The aim of this study was to investigate the antimicrobial activity of currently available topical skin pharmaceuticals against *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*. The agar dilution assay was used to determine the minimal inhibitory concentration for cream formulations and their active substances. Corticosteroid formulations with the antiseptics clioquinol or halquinol were active against all microbes. The hydrogen peroxide formulation was primarily active against staphylococci. Clotrimazole, miconazole and econazole showed an effect against staphylococci in addition to their effect on *C. albicans*.

In contrast, terbinafine had no antibacterial effect. Fusidic acid was active against staphylococci, with slightly weaker activity against *S. pyogenes* and no activity against *C. albicans* or *E. coli*. In summary, some topical skin pharmaceuticals have broad antimicrobial activity *in vitro*, clioquinol and halquinol being the most diverse. In limited superficial skin infection topical treatment can be an alternative to systemic antibiotics and should be considered. With the global threat of multi-resistant bacteria there is a need for new, topical, non-resistance-promoting, antimicrobial preparations for the treatment of skin infections. *Key words: azoles; clioquinol; fusidic acid; halquinol; hydrogen peroxide; skin infection; terbinafine.*

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Skin and soft tissue infections (SSTIs) are common conditions causing considerable morbidity. Microbes such as bacteria and yeasts often co-contribute to these infections. An appealing way of treating superficial skin infections is through the use of topical antimicrobials. Topical treatment delivers a high concentration of a drug to the desired area with limited or no systemic effects. Unfortunately, some topical antibiotics, such as fusidic acid and mupirocin, have proved to be promoters of resistance and restrictive use is advised (1–3). In contrast, topical azoles and allylamines against dermatophyte and yeast infections have been prescribed extensively with less development of resistance (4, 5).

Biocides are broad-spectrum chemical agents that inactivate microbes. When they are used on living tissue they are usually referred to as antiseptics. These agents, for instance chlorhexidine and triclosan, represent another group of topical antimicrobials that are used in modern healthcare and in consumer products. Antiseptics have a broader spectrum of activity than antibiotics and often act on multiple cellular targets (6).

Resistance to antibiotics used in the treatment and prevention of infectious disease in humans and animals is a rapidly increasing global problem. In the past two decades there has been a large number of reports of multi-resistant bacteria causing considerable morbidity and mortality. Antibiotic overuse in humans and animals and dissemination of bacteria through the food chain, trade and human migration has lead to the current, highly worrying, situation. In dermatology, the resistance pattern of *Staphylococcus aureus*, one of the most important skin pathogens, has been of special interest. Methicillin-resistant *S. aureus* (MRSA) have resisted eradication attempts and have spread successfully from hospitals into the community. In the USA and Europe community-associated MRSA (CA-MRSA), first recognized in the mid-1990s, are causing necrotic SSTIs in dermatology out-patients (7–9).

In the late 1990s outbreaks of bullous impetigo among children were observed in Sweden and Norway (10, 11). These outbreaks were caused by a fusidic acid-resistant clone of *S. aureus* (FRSA) and its emergence was associated with an increase in the use of topical fusidic acid. The FRSA has since flourished in several European countries and there are some reports of high frequencies of FRSA in patients with atopic dermatitis (12–16).

Microbes are believed to trigger, exacerbate or sustain inflammatory skin disease, such as atopic dermatitis, seborrhoeic dermatitis and psoriasis (17). Due to this, treatment of skin disease often requires antimicrobial therapy. At the same time it is vital, whenever possible, to limit and carefully target the use of antibiotics. Faced with this dilemma, dermatologists, general practitioners and other healthcare professionals dealing with superficial skin infections need to be aware and make use of the anti-microbial potential of already existing topical skin pharmaceuticals. For instance, it is known...
that some of the azoles, originally developed as antifungals, also have activity against certain Gram-positive bacteria (18). It can also be hypothesized that diols, preservatives and other additives in skin creams may have an antimicrobial effect of their own. Topical skin pharmaceuticals with broad, non-resistance-promoting activity against staphylococci, streptococci, dermatophytes or yeasts can be of great use in dermatology where infections are often mixed.

The aim of this study was to investigate the antimicrobial properties of currently available antibacterial, antifungal and anti-inflammatory topical formulations. We have adopted an unconventional approach whereby we test the formulations in their marketed form and not merely the designated active substance of each formulation. With the agar dilution assay we have determined the minimal inhibitory concentration (MIC) against common skin pathogens for a panel of formulations and substances.

MATERIALS AND METHODS

Topical skin pharmaceuticals

The following skin pharmaceuticals were tested. All formulations were creams.

**Antibiotic formulation.** Fucidin® 2% (fusidic acid) (Leo Pharma, Malmö, Sweden).

**Antifungal formulations.** Canesten® 1% ( clotrimazole) (Bayer, Solna, Sweden), Dakta® 2% ( miconazole) (Janssen-Cilag, Sollentuna, Sweden) and Lamisil® 1% (terbinafine) (Novartis, Täby, Sweden).

**Antifungal formulation with corticosteroid.** Daktacort® (miconazole 20 mg/g + hydrocortisone 10 mg/g) (Janssen-Cilag, Sollentuna, Sweden).

**Antiseptic formulation.** Microcid® 1% (hydrogen peroxide) (Bioglan Pharma, Malmö, Sweden).

**Corticosteroid formulation.** Betnovat® (betamethasone), Emovat® 0.05% (clobetasone butyrate) and Dermovat® 0.05% (clobetasol), all from GlaxoSmithKline, Solna, Sweden and Hydrokortison CCS® 1% (hydrocortisone) (Clear Chemical Sweden, Borlänge, Sweden).

**Antiseptic formulations with antiseptic agent.** Betnovat® with chinoform (betamethasone 1 mg/g + clioquinol 30 mg/g) (GlaxoSmithKline, Solna, Sweden) and Kenacutan® (triamcinolone 1 mg/g + halquinol 7.5 mg/g) (Bristol-Myers Squibb, Bromma, Sweden). Clioquinol is 5-chloro-7-iodo-8-quinolinol and halquinol is a mixture of 5,7-dichloro-, 5-chloro- and 7-chloro-8-quinolins.

Active substances

The following active substances were tested; clioquinol (5-chloro-7-iodo-8-quinolinol), econazole nitrate salt, fusidic acid, ketoconazole and miconazole nitrate salt, all from Sigma-Aldrich, Stockholm, Sweden and terbinafine hydrochloride (Biomol, Immunkemi F & D, Järfalla, Sweden).

Microbes

The following reference strains were used: Candida albicans (CCUG 5594), Escherichia coli (CCUG 24), Staphylococcus aureus (CCUG 17621), Staphylococcus epidermidis (CCUG 39508) and Streptococcus pyogenes (CCUG 4207).

Agar dilution assay

MICs of the formulations and substances were determined by agar dilution assay. Dilution series for each formulation/substance (individual plates for each dilution step) were prepared in blood agar medium (Columbia agar (Oxoid, Basingstoke, UK) + 5% defibrinated horse blood) for S. pyogenes and in Diagnostic Sensitivity Test (DST) agar (Oxoid) for C. albicans, E. coli, S. aureus and S. epidermidis. All dilution series were made with freshly prepared and autoclaved agar that had been allowed to cool to a temperature of 48–50°C in an oven before a formulation or substance was added.

Formulations were weighed and dissolved directly in agar for the concentrations 20% (10 g cream in a final volume of 50 ml agar, weight/volume (w/v)), 10% (w/v), 5% (w/v) and 1% (w/v). For the lower concentrations a stock solution of 1% (0.5 g cream in a final volume of 50 ml agar) was prepared and further diluted with agar to obtain concentrations 0.5–0.0001% (w/v). A pre-warmed oven with a constant temperature of 50°C kept the stock solution at an appropriate temperature during experimental procedures. Final concentration ranges were; Betnovat® 1–20% (w/v), Betnovat® with chinoform 0.005–20% (w/v), Emovat® 1–20% (w/v), Dermovat® 1–20% (w/v), Canesten® 1% 0.001–20% (w/v), Dakta® 2% 0.001–5% (w/v), Daktacort® 0.001–20% (w/v), Fucidin® 2% 0.0001–20% (w/v), Hydrokortison CCS® 1% 1–20% (w/v), Kenacutan® 0.005–20% (w/v), Lamisil® 1% 0.001–20% (w/v) and Microcid® 1% 0.001–20% (w/v).

Substances were dissolved in dimethylformamide (DMF) to prepare a stock solution of 10 mg/ml and this solution was further diluted and mixed with agar. When testing S. pyogenes, the stock solution was instead prepared with ethanol 99.5% (Kemetyl AB, Haninge, Sweden) because DMF was no longer recommended for laboratory use. Final concentration ranges were; clioquinol 0.125–100 µg/ml, econazole nitrate salt 1.0–50 µg/ml, fusidic acid 0.03125–25 µg/ml, ketoconazole 0.125–100 µg/ml, miconazole nitrate salt 0.125–100 µg/ml and terbinafine hydrochloride 0.5–50 µg/ml. The MICs against all microbes for DMF (range tested 0.625–10% (volume/volume = v/v)) or ethanol (range tested 0.625–10% (v/v)) were also determined to ensure that antimicrobial effect could not be attributed to the solvent for the active substances.

Standard Petri dishes (92 × 16 mm (diameter × height)) were used and the agar volume in each plate was 25 ml. After the agar was poured, plates were allowed to cool and were then used immediately. There were duplicate plates for all dilution steps.

Bacterial colonies from blood agar plates were used for prepa-ration of the inoculums. C. albicans was grown on Sabauroud’s agar medium. Colonies were diluted in phosphate-buffered saline (PBS) and suspensions were adjusted to approximately 2 × 10^6 colony forming units (CFU)/ml for C. albicans, 2 × 10^4 CFU/ml for E. coli, 2 × 10^1 CFU/ml for S. aureus, 8 × 10^2 CFU/ml for S. epidermidis and 6 × 10^2 CFU/ml for S. pyogenes using a turbidimeter (DEN-1 McFarland Densitometer, Biosan). The surface of the agar plates were inoculated with a 20 µl spot using a replicator and incubated at 37°C for 24 h. The inoculum size for each microbe was chosen to allow sufficient growth in the absence of formulation/substance within the incubation period. MIC was the lowest concentration of the formulation/substance inhibiting growth determined by visual inspection. MIC for the active substance as part of a cream was calculated as the concentration of active substance in the cream multiplied by the MIC for the entire cream.
Antimicrobial activity of skin pharmaceuticals

RESULTS

Antibiotic formulation and substance

The MICs for Fucidin® 2% cream (fusidic acid) and fusidic acid are presented in Table I. The strongest activity was seen against *S. aureus* and *S. epidermidis* (MIC 0.1 µg/ml for fusidic acid as part of the cream or alone and 5 µg/ml for the entire cream), but there was also an effect on *S. pyogenes* (MIC 10 µg/ml for fusidic acid as part of the cream, 6.25 µg/ml for fusidic acid alone and 500 µg/ml for the entire cream). Fusidic acid did not inhibit growth of *C. albicans* or *E. coli*.

Antifungal formulations and substances

Clotrimazole as part of Canesten® 1% and miconazole nitrate, econazole nitrate and terbinafine hydrochloride were effective against *C. albicans* (MICs 3.125–25 µg/ml).

Miconazole in Daktar® 2% and Daktacort® had a high MIC against *C. albicans* (1000 µg/ml for miconazole as part of the cream, 50,000 µg/ml for the entire cream) compared with miconazole nitrate alone (MIC 3.125 µg/ml). The same pattern was noted for terbinafine in Lamisil® 1% (MIC 2000 µg/ml for terbinafine as part of the cream, 200,000 µg/ml for the entire cream) and terbinafine hydrochloride alone (MIC 25 µg/ml).

Clotrimazole, miconazole and econazole nitrate showed an effect against staphylococci (MIC 1.0 µg/ml for clotrimazole and miconazole as part of creams, MIC 1.5625 µg/ml for miconazole nitrate alone and MIC 1.0 µg/ml for econazole nitrate alone).

Activity against *S. pyogenes* was variable. Lamisil® 1% had the strongest effect, with a MIC of 10 µg/ml against *S. pyogenes*, but terbinafine hydrochloride alone did not have any effect.

There was no effect on *E. coli* for any of the antifungal formulations or substances (Table II).

Antiseptic formulation

The antiseptic cream Microcid® 1% (hydrogen peroxide) had effect primarily on *S. aureus* (MIC 1.0 µg/ml for hydrogen peroxide as part of the cream and 100 µg/ml for the entire cream) and *S. epidermidis* (MIC 0.5 µg/ml for hydrogen peroxide as part of the cream and 50 µg/ml for the entire cream) with weaker activity against *E. coli* and *S. pyogenes* and even less activity against *C. albicans* (Table III).

Corticosteroid formulations

The corticosteroid creams showed high MICs against all microbes. In some cases there was still growth of microbes on agar plates with 20% (w/v) of cream as illustrated by Table IV.

| Table I. Minimal inhibitory concentrations (MICs) for antibiotic cream and substance. MIC expressed as µg/ml of active substance as part of cream (ASPC), entire cream (EC) and active substance alone (AS) |
|---|---|---|---|---|---|---|---|---|---|---|
| | C. albicans | E. coli | S. aureus | S. epidermidis | S. pyogenes |
| | ASPC/AS | EC | ASPC/AS | EC | ASPC/AS | EC | ASPC/AS | EC |
| Fucidin® 2% (fusidic acid) | No effect | No effect | No effect | No effect | 0.1 | 5.0 | 0.1 | 5.0 | 10 | 500 |
| Fusidic acid | No effect | – | No effect | – | 0.13 | – | 0.13 | – | 6.3 | – |

MIC of active substance as part of cream (ASPC) is calculated as the concentration of active substance in the cream multiplied by MIC for the entire cream.

| Table II. Minimal inhibitory concentrations (MICs) for antifungal creams and substances. MIC expressed as µg/ml of active substance as part of cream (ASPC), entire cream (EC) and active substance alone (AS) |
|---|---|---|---|---|---|---|---|---|
| | C. albicans | E. coli | S. aureus | S. epidermidis | S. pyogenes |
| | ASPC/AS | EC | ASPC/AS | EC | ASPC/AS | EC | ASPC/AS |
| Canesten® 1% (clotrimazole) | 10 | 1000 | No effect | No effect | 1.0 | 100 | 1.0 | 100 | 500 | 50,000 |
| Daktar® 2% (miconazole) | 1000 | 50,000 | No effect | No effect | 1.0 | 50 | 1.0 | 50 | 1000 | 50,000 |
| Daktacort® (miconazole) | 1000 | 50,000 | 4000 | 200,000 | 1.0 | 50 | 1.0 | 50 | 1000 | 50,000 |
| Miconazole nitrate | 3.1 | – | – | – | 1.6 | – | 1.6 | – | 25 | – |
| Lamisil® 1% (terbinafine) | 2000 | 200,000 | No effect | No effect | No effect | No effect | 2000 | 200,000 | 10 | 1000 |
| Terbinafine HCl | 25 | – | – | – | No effect | – | No effect | – | No effect | – |
| Econazole nitrate | 3.1 | – | – | – | 1.0 | – | 1.0 | – | 25 | – |
| Ketoconazole | 3.1 | – | – | – | 25 | – | 13 | – | – |

MIC of active substance as part of cream (ASPC) is calculated as the concentration of active substance in the cream multiplied by MIC for the entire cream.

MIC 50 µg/ml. At 50 µg/ml of miconazole nitrate, terbinafine HCl, econazole nitrate and ketoconazole there is 5% DMF in agar. MIC for DMF against *E. coli* is 5%.

MIC 50 µg/ml. At 50 µg/ml of ketoconazole there is 5% ethanol in agar. MIC for ethanol against *S. pyogenes* is 5%.

*Acta Derm Venereol 90*
Determined.

activity against all microbes with the exception of chinoform (betamethasone with clioquinol) had similar true effect of clioquinol against S. pyogenes there was 5% ethanol in the agar and no growth of alone had comparable effects. At 50 µg/ml of clioquinol for clioquinol as part of the cream). Clioquinol tested (MIC 15 µg/ml and 150 µg/ml, respectively, for clioquinol as part of the cream). Clioquinol tested alone had comparable effects. At 50 µg/ml of clioquinol no growth of S. pyogenes was seen. However, at 50 µg/ml of clioquinol there was 5% ethanol in the agar and MIC for ethanol against S. pyogenes was 5%, thus the true effect of clioquinol against S. pyogenes could not be determined.

Kenacutan® (triamcinolone with halquinol) was similarly effective against all microbes (MIC 7.5 µg/ml for halquinol as part of the cream) except S. pyogenes. MIC of Kenacutan® against S. pyogenes was not determined because this cream was discontinued during the study.

**DISCUSSION**

We have investigated the in vitro activity of a number of widely used topical skin pharmaceuticals. The choice of formulations was not limited to those marketed as antimicrobials but also included topical corticosteroids.

For the purpose of this study we chose the agar dilution assay, which is a standard method in Swedish susceptibility testing (19). The method is extensively used in our research laboratory and is carefully standardized. The agar dilution assay allows for the testing of entire formulations that can be dissolved easily in the agar. When dissolving a cream formulation in agar the turbidity is affected when concentrations are high. Despite this, bacterial or yeast colonies are still easily recognizable when present on the plates and MICs for derivatives or other additives in these formulations.

out antiseptic additives had weak or no antimicrobial activity at all in the ranges tested, but it was important to explore any potential antimicrobial effect of preservatives or other additives in these formulations.

The hydrogen peroxide cream Microcid® showed activity against S. aureus and S. epidermidis, but less so against S. pyogenes and E. coli and the effect was even weaker against C. albicans. However, the highest MIC for hydrogen peroxide was 500 µg/ml (against
C. albicans), which is equivalent to 50,000 µg/ml or 0.05 g/ml of Microcid® cream. A concentration of this magnitude can possibly be reached in vivo by applying a relatively modest amount of cream.

Fusidic acid was effective against S. aureus, S. epidermidis and S. pyogenes, whereas E. coli and C. albicans were unaffected. Fusidic acid is primarily an anti-staphylococcal drug, but activity against streptococci has been described in vitro. Leclercq et al. (22) obtained MIC levels ranging from 8 µg/ml to 16 µg/ml (depending on the type of medium used) for fusidic acid when 242 strains of streptococci isolated from skin and soft tissue infections were evaluated. However, the spread of a fusidic acid-resistant clone of S. aureus (FRSA) causing bullous impetigo in a number of European countries in recent years argues strongly against the use of topical fusidic acid (10, 16, 23–25).

It is known that members of the azole class of antifungal drugs possess antibacterial properties against some Gram-positive bacteria (26, 27). Clotrimazole and econazole have been shown to be effective against Mycobacterium smegmatis and Streptomyces strains in vitro (28). These bacteria contain P450 mono-oxygenases (P450s), some of which are homologues to 14α-sterol demethylase, the target enzyme of azoles. In contrast to their effect against staphylococci, both as part of formulations and as single substances. It can be speculated that this effect was mediated through 14α-sterol demethylase homologues in staphylococci. In contrast to their effect against staphylococci, clotrimazole and miconazole in formulations were much less effective against S. pyogenes (MIC 500–1000 µg/ml). MIC for miconazole nitrate against S. pyogenes was, however, 25 µg/ml. It is difficult to assess whether MICs in the range of 500–1000 µg/ml represent any clinically relevant antimicrobial effect. In vivo effects cannot be ruled out, because cream application on the skin theoretically generates relatively high local concentrations of the active substance; however, this is difficult to measure.

Interestingly, the effect of miconazole against C. albicans as part of Daktar® or Daktacort® was markedly different from the effect of miconazole nitrate tested as a single substance. The MIC for miconazole in Daktar® and Daktacort® against C. albicans was surprisingly high (1000 µg/ml), but only 3.125 µg/ml for miconazole nitrate. The reason for this is not evident (29). Raab & Gmeiner (30) have reported on the protective action of glucocorticoids in high concentrations against econazole nitrate in yeasts but not in staphylococci in vitro. This may explain the higher MIC for miconazole in Daktacort®, which is a combination of miconazole and hydrocortisone, but not the MIC of Daktar®, which does not contain any glucocorticoid. Similarly, terbinaf none as part of Lamisil® 1% cream exhibited MIC 2000 µg/ml against C. albicans, while terbinafine hydrochloride alone had a MIC of 25 µg/ml. It can be speculated that conditions in the in vitro situation, such as interactions between cream and agar components, can account for this discrepancy.

One problem with this and other MIC studies is the difficulty of interpreting and applying in vivo data to the in vitro situation. Specifically, it is hard to transform a MIC value in µg/ml obtained with the agar dilution assay into suggestions on the amount of cream needed to be applied in order to reach a similar concentration on the skin. Even if such a transformation was easily made it is important to keep in mind that the in vitro and in vivo environments can differ greatly with regards to several factors such as pH, salt concentrations and temperature. The MIC value of a formulation has to be viewed in relation to the MIC values of other formulations tested with the same assay and, most importantly, to clinical experience.

One aim of this investigation was to test whether base components of a formulation had additive or synergistic effects with the active substance of that formulation. Our data does not support the presence of such effects. MIC values for the active substance as part of a formulation and for the same substance tested separately were roughly the same, with the exception of the discrepancies for miconazole and terbinaf none discussed above.

This study adds in vitro MIC data for currently available topical antimicrobial and anti-inflammatory skin pharmaceuticals. The data can be of importance in the management of superficial skin infections and in the

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**Table V. MICs for corticosteroid creams with antiseptic agent. MIC expressed as µg/ml of active substance as part of cream (ASPC)*, entire cream (EC) and active substance alone (AS)**

<table>
<thead>
<tr>
<th></th>
<th>C. albicans</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>S. epidermidis</th>
<th>S. pyogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASPC/AS</td>
<td>EC</td>
<td>ASPC/AS</td>
<td>ASPC/AS</td>
<td>ASPC/AS</td>
</tr>
<tr>
<td>Betnovat® with chinoform (cloquinol)</td>
<td>15</td>
<td>500</td>
<td>15</td>
<td>500</td>
<td>15</td>
</tr>
<tr>
<td>Clioquinol</td>
<td>6.3</td>
<td>–</td>
<td>13</td>
<td>–</td>
<td>6.3</td>
</tr>
<tr>
<td>Kenacutan® (halquinol)</td>
<td>7.5</td>
<td>1000</td>
<td>7.5</td>
<td>1000</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*Not investigated because Kenacutan® was taken off the market during this work.
pursuit of limiting the use of systemic antibiotics. It also highlights the need for new, topical, non-resistance promoting, antimicrobial treatment alternatives for skin infections. Recently, a novel pleuromutilin antibiotic, retapamulin, primarily active against staphylococci and streptococci, has been introduced for topical treatment of impetigo. So far there are few reports of resistance to retapamulin, which inhibits protein synthesis through interaction with the 50S ribosomal subunit and is structurally distinct from other ribosomal inhibitors (31–33).

Antiseptics have broad antimicrobial effects and can be suitable candidates for novel drugs. We have previously explored the antimicrobial properties of pentane-1,5-diol in vitro and in vivo with promising results (34–37). Since antiseptics usually target several different cellular mechanisms they are less likely to promote resistance, although it is important to note that there are reports of resistance development (6).

In view of increasing antibiotic resistance the development of new antiseptic and antibiotic topical preparations is an important task for the future. Responsible use of existing and future formulations is essential for the successful management of skin infections.

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