² Each sampling area was marked in such a way that the standard size of 5 cm² was clearly visible. A cotton swab was soaked in tryptic soy broth (TSB). The sampling area was rigorously rubbed as described before.⁷ Meticulous care was taken to ensure that the swab was only rubbed within the marked skin area. Then, the swab was transferred into 5 ml of TSB containing a combination of neutralizing agents for inactivation of residual microbiocidal activity. The following neutralizing agents were used: 3% Tween 80, 3% saponine, 0.1% histidine and 0.1% cysteine. The combination of neutralizing agents was previously found to be valid for neutralization of 85% ethanol. The tube was vortexed for

30 sec at high frequency. A serial dilution was done in TSB. From appropriate dilution steps, aliquots of 1 ml were spread on tryptic soy agar (TSA) in duplicate.

Calculation of bacterial reduction

The plates were incubated for a total of 48 h at 36°C, and the colony-forming units (cfu) from plates were counted. For calculation purposes, plate count values ≤ 300 cfu were accepted. Plate count values of zero were reset to 1, as the \log_{10} of 0 is undefined, but the \log_{10} of 1 = zero. The weighted mean of cfu was calculated taking into account the number of cfu per plate and the corresponding dilution step. The weighted mean was multiplied by the dilution factor in order to obtain the number of cfu per ml in the sampling liquid. All pre- and post-values were expressed as \log_{10} values. For each experiment, the logarithmic reduction factor (\log_{10} RF) was calculated as the difference between the \log_{10} baseline value and the \log_{10} post-values.

Statistics

A comparison of multiple mean \log_{10} RFs was done using Friedman's ANOVA. In addition, based on the DGHM test method for skin antisepsis a product is considered effective for skin antisepsis if the mean is not significantly lower than the corresponding mean \log_{10} RF of the 10 min reference treatment (one sided Wilcoxon matched-pairs signed-ranks test). Differences of all other means were investigated by the two sided Wilcoxon matched-pairs signed-ranks test.² A P-value < 0.05 was chosen to indicate a significant difference.

Results

Skin antiseptics on the forehead

Product A was most effective after 5 min and less effective after 4 and 3 min (Table I). The difference between all four treatments was significant ($P = 0.007$; Friedman's ANOVA). A pair-wise analysis did not reveal a statistically significant difference in the efficacy between any of the application times of product A in comparison to the reference treatment ($P > 0.05$; two sided Wilcoxon matched-pairs signed-ranks test).

Product B was also most effective after 5 min and less effective after 4 and 3 min (Table I). The difference between all four treatments was significant ($P < 0.001$; Friedman's ANOVA). A pair-wise analysis revealed a significant lower efficacy for any of the application times of product B in comparison to the reference treatment ($P < 0.005$; one sided Wilcoxon matched-pairs signed-ranks test).

Product C also showed the highest efficacy after 5 min and a lower efficacy after 4 and 3 min (Table I). The difference between all four treatments was significant ($P < 0.001$; Friedman's ANOVA). A pair-wise analysis revealed only a significant lower efficacy for a 3 min application time of product C in comparison to the reference treatment ($P = 0.001$; one sided Wilcoxon matched-pairs signed-ranks test).

Product D showed also the best efficacy after 5 min and a lower efficacy after 4 and 3 min (Table I). The difference between all four treatments was significant ($P < 0.019$; Friedman's ANOVA). An application time of 5 min was significantly more effective in comparison to the reference treatment ($P = 0.023$; two-sided Wilcoxon matched-pairs signed-ranks test). For the other exposure times the efficacy was equal to the reference.

Product E showed the best efficacy after 5 min and a lower efficacy after 4 and 3 min (Table I). The difference between all four treatments was not significant ($P < 0.218$; Friedman's ANOVA).

Skin antisepsis of lower back in volunteers

Product A, product B, product C, and product D were equally effective as compared to the reference treatment (Table II). The difference between all four treatments each were not significant ($P = 0.735$; $P = 0.902$; $P = 0.531$; $P = 0.715$; Friedman's ANOVA), respectively.

Product E was also equally effective in comparison to the reference treatment (Table II). The difference between all four treatments, however, was significant ($P = 0.009$; Friedman's ANOVA). A pair-wise comparison between product E and the reference treatment revealed no significant difference for any of the application times ($P > 0.05$; one sided Wilcoxon matched-pairs signed-ranks test). **No statistical difference was observed in pre- and post results for healthy volunteers and patients on the lower back ($P > 0.05$; two sided Wilcoxon matched-pairs signed-ranks test).**

Discussion

Based on our results it seems not to be necessary to insist on a standard 10 minute application time for skin antiseptics on the forehead or the lower back as it is currently required by the test method of the German Society for Hygiene and Microbiology.⁸ Our data revealed a strong difference between the efficacy of skin antiseptics on forehead and lower back. On the lower back, all tested skin antiseptics were as effective as the reference alcohol within 3 minutes, while on the forehead two of the five preparations were significantly less effective within 3 min, as compared to the reference alcohol. The reason for this finding is the different baseline bacterial density and probably the different density of the number of sebaceous glands per cm^2 (2.1 vs. 3.7 log₁₀). Similar baseline values have been reported before.^{9,10}

Application of skin antiseptics for 4 minutes on the lower back of volunteering patients yielded a reduction of resident bacteria equivalent to the 10 min reference antiseptics. It is likely that even shorter application times might reveal an equivalent efficacy on the lower back, however, as we did not investigate shorter application times and therefore no proof, this remains speculation at this stage. Using product D, for example, the bacterial log log₁₀ RF was 1.14 in healthy volunteers and 0.88 in volunteering patients (sub-group data not shown in table II). Particularly the ethanol-based skin antiseptics are likely to be effective even after shorter application times, as recent data show that on the forehead an application time of 2.5 min still was equally effective to the 10 min reference procedure.⁶

The efficacy of all antiseptics was lower on the lower back than on the forehead. This finding was expected as the mean baseline bacterial density was quite high with bacterial densities of 3.7 log₁₀ on the forehead and remarkably lower on the lower back with 2.1 log₁₀. Resident bacteria apparently can be reduced to a maximum level which can be described as the “irreducible minimum”. Prolongation of the application time will not provide any increase of

efficacy, which also was also recently shown, albeit for surgical hand disinfection.^{4,5}

Of importance are our results showing that the mean efficacy of two skin antiseptics applied for 4 minutes on the lower back was equal for both, healthy volunteers and volunteering patients. Similar results between volunteers and patients have been described earlier by Kurth et al.¹¹ Testing the efficacy of skin antiseptics on healthy volunteers can therefore be considered to be a valid method for assuming an efficacy on patients' skin as well.

Since the baseline bacterial density and the resulting reduction factors between forehead and lower back differ, skin antiseptics should be tested on the anatomic sides for which a specific product is intended, and not on the forehead alone. Further research therefore should also include other skin sites such as the perineum, breast, joints and the foot.

Conclusions

Short application times are desirable for skin antiseptics to ensure maximum compliance and efficacy, even under emergency conditions. Application times for skin antiseptics on the forehead or the lower back should be based on their "true application time" or the time, when they are equally effective as compared to the reference skin antiseptic using propan-2-ol (70%, v/v) for 10 minutes application time. Alcohol-based skin antiseptics do not require a standard 10 min application time, especially on the lower back. Most antiseptics surpass the mean log₁₀ reduction factor of the reference alcohol within 10 minutes application time already after 3 or 4 min on the forehead and lower back, respectively. For all of the tested antiseptics a minimum application time of 3 minutes on skin containing sebaceous glands can be recommend.

List of abbreviations

TSB	Trypton-Soya-Broth
TSA	Trypton-Soya-Agar
cfu	Colony-Forming Units
log ₁₀ RF	logarithmic Reduction Factor

Competing Interests

The study was sponsored by Bode Chemie GmbH & Co. KG, Hamburg, Germany and Antiseptica chem.-pharm. Produkte GmbH, Pulheim/Brauweiler, Germany. The sponsors had no participation in the study design, analysis and interpretation of data. The authors declare no competing interests.

Authors' contributions

NOH, SAG and AK designed the study, NOH, OA, MD and SAG analysed the data, OA, NOH, MD and AK wrote the manuscript; All authors read and approved the final manuscript.

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Table I Mean log₁₀-reduction of resident skin flora obtained with four skin antiseptics on the forehead of 20 healthy volunteers applied for 3, 4 and 5 min in comparison to a 10 min reference skin antiseptics.

Test product	Mean RF ± standard deviation			
	Reference (10 min)	Preparation (5 min)	Preparation (4 min)	Preparation (3 min)
A (n = 20)	1.57 ± 0.66	2.05 ± 0.59	1.83 ± 0.62	1.57 ± 0.46
B (n = 20)	2.47 ± 0.53	2.15 ± 0.69	1.93 ± 0.67	1.60 ± 0.52
C (n = 20)	1.88 ± 0.74	2.05 ± 0.63	1.68 ± 0.62	1.48 ± 0.58
D (n = 20)	1.67 ± 0.75	2.16 ± 0.79	1.82 ± 0.70	1.75 ± 0.69
E (n = 20)	1.41 ± 0.80	1.92 ± 0.82	1.55 ± 0.74	1.59 ± 0.95

Table II Mean log₁₀-reduction of resident skin flora obtained with four skin antiseptics on the lower back of 10 healthy volunteers and 10 patients each, applied for 3, 4 and 5 min in comparison to a 10 min reference antiseptics.

Test product	Mean RF ± standard deviation				P-value
	Reference (10 min)	Preparation (5 min)	Preparation (4 min)	Preparation (3 min)	
A (n = 20)	1.24 ± 0.32	1.22 ± 0.36	1.24 ± 0.32	1.09 ± 0.65	0.735
B (n = 20)	1.23 ± 0.35	1.20 ± 0.36	1.20 ± 0.36	1.22 ± 0.35	0.902
C (n = 20)	0.99 ± 0.47	0.98 ± 0.47	0.95 ± 0.40	0.95 ± 0.40	0.531
D (n = 20)	1.15 ± 0.44	1.11 ± 0.40	1.14 ± 0.42	1.00 ± 0.55	0.715
E (n = 20)	1.14 ± 0.44	0.87 ± 0.52	0.95 ± 0.44	1.07 ± 0.36	0.009