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Research Article

Efficacy of two iso-propanol-based skin antiseptics applied to human skin with many sebaceous glands for 2 and 10 minutes

Wirksamkeit von zwei Hautantiseptika auf Basis von Propan-2-ol auf talgdrüsenreicher Haut in 2 und 10 Minuten

Abstract

Background: Recent research suggests that specific ethanol-based skin antiseptics exhibit their efficacy on the resident skin flora of the forehead in only 2.5 minutes. We have now looked at the efficacy of two skin antiseptics based on 63% (w/w) propan-2-ol (iso-propanol) and applied for 10 and 2 minutes on skin with a high density of sebaceous glands.
Methods: Each experiment was performed in a reference-controlled cross-over design with at least 20 participants. Application of isopropanol (70%, v/v) for 10 minutes to the forehead served as the reference treatment. Pre-values and post-values (immediately after the application and after 30 min) were obtained by swabbing a marked area of 5 cm^2 for about 10 s. Swabs were vortexed in tryptic soy broth containing valid neutralizing agents. After serial dilution aliquots were spread on tryptic soy agar. Colonies were counted after incubation of plates at 36 °C for 48 h. The mean log_{10} reduction of bacteria was calculated. The Wilcoxon matched-pairs signed-ranks test was used for a comparison of treatments.
Results: Skin antiseptic A applied for 10 min (one experiment) was equally effective to the reference treatment. When applied for 2 min (two experiments) it was still equally effective to the reference treatment immediately after application (e.g. 1.6 versus 1.4 log_{10} reduction) and after 30 min (1.7 versus 1.4 log_{10} reduction). Skin antiseptic B applied for 10 and 2 min (one experiment each) was also equally effective to the reference treatment both immediately after application and after 30 min.
Conclusions: The clear and coloured skin antiseptics applied for 2 min on the skin of the forehead fulfilled the national efficacy requirements for skin antisepsis. The shorter application time on skin with a high density of sebaceous glands will allow acting more efficiently in clinical practice.

Keywords: skin antiseptic, propan-2-ol, isopropanol, efficacy, forehead

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Zusammenfassung

Zielsetzung: Aktuelle Forschungsergebnisse zeigen, dass bestimmte ethanolische Hautantiseptika ihre Wirksamkeit auf die residente Flora der Stirn in nur 2,5 Minuten ausüben. Wir haben nun die Wirksamkeit von zwei Hautantiseptika auf Basis von 63% (w/w) Iso-Propanol bei Einwirkzeiten von 2 und 10 Minuten auf talgdrüsenreicher Haut untersucht.
Methode: Jeder Versuch wurde im Referenz-kontrollierten Überkreuzdesign mit mindestens 20 Teilnehmern durchgeführt. Das Referenzverfahren war die Anwendung von 70% Iso-Propanol (v/v) über 10 Minuten auf die Stirn. Vorwerte und Nachwerte (unmittelbar nach der Anwendung sowie nach 30 Minuten) wurden von dem markierten Areal (5 cm^2)

mittels Tupferabstrichmethode über 10 Sekunden bestimmt. Die Tupfer wurden in Trypton-Soya-Bouillon mit validierten Neutralisierungssubstanzen geschüttelt. Aus der anschließenden Verdünnungsreihe wurden Aliquots auf Trypton-Soya-Agar ausgespatelt. Nach der Bebrütung der Platten über 48 h bei 36°C wurden die Kolonien gezählt. Die mittlere log_{10}-Reduktion der Bakterien wurde berechnet. Der Wilcoxon matched-pairs signed-ranks Test wurde zum Vergleich von Behandlungen verwendet.

Ergebnisse: Die Anwendung von Hautantiseptikum A über 10 Minuten (1 Versuch) war im Vergleich zum Referenzverfahren gleichwertig wirksam. Bei Anwendung über 2 Minuten (2 Versuche) war das Hautantiseptikum A noch immer zum Referenzverfahren gleichwertig wirksam, sowohl unmittelbar nach der Anwendung (z.B. 1,6 versus 1,4 log_{10} Reduktion) und nach 30 Minuten (1,7 versus 1,4 log_{10} Reduktion). Hautantiseptikum B war nach 10 und 2 Minuten (jeweils 1 Versuch) ebenso zum Referenzverfahren gleichwertig wirksam, sowohl unmittelbar nach der Anwendung als auch nach 30 Minuten.

Fazit: Die ungefärbten und gefärbten Hautantiseptika erfüllten bei einer Einwirkzeit von 2 Minuten die nationalen Anforderungen an die Wirksamkeit von Hautantiseptika. Die kürzere Einwirkzeit auf talgdrüsenreicher Haut wird eine rationellere klinische Praxis ermöglichen.

Schlüsselwörter: Hautantiseptik, Propan-2-ol, Isopropanol, Wirksamkeit, Stirn

Background

It has been reported recently that two skin antiseptics based on 85% ethanol are equally effective in 10 and 2.5 minutes on the skin of the forehead [1]. In clinical practice the actual application time for a skin antiseptic is often less than 10 minutes on skin with a high density of sebaceous glands. That is why we wanted to find out if two skin antiseptics based on 63% propan-2-ol (isopropanol) and so far studied with an application of 10 minutes are equally effective on the resident flora of the forehead with an application time of 2 min.

Methods

The efficacy was evaluated using the test method of the German Society for Hygiene and Microbiology. It was first established in 1991 [2] and includes a 10 minute reference procedure which was established based on data by Christiansen et al. in 1987 and justified by its optimum efficacy on the resident skin flora [3]. In Germany, a 10 minute application time on skin with a high density of sebaceous glands was the standard application time until 2008 when shorter application times as short as 1 min were allowed [4]. The data sets with a 10 minute application time were obtained using the method version of 1991 [2], all other data sets were obtained using the last method version of 2002 [5]. Each experiment was performed in a reference-controlled cross-over design. A minimum of 20 volunteers was recruited per experiment. The skin of the forehead was randomly divided into five areas of approximately five cm^2. One area was chosen to determine the baseline bacterial density. Two areas were used for application of the reference alcohol (10 minutes), one for each of the different sampling times. Another two test areas were used for application of the skin antiseptic (2 or 10 minutes), one for each of the different sampling times. A cotton swab was soaked with the skin antiseptic and swabbed over the marked test field. The procedure was repeated up to five times in order to keep the skin moist with the skin antiseptic for the entire application time. Ethical approval for studying the efficacy for skin antisepsis was obtained from the ethics committee of the University Hospital Kiel, Germany (1991). The study was conducted in accordance with the ethical principles that have their origins in the current version of the Declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland, October 2000). Informed consent was obtained from each participant.

The following preparations were used: propan-2-ol (70%, v/v) as the reference alcohol, a clear skin antiseptic based on 63% [w/w] iso-propanol (Cutasept F, Bode Chemie GmbH, Hamburg, Germany; “skin antiseptic A”), and a coloured skin antiseptic based on 63% [w/w] iso-propanol (Cutasept G, Bode Chemie GmbH, Hamburg, Germany; “skin antiseptic B”).

Each sampling area was marked so that the standard size of 5 cm^2 was clearly visible. A cotton swab was soaked in tryptic soy broth (TSB). The sampling area was rigorously rubbed for about 10 s as described before [6]. The swab was transferred into 5 ml TSB containing a combination of neutralizing agents for inactivation of residual biocidal activity [7]. The following neutralizing agents were used: 3% Tween 80, 3% saponine, 0.1% histidine and 0.1% cysteine. This combination of neutralizing agents was found to be valid for neutralization of the skin antiseptics (data not shown). The tube was vor-

Table 1: Reduction (mean and stdev) of resident skin bacteria on the forehead of volunteers; skin antiseptics based on 63% (w/w) iso-propanol were applied for 2 and 10 minutes, the reference alcohol was applied for 10 min.

Skin antiseptic	Application time of skin antiseptic	Sampling time	Mean log_{10}-reduction obtained by skin antiseptic	Mean log_{10}-reduction obtained by 10 min reference procedure	p-value*
A	10 min	Immediate effect	1.67 ± 0.68	1.75 ± 0.69	0.862
		After 30 min	1.69 ± 0.59	1.73 ± 0.72	0.670
	2 min	Immediate effect	1.63 ± 0.93	1.40 ± 0.93	n.a.
		After 30 min	1.68 ± 0.96	1.44 ± 0.74	n.a.
	2 min	Immediate effect	1.27 ± 0.79	1.43 ± 0.52	0.327
		After 30 min	1.84 ± 0.70	1.78 ± 0.75	n.a.
B	10 min	Immediate effect	1.26 ± 0.40	1.25 ± 0.36	n.a.
		After 30 min	1.28 ± 0.51	1.29 ± 0.35	0.968
	2 min	Immediate effect	1.68 ± 0.50	1.87 ± 0.77	0.255
		After 30 min	1.75 ± 0.74	1.80 ± 0.83	0.492

*Wilcoxon matched-pairs signed ranks test

texed for 30 s. A serial dilution was done in TSB. From appropriate dilution steps aliquots of 1 ml were spread on tryptic soy agar (TSA) in duplicate.

Two marked skin areas on the forehead were treated with the reference alcohol, two other ones with one of the two skin antiseptics. After each type of treatment two samples were taken (post-values). The first sample was taken immediately after completion of the application (10 or 2 min after beginning of the application). The second sample was taken 30 minutes after beginning of the application (20 or 28 min after completion of application). Between each product application, a rest period of at least one week elapsed in order to allow the reconstitution of normal skin flora.

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 0 were reset to 1, because the log_{10} of 0 is undefined, and the log_{10} of 1 = 0. 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 sample from each volunteer, the logarithmic reduction factor (RF) was calculated as the difference between the log_{10} baseline value and the log_{10} post-values. A product is considered effective for skin antisepsis if the mean RF at both sampling times is not significantly lower than the corresponding mean RF of the 10 min reference treatment (one sided Wilcoxon matched-pairs signed-ranks test; p>0.1).

Results

Skin antiseptic A applied for 10 min reduced the bacterial density on the forehead by 1.67 log_{10}-steps (10 min after beginning of the application) and 1.69 log_{10}-steps (30 min after beginning of the application) and was equally effective in comparison to the reference procedure (Table 1). Two experiments were performed with the clear skin antiseptic A applied for 2 minutes. In both experiments, skin antiseptic A was equally effective at both sampling points (p>0.1).

Skin antiseptic B was studied in two experiments (Table 1). Application for 10 minutes was equally effective than the 10 min reference procedure in the immediate effect (1.26 versus 1.25 log_{10} reduction) and after 30 min (1.28 versus 1.29 log_{10} reduction; p=0.968). When applied for 2 minutes it was also equally effective immediately after the application (1.68 versus 1.87 log_{10} reduction; p=0.255) and 30 min after beginning of the application (1.75 versus 1.80 log_{10} reduction; p=0.492).

Discussion

For the first time we were able to demonstrate that the efficacy of two iso-propanol-based skin antiseptic is achieved on the forehead within 2 min and does not require a 10 min application time. This could be shown for a clear and a coloured skin antiseptic both based on 63% (w/w) iso-propanol. Extending the application time with any of the two skin antiseptics beyond 2.5 min has, based on our data, no additional effect on the bacterial density and can therefore be regarded as unnecessary.

Although the efficacy of iso-propanol on the resident skin is considered to be weaker compared to ethanol and n-propanol [8], it is nevertheless one of the most commonly used types of alcohol for skin antisepsis both in

Europe and the US and is considered to be safe and effective for this purpose [9]. Its efficacy is often underestimated especially when a combination with other active agents is used [10].
The clinical advantage is substantial if the application time on skin with a high density of sebaceous glands is reduced from 10 to 2 min without any loss of efficacy. Although data were obtained on the forehead other skin areas are also known to have a high density of sebaceous glands such as the skin on back or the chest [11] which underlines the relevance of our data especially in spine surgery or open thoracic and cardiac surgery. But this will only be possible for preparations with sound evidence that a short application time yields the same efficacy as a long application time.

Conclusions

The clear and coloured skin antiseptics which were tested in our study and which were applied for 2 min on the skin of the forehead fulfilled the efficacy requirements for skin antisepsis. The shorter application time on skin with a high density of sebaceous glands will allow acting more efficiently in clinical practice.

List of abbreviations

CFU – Colony-forming units
RF – Reduction factor
TSA – Tryptic soy agar
TSB – Trypric soy broth

Notes

Competing interests

The first and last authors are paid employees of Bode Chemie GmbH, Hamburg, Germany.

Authors' contributions

GK and NN designed the study, all authors analysed the data, GK wrote the manuscript, and all authors read and approved the final manuscript.

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