Antimicrobial efficacy in vivo of a new formulation of 2-butanone peroxide in n-propanol: comparison with commercial products in a cross-over trial


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Received 25 June 2008; accepted 7 November 2008

KEYWORDS
E. coli; Hand rub; Peroxide 2-butanone; UNE-EN-1500

Summary The use of hand rub to obtain maximum decrease in bacterial load is important because the reduction needed to avoid transmission is unknown. The monomer of 2-butanone peroxide is a peroxygen derivative with potential biocidal use in hospitals. The aim of this study was to compare the efficacy of hand rub with an alcoholic solution of peroxide 2-butanone versus five antiseptic products, against E. coli K12 (CECT 433) transient flora acquired by hand immersion in a broth culture following the UNE-EN-1500 standard. Isopropanol 60% (control) obtained 99.99% reductions, driving down the bacterial load from 10^6 cfu/mL in the initial inocula to <100 cfu/mL. Products A, B and C (different alcoholic solutions ranging from 65% to 75% with low amounts of biguanidines and/or quaternary ammonium compounds) resulted in significantly lower amounts, reducing initial inocula to ~500 cfu/mL. Products D and E (70–75% alcohol solutions containing higher amounts of different quaternary ammonium compounds and triclosan in the case of product E) produced reductions similar to that of isopropanol, with significantly larger reductions than products A, B and C. The product with the solution of 2-butanone peroxide...
produced the same effect as products D and E with mean reductions of $\approx 4 \log_{10} (99.99\%)$, driving the initial inocula down to $\leq 100 \text{ cfu/mL}$, despite the low concentration (35%) of propanol in the solution. This novel peroxygen biocide offers high in-vivo cidal activity against acquired \textit{E. coli} transient flora, offering an alternative to products with higher alcohol concentrations.

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Introduction

Most nosocomial infections are transmitted via hands of healthcare workers.\textsuperscript{1} Resident flora on hands includes mainly Gram-positive, low virulence micro-organisms rarely transmitted by hand contact that are not easily removed through hand washing.\textsuperscript{2} By contrast, transient flora consists mainly of Gram-negative bacteria that are an important cause of nosocomial infections, but that can be removed by hand washing.\textsuperscript{2} The purpose of hand washing is to remove the transient flora acquired through contact with patients and equipment.\textsuperscript{3}

It has been estimated that a twofold increase in the compliance of hand washing would result in nearly 50% decrease in nosocomial infections, but a recent report showed that a compliance increase from 37–38% to 68–69% was not associated with detectable changes in the incidence of healthcare-associated infections.\textsuperscript{4,5} In hospital daily practice, compliance with hand-washing recommendations does not exceed 50%, and thus new methods and antiseptics are continuously being developed.\textsuperscript{6,7}

Recently Neochemical S.A. has discovered and patented a series of dialkylketone peroxides as biocides, one of which, 2-butanone peroxide, is intended for use in skin, instruments and environment and thus has been successfully incorporated as an active substance into several antiseptic formulations.\textsuperscript{8} The monomer of 2-butanone peroxide has shown in-vivo biocidal activity against species of the genus \textit{Escherichia}, \textit{Pseudomonas}, \textit{Enterococcus} and \textit{Staphylococcus} spp. as well as mycobactericidal, fungicidal and virucidal activity.\textsuperscript{8}

The aim of the present study was to determine the efficacy of a 2-butanone peroxide solution as hand rub in reduction of transient \textit{E. coli} flora in comparison with common antiseptics used for this aim in daily practice in Spain.

Table I  Formulation of study products

<table>
<thead>
<tr>
<th>Product</th>
<th>Active components</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Bisbiguanide hydrochloride polyhexamethylene (0.2%) Ethanol (49.9%) 2-Propanol (15.8%)</td>
</tr>
<tr>
<td>B</td>
<td>Chlorhexidine digluconate (0.6%) Phenoxethanol (1%) Benzalkonium chloride (0.1%) Ethanol (70%)</td>
</tr>
<tr>
<td>C</td>
<td>n-Duopropenide (0.46%) Ethanol (60%) 2-Propanol (15%)</td>
</tr>
<tr>
<td>D</td>
<td>Mecetronium ethylsulphate (0.2%) 2-Propanol (45%) n-Propanol (30%)</td>
</tr>
<tr>
<td>E</td>
<td>Mecetronium ethylsulphate (0.2%) Triclosan (2%) Phenoxethanol (1%) Benzalkonium chloride (0.1%) Ethanol (70%)</td>
</tr>
<tr>
<td>F</td>
<td>2-Butanone peroxide (2%) n-Propanol (35%)</td>
</tr>
</tbody>
</table>

Methods

Products

2-Butanone peroxide 2% in a 35% alcohol solution (Neostex-Plus\textsuperscript{6}) plus five antiseptic solutions of common use for hand rub in the hospital setting were evaluated in this study (Table I). As control for biocidal activity, 2-propanol 60% (v/v) was used.

Strain

An 18–24 h growth broth culture of \textit{Escherichia coli} K12 (CECT 433) was used.

Design

A cross-experimental design with 12 healthy volunteers (24 hands as observational unit) was used. Subjects were divided into two subgroups of six
volunteers each: group 1 applied the control product as first hand rub and one of the test products as second hand rub; group 2 did the contrary in order to test each control/product pair on the same experimental day. A washout period of two weeks was left between each of the six antiseptic products tested.

**Measurements of bactericidal activity**

Biocidal activity was assessed following the UNE-EN-1500 standard, commonly used for validation of alcoholic formulations for hand-rub products in conditions similar to those in daily practice. Briefly, each experiment was done as follows: hands were washed with a soft soap for 1 min, immersed halfway to the metacarpals in the broth culture for 5 s, removed from the broth culture, excess fluid was drained off, and hands were dried in the air for 3 min. Bacterial recovery for the initial value was obtained by kneading the fingertips of each hand separately for 60 s in tryptic soy broth (TSB) without neutralisers. The hands were removed from the broth and disinfected for 60 s with standard 60% isopropanol or the test product. Hands were rinsed in running water for 5 s and water was drained off. Fingertrips of each hand were kneaded separately in 10 mL of TSB with added neutralisers. Broths were appropriately diluted and plated. Colony counting was performed after 24–48 h incubation at 37 °C. Tests with the reference standard (60% isopropanol) and each test product were carried out within an interval of 3 h.

As neutraliser a mixture of triptone (5 g), yeast extract (2.5 g), dextrose (10 g), sodium tioglicolate (1 g), sodium bisulphite (2.5 g), soy lecithin (7 g) and polysorbate (5 g) in 1000 mL distilled water was used.

**Statistical methods**

Initial colony counts in the different tests carried out with controls or with study products were compared by the non-parametric Kruskal–Wallis test.

Differences between initial and final log10 bacterial counts were calculated and mean ± SD values determined. For paired comparisons between log10 reductions obtained with controls versus those obtained with tested products the non-parametric Wilcoxon test was used, and $P \leq 0.05$ was considered significant.

The reduction difference between product/control pairs ($\log_{10}$ reduction with control − $\log_{10}$ reductions with products) was calculated. For multiple comparisons of these reduction differences, the Kruskal–Wallis test with Mann–Whitney for two-by-two comparisons was used, and $P \leq 0.003$ was considered as significant by using the Bonferroni correction.

**Results**

No significant differences were found in the initial colony counts (mean values ranging from $5.8 \log_{10}$ to $6.2 \log_{10}$) (Table II) in the different experiments carried out with controls or with study products, or between the initial colony counts for controls versus products in each experiment.

Table II shows initial counts and reductions for each control/product paired experiment. Significantly lower reductions ($P < 0.05$) for products A, B and C (mean values ranging from $-3.4 \log_{10}$ to $-3.7 \log_{10}$) versus their controls (mean values ranging from $-4.4 \log_{10}$ to $-4.7 \log_{10}$) were found. Ten times mean lower reductions were obtained for these products versus controls. Similar ($P > 0.05$) reductions (mean values ranging from $-3.9 \log_{10}$ to $-4.3 \log_{10}$) were obtained with products D, E and F and their controls (mean values ranging from $-3.8 \log_{10}$ to $-4.3 \log_{10}$).

With respect to the reduction difference ($\log_{10}$ reduction control − $\log_{10}$ reduction product), it was similar for products A, B and C (mean reduction difference values ranging from $1.0 \log_{10}$ to $1.2 \log_{10}$), without differences ($P > 0.05$) between them. Products D, E and F showed similar ($P > 0.05$) reduction differences (mean reduction difference values ranging from $-0.1 \log_{10}$ to $-0.2 \log_{10}$). The reduction difference was significantly lower ($P \leq 0.002$) for products D, E and F versus A, B and C (Table II).

Products D, E and F showed a reduction profile of initial inocula similar to controls, whereas products A, B and C showed a significantly lower reduction profile than controls (Figure 1).

**Discussion**

Antiseptics are biocides that destroy or inhibit the growth of micro-organisms on living tissue (e.g. healthcare personnel handwashes). The method most used in Europe to assess the efficacy of hand hygiene agents is the European Standard UNE-EN-1500 using as control the biocidal activity of 2-propanol 60% v/v. Alcohol efficacy depends on the alcohol type and its concentration. Alcohols at concentrations of 60–95% exhibit typically 3.5–5.0 $\log_{10}$ reductions of test bacteria from artificially contaminated hands after 30–60 s of
application, but they do not have appreciably persistent (i.e. residual) activity.\textsuperscript{10,12} For this reason many alcohol products include other biocides, such as chlorhexidine, quaternary ammonium compounds or triclosan, that can remain in the skin following evaporation of alcohol.\textsuperscript{11} In this sense, studies have demonstrated that formulations containing 60–95% alcohol (alone) or 50–95% (when combined with limited amounts of quaternary ammonium compounds, hexachlorophen or chlorhexidine) obtain lowered bacterial counts more effectively than other agents, compensating for the fact that chlorhexidine or quaternary ammonium compounds are less effective against Gram-negative bacteria.\textsuperscript{10} Being conservative is important to obtain the maximum reduction in bacterial content through hand washing to reduce transmission of pathogens in healthcare facilities because the reduction (90%, 99%, 99.9% or 99.99%) needed to reduce transmission is unknown.\textsuperscript{12,13}

This study compares the in-vivo efficacy of different biocides in alcoholic solutions (containing $\geq$65% alcohol) with the biocide 2-butanone peroxide 2% in a 35% alcohol solution, to explore differences in their capability to reduce a $10^6$ cfu/mL initial inoculum of the \textit{E. coli} reference strain after hand rub using the recommended volumes and timings.

Isopropanol 60% obtained reductions of 99.99%, reducing the initial inocula from $10^6$ to <100 cfu/mL, which can conceptually be considered as eradication. Nevertheless products A, B and C obtained significantly lower reductions down to $\sim$500 cfu/mL, despite containing different alcoholic solutions ranging from 65% to 75% with low amounts of biguanidines and/or quaternary ammonium compounds.

Products D and E, with 70–75% alcohol solutions containing higher concentrations of different quaternary ammonium compounds and the biphenol triclosan (in the case of product E), produced similar reductions to isopropanol, with statistically significant higher reductions than products A, B and C.

Product F, the solution of 2-butanone peroxide 2% (Neostex-Plus\textsuperscript{®}), produced the same effect as products D and E with mean reductions of $\sim$4 log\textsubscript{10} (99.99%), reducing the initial inocula from $10^6$ to $\leq$100 cfu/mL. It should be noted that in contrast to products D and E, Neostex-Plus\textsuperscript{®} only contained 35% propanol. This is important in a field where the general assumption is that antimicrobial activity of alcohols is significantly lowered at concentrations below 50%, and optimal in the 60–90% range.\textsuperscript{11}

In addition to the quantitative limitation of the lack of scientific studies establishing which micro-organism counts on hands need to be reduced to minimise pathogen transmission,\textsuperscript{10} there is a qualitative limitation in this study; following Standard UNE-EN-1500 methodology, only the \textit{E. coli} reference strain was used as contaminating bacterium. This does not permit generalisation of in-vivo efficacy against other micro-organisms, and only

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**Table II**  Initial counts (log\textsubscript{10}) and reductions (log\textsubscript{10}) for control and products, and reduction difference (log\textsubscript{10} reduction control – log\textsubscript{10} reduction product)

<table>
<thead>
<tr>
<th>Control (isopropanol 60%)</th>
<th>Study product</th>
<th>Reduction difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial inocula</strong></td>
<td><strong>Reduction</strong></td>
<td><strong>Initial inocula</strong></td>
</tr>
<tr>
<td>6.0 ± 0.5</td>
<td>–4.4 ± 1.5</td>
<td>A</td>
</tr>
<tr>
<td>6.1 ± 0.2</td>
<td>–4.7 ± 1.6</td>
<td>B</td>
</tr>
<tr>
<td>6.2 ± 0.3</td>
<td>–4.7 ± 1.4</td>
<td>C</td>
</tr>
<tr>
<td>6.1 ± 0.1</td>
<td>–4.3 ± 1.6</td>
<td>D</td>
</tr>
<tr>
<td>6.0 ± 0.6</td>
<td>–4.0 ± 1.3</td>
<td>E</td>
</tr>
<tr>
<td>5.8 ± 0.8</td>
<td>–3.8 ± 1.3</td>
<td>F</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Significantly ($P < 0.005$) lower product reductions versus control reductions.
\textsuperscript{b} Significantly ($P < 0.02$) higher differences in log\textsubscript{10} reductions versus control (log\textsubscript{10} reduction control – log\textsubscript{10} reduction product) for D or E or F versus A, or B or C.

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**Figure 1** Differences in log\textsubscript{10} reduction between control (2-propanol 60%) and products (log\textsubscript{10} reduction control – log\textsubscript{10} reduction product).
Antimicrobial efficacy of 2-butanone peroxide in n-propanol

In previous peroxygen compounds 18) together these last three cidal activities were not present well as sporicidal, fungicidal and virucidal activity (these last three cidal activities were not present in previous peroxygen compounds 18) together with favourable results in toxicity tests. 8 By contrast, alcohols/phenols lack sporicidal activity, phenols exhibit little activity against Pseudomonas and moulds, aldehydes lack mycobactericidal activity, anilides exhibit low activity against Gram-negative bacteria and fungi, biguanides (chlorhexidine) do not exhibit sporicidal and mycobactericidal activity or activity against poliovirus, and quaternary ammonium compounds do not exhibit activity against mycobacteria and non-enveloped viruses. 11,19–21

In conclusion, the results of this study show that the solution of 2-butanone peroxide 2% in 35% alcohol offers high in-vivo cidal activity against acquired E. coli transient flora, offering an alternative to products with higher alcohol concentrations.

Acknowledgements

We thank M.J. Giménez (PRISM-AG, Madrid, Spain) for her critical review of the manuscript, and A. Coronado (Neocheical, Madrid, Spain) for his continuous support.

Conflict of interest statement

A.L. is employee of Neocheical S.A. Madrid, Spain. Rest of authors: None to declare.

Funding sources

This study was supported by an unrestricted grant from Neocheical, S.A., Madrid, Spain.

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