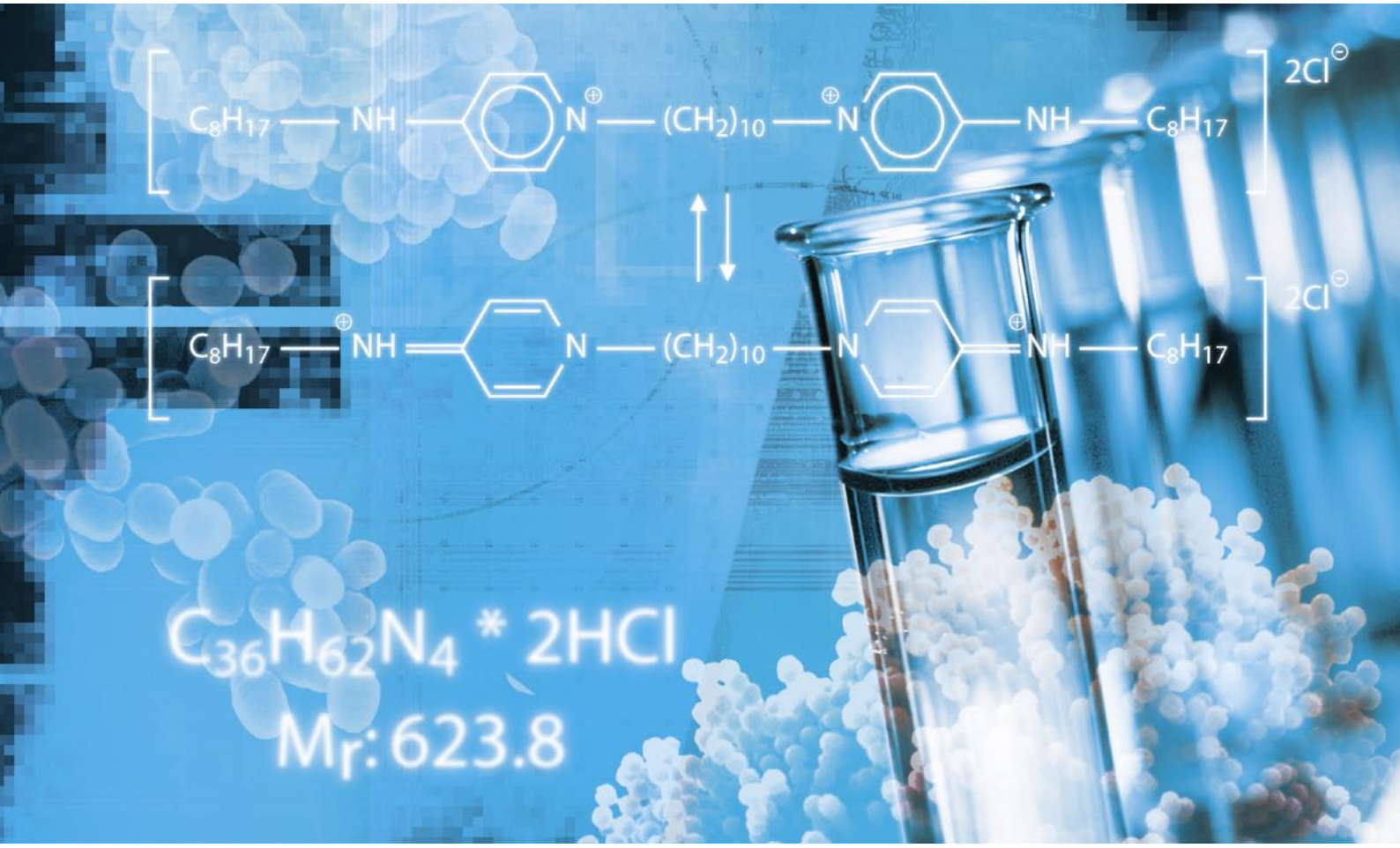

Octenidine dihydrochloride

A new topical antimicrobial for local treatment of skin, mucous membranes and wounds

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Properties, Efficacy and Tolerance

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1 Summary

Topical antimicrobial agents are typically used for skin, mucous membrane and open wound decontamination.
Due to its specific capability to adhere and form complexes with chemical cell components and whole cells along with its high antimicrobial efficacy, octenidine dihydrochloride (octenidine) may be considered as a unique antimicrobial agent exerting its activity through non-cytotoxic complexes on the site of action. Therefore, octenidine may not be considered as a typical antiseptic agent such as chlorhexidine or PVP-iodine.

Octenidine shows properties of positively charged (cation-active) chemical compounds. However in contrast to chlorhexidine and benzalkonium chloride it belongs to a completely different class of chemicals: the bispyridinamines. Therefore, octenidine displays unique properties not common to other cationic antiseptic substances.

Octenidine exhibits a broad spectrum of antimicrobial efficacy against Gram-positive and Gram-negative bacteria and fungi. The efficacy is not adversely affected by interfering substances (e.g. blood, mucous). Octenidine shows a strong residual effect on skin which can still be observed 24 hr after application. It has been shown that mechanisms known to cause resistance to other cation active antimicrobials do not apply to octenidine.

Toxicologically, octenidine has been extensively characterised and poses no risk in terms of acute, subchronic and chronic toxicity, carcinogenicity, reproduction, irritation and allergenicity.

Octenidine is not absorbed through intact or broken skin and therefore is not systemically available.

Due to its excellent antimicrobial efficacy and tissue compatibility, octenidine may be used for different topical applications where fast action and a long lasting effect are required. Examples for such applications are the decontamination of skin of patients colonised with MRSA, the treatment of acute and chronic wounds heavily colonised or locally infected by pathogenic bacteria and the care of central venous catheter insertion sites.

With octenidine a highly effective and compatible innovative antimicrobial compound for topical use is available.

2.1 History

The use of disinfectants and antiseptics preceded the understanding of their action, and seems to have arisen from the observation that certain substances stopped putrefaction of meat or rotting of wood. John Pringle (1707 – 1782) apparently first used the term „antiseptic“ (Greek for „against putrefaction“) in 1750 to describe substances that prevent putrefaction. The idea was eventually applied to the treatment of suppurating wounds. Mercuric chloride was used by Arabian physicians in the Middle Ages for preventing sepsis in open wounds.

However, it was not until the nineteenth century that antiseptics came into general use in medicine. Chlorinated soda, essentially hypochlorite, was introduced in 1825 for the treatment of infected wounds, and the tincture of iodine was first introduced in 1839. These pioneer attempts at antisepsis were not generally accepted until Pasteur (1822 – 1895) published his article in 1863 on the microbial origin of putrefaction. This led to an understanding of the origin of infection and provided the rationale for its prevention. As so often in the history of medicine a change of practice depended on the persistence of one man.

For antiseptics, this man was John Lister (1827 – 1912). He chose phenol and applied it vigorously in surgery. A 2.5% solution was used for dressing wounds and twice that concentration for sterilizing instruments. The effect of Lister's practices was revolutionary and the antiseptic technique opened the way to great surgical advances. Even at this time around 1870, the use of antiseptics was still empirical.

Since that time, antiseptics have seen steady but unspectacular improvement. Many of the traditional antiseptics have had continued use in refined forms. The phenols have been modified and made more acceptable for general use, nevertheless local intolerance and systemic side effects were common and resulted in serious draw backs.

Acriflavine, introduced in 1913, was the first of a number of basic antiseptics. It has had many years of use but has been largely displaced in the last three decades by colourless cationic antiseptics with a broad spectrum of antimicrobial efficacy and low toxicity.

Alcohols (i.e. ethanol, 1-propanol, 2-propanol) in high concentrations are the agents of choice for fast action on intact skin but are not qualified for the use on mucous membranes or wounds due to their potential for irritation.

2.2 Rationale and indications of local antimicrobial antisepsis

Colonisation of skin

The average adult has a skin area of approximately 1.75 m^2. The superficial part of the skin, the epidermis, has five layers with the stratum corneum being the outermost layer (which itself is made of up to 15 layers). With this structure the skin differs from mucosa in several aspects. Table 1 summarises the principle differences between skin and mucosa.

Table 1: Comparison of skin and mucosa

	Skin	Mucosa
Histology	keratinised squamous epithelium	non-keratinised squamous epithelium
Moisture	dry	mucous exudation
Microbiology	low microbial density	high microbial density
Absorption Capacity	low	high
Sensitivity	low	pronounced

Sources of wound infections

Surface tissues such as skin and mucous membranes, which are constantly in open contact with the environment are colonised by many different microbial strains and species. The mixture of these organisms regularly found at any episomatic site is referred to as the resident flora.

The resident flora is exceedingly complex and consists of more than 200 species of bacteria, and a few eukaryotic fungi. The main representatives and the qualitative distribution of the bacterial flora of humans are shown in Table 2 (Larson 2001).

Table 2: Bacteria commonly found on the surface of the human body

Bacterium	Skin	Lower intestine	Vagina
Staphylococcus epidermidis	++	+	++
Staphylococcus aureus	+	++	+
Streptococcus mitis	-	+/-	+
Enterococcus faecalis	-	++	+
Streptococcus pneumoniae	-	-	+/-
Streptococcus pyogenes	+/-	+/-	+/-
Neisseria spp.	-	-	+
Neisseria meningitidis	-	-	+
Veillonella spp.	-	+/-	-
Enterobacteriaceae (Escherichia coli)	-	++	+
Proteus spp.	-	+	+
Pseudomonas aeruginosa	-	+	-
Bacteroides spp.	-	++	+/-
Bifidobacterium bifidum	-	++	-
Lactobacillus spp.	-	++	++
Clostridium spp.	-	++	-
Clostridium tetani	-	+/-	-
Corynebacteria	++	+	+
Mycobacteria	+	+	-
Spirochetes	-	++	-
Mycoplasmas	-	+	+

Abbr.		
	+	occasional
	++	common
	-	absent

Pathogenic microorganisms

As long as the skin or mucosa is intact and the human being has their full immunocompetence, infections will rarely occur, although some of the bacteria listed in Table 2 are pathogens or opportunistic pathogens.
Since the colonisation density of microorganisms on mucosa is several-fold higher than those on skin, even small injuries might induce bacterial infections caused by the physiological (resident) skin flora or by environmental (transient) flora (Larson 2001).

- **Staphylococcus aureus** is a potential pathogen and the leading cause of bacterial disease in humans.
- **Streptococcus pneumoniae** causes about 95 % of all bacterial pneumonia.
- **Neisseria meningitidis** is an important cause of bacterial meningitis.
- **Escherichia coli** can cause intestinal infections, urinary tract infections and neo-natal meningitis.
- **Pseudomonas aeruginosa**, an opportunistic pathogen can invade virtually any tissue and is the leading cause of hospital-acquired (nosocomial) Gram-negative infections.
- Potential wound pathogens are the Gram-positive β-haemolytic streptococci (most common: **Streptococcus pyogenes**), **Enterococcus faecalis**, multi-resistant **S. aureus**, the Gram-negative **P. aeruginosa**, **Enterobacter spp.**, **E. coli**, **Klebsiella spp.**, **Proteus spp.**, the anaerobes **Bacteroides spp.** and **Clostridium spp.**, and the fungi **Candida albicans** (and **non-C. albicans**) and **Aspergillus spp.**

Antimicrobial substances should effectively kill all relevant bacteria and fungi within a short contact time without irritating the skin or mucosa and without showing any systemic toxicity in the case of resorption.

The goal of any topical antimicrobial treatment is to:

- Prophylactically reduce the risk of an undesired colonisation, and thus reducing the risk of infection, in situations when an increased risk has to be postulated. This includes e.g. catheterisation of the bladder, surgically exposed or opened areas of, or, within the body, including wounds.
- Prophylactically reduce the risk of transmission of pathogens, e.g. protection of neonates against infections by pathogens which may colonise the birth canal during delivery (**Candida spp.**, **group-B streptococci**, but also **HIV** or **Herpes genitalis** virus) or transmission of Methicillin resistant **S. aureus** (MRSA) by wounds colonised with these antibiotic-resistant bacteria.
- In addition to these more prophylactic or preventive applications, antimicrobial substances may also be used as local therapeutic agents to treat symptoms induced e.g. by local bacterial wound infections, thus avoiding potential side effects by systemic antibiotics.

Due to the unspecific mode of action of local antimicrobial agents, a selection of resistant pathogens in comparison to antibiotics is highly unlikely.

2.3 Properties of antiseptics

Definition of antiseptics

An antiseptic is a substance that prevents or arrests the growth or action of microorganisms either by inhibiting their activity or by destroying them. The term is used especially for preparations applied topically to living tissue.

The legal definition (Federal Food, Drug, and Cosmetic Act, 1938) states:
"The representation of a drug in its labelling as an antiseptic shall be considered to be a representation that it is a germicide, except in the case of a drug purporting to be, or represented as, an antiseptic for inhibitory use as a wet dressing, ointment, dusting powder, or other such use as involves prolonged contact with the body."

Requirements

Given the partial bad reputation from history antiseptics should fulfil the following requirements:

A Efficacy
- broad-spectrum antimicrobial efficacy
- fast onset of effect
- organic substances (e.g. blood, proteins, mucin) should not exhibit a negative influence on efficacy
- residual effect after application
- no induction of bacterial resistance

B Tolerance
- toxicologically safe also when used repeatedly or long term
- no local irritation, particularly no impairment of wound healing
- no or low sensitisation potential
- low percutaneous absorption or no systemic side-effects

Mode of action of cation-active antimicrobials

- Antiseptics bind readily to bacteria; the amount absorbed depends on the concentration of active ingredient in the solution. The most important site of absorption for cation-active compounds is the cytoplasmic membrane.

- The extent of killing the bacteria is governed by three principal factors: (a) concentration of the antiseptic, (b) bacterial cell density, and (c) time of contact. The absorption of a given amount of the compound per cell leads to killing a definite fraction of the bacterial population in a chosen time interval.

The essential characteristic of antiseptics compared to antibiotics is their unspecific microbiocidal action. The compound usually penetrates the cell and brings about extensive ill-defined disruption of normal cellular functions (Block 2001).

2.4 Active principle of octenidine dihydrochloride

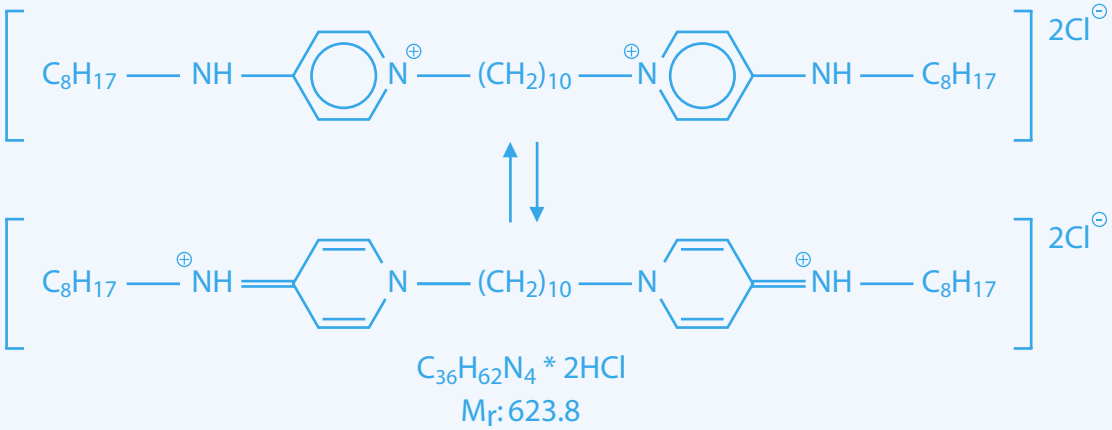
N,N'-(1,10 decanediyldi-1[4H]-pyridinyl-4-ylidene) bis-(1-octanamine) dihydrochloride (= octenidine dihydrochloride): mol. wt. 623.8, CAS number 70775-75-6 is a chemical substance with two cation active centres in its molecule not interacting with each other due to the fact that they are separated by a long aliphatic hydrocarbon chain (10 CH_2 groups).

Octenidine was developed approximately 20 years ago. As a cation-active substance octenidine dihydrochloride (octenidine) binds readily to the negatively charged bacterial cell envelope, consequentially disrupting the vital functions of the cell membrane and killing the cell. Preliminary results point to a strong adherence particularly to lipid components (e.g. cardiolipin) prominent in bacterial cell membranes explaining the high antimicrobial efficacy without adversely affecting human epithelia or wound tissue (unpublished).

Recently, it has been shown that cells of **S. aureus** display decreased net negative surface charge by esterification of the teichoic acid with D-alanine which is a pH-dependent reaction. This modification of the teichoic acid resulted in higher resistance to human cationic antimicrobial peptides. Antimicrobial activity of octenidine, benzalkoniumchloride, and chlorhexidine was tested in quantitative suspension tests at different concentrations with **S. aureus** ATCC 6538, the mutant strain **S. aureus** AG1 lacking the dltA gene, the isogenic wild type Sa 113, **Kocuria rhizophila** DSM 348, and **Enterococcus faecium** ATCC 6057 pre-cultured in TSB at pH 5 and 8 for 18 h at 36 °C. The teichoic acids of the mutant AG1 lacked D-alanine, as a result of which the cells displayed an increased negative surface charge. Live/dead staining was performed after treating staphylococcal cells with octenidine to investigate the effect of octenidine on the cell membrane.

The results obtained by quantitative suspension tests showed clearly that the cells of all Gram-positive species pre-cultured at pH 5 were more resistant than cells grown at pH 8. At the lowest concentration of octenidine and benzalkoniumchloride tested, a 5 log drop in cell viability was already achieved within 1 minute if the cells had been pre-cultured at pH 8. In the case of pre-culturing the cells at pH 5, a similar loss of viability could not be obtained even after 10 minutes. The difference became smaller or did not exist at increasing concentrations of active ingredients or with longer contact times at a given concentration. The susceptibility of the dlt mutant AG1 of **S. aureus** to octenidine was not affected by growing the cells at pH 5 in contrast to the isogenic wild type.

This data shows that the amount of the net negative charge of Gram-positive cell walls has an impact on the antimicrobial activity of cationic substances but does not prevent its penetration through the peptidoglycan layer or the damage to the cell membrane as demonstrated with octenidine (Goroncy-Bermes 2006).



Due to this structure, octenidine differs explicitly from quaternary ammonium compounds e.g. benzalkonium chloride and bisguanidines e.g. chlorhexidine. Amide or ester structures are not part of the molecule. Therefore, octenidine is stable under different physical and chemical conditions and not prone to hydrolysis.

Stability tests have shown that octenidine is stable in a pH range of 1.6 to 12.2. It is not sensitive to light and can be sterilised in aqueous solution up to 130 °C without loosing its integrity or decreasing its efficacy (Harke 1989).

4 Antimicrobial efficacy

4.1 Immediate effect in different applications

Numerous studies indicate that octenidine exhibits a broad spectrum of antimicrobial activity against a variety of Gram-positive and Gram-negative bacteria (Bailey et al 1984, Sedlock and Bailey 1985) and that this compound is also effective against plaque-producing organisms such as **Actinomyces viscosus**, **Actinomyces naeslundii**, **Streptococcus mutans** and **S. sanguis** (Slee and O'Connor 1983).
Octenidine shows greater effectiveness as an inhibitor to plaque-forming enzymes of **S. mutans** than chlorhexidine or alexidine (Bailey et al 1984).

The antimicrobial action of octenidine against plaque-forming and other microorganisms has been examined and compared to that of other antimicrobial agents.
The MIC-values of octenidine in comparison to chlorhexidine and alexidine for different facultativly pathogenic bacteria are presented in Tab. 3.

Table 3: Antibacterial minimum inhibitory concentration (MIC) of octenidine [µg/ml] (data from Bailey et al 1984)

	Staphylococcus aureus	Escherichia coli	Klebsiella pneumoniae	Proteus mirabilis	Pseudomonas aeruginosa
Octenidine	1.0	1.0	2.0	2.0	3.9
Chlorhexidine	0.2	0.5	3.9	15.6	15.6
Alexidine	0.5	2.0	3.9	31.3	15.6

In animal studies octenidine was generally found to be more effective than chlorhexidine in producing plaque reduction (Emilson et al 1981, Shern et al 1985).
In clinical trials octenidine has been shown to prevent plaque formation in humans over a seven-day period (Patters et al 1983) and significantly inhibited the development of plaque and gingivitis when used as the only means of oral hygiene for 21 days (Patters et al 1986).

The MIC-values of octenidine for different yeast strains (**Candida albicans**, **C. tropicalis**, **C. pseudotropicalis**) have been shown to be between 1.5 and 3.0 µg/ml and therefore in the range of those for bacteria (Ghannoum et al 1990). The same low concentrations of octenidine held true for MIC values to different fungi (Tab. 4). The MIC values are much lower (approx. 10-fold) than those for chlorhexidine (Harke 1989).

Table 4: Growth inhibiting effect against fungi in a serial dilution test. MIC values in %. + : growth, - : no growth (Harke 1989)

Conc (%)	0.0001	0.001	0.001	0.005	0.01	0.05
	Octenidine		Chlorhexidine			
Candida albicans	+	-	+	-	-	-
Trichophyton mentagrophytes	+	-	+	+	+	-
Microsporum gypseum	+	-	+	+	-	-
Epidermophyton floccosum	+	-	+	-	-	-

Octenidine at a concentration of 1.5 mM (= 0.94 µg/ml) (in vitro) produced a reduction > 99 % in survival tests of the following organisms following a 15 minute exposure: **S. epidermidis**, **S. aureus**, **P. mirabilis**, **S. pyogenes**, **K. pneumoniae**, **E. coli**, **P. aeruguinosa** and **C. albicans**. **S. epidermidis** was found to be the most susceptible of the test organisms, and **E. coli** and **C. albicans** were the least susceptible. In addition, octenidine was more active than chlorhexidine against each test strain (Sedlock and Bailey 1985).

These results were confirmed by Harke (1989) who showed that the concentration of octenidine necessary to achieve complete inactivation of bacteria and yeasts is (as already shown for MIC values of fungi) much lower (approx. 10-fold) than for chlorhexidine (Tab. 5).

Table 5: Microbiocidal effect in qualitative suspension test of octenidine in comparison to chlorhexidine after 5 min and 60 min contact time, effective concentration in % (Harke 1989)

	Octenidine (%)		Chlorhexidine (%)	
	5 min	60 min	5 min	60 min
S. aureus	0.025	0.005*	> 0.2	0.1
E. coli	0.025	0.005*	0.1	0.025
P. mirabilis	0.025	0.01	> 0.2	0.2
P. aeruginosa	0.025	0.005*	> 0.2	0.1
C. albicans	0.01	0.005*	0.025*	0.025*

* lower concentration was not tested

Studies which examined the skin decontamination capacity of octenidine indicate that it is effective against the common nosocomial pathogens found on the skin. The activity of this compound was found to be comparable or superior to that of various commercially available skin decontamination agents (Sedlock and Bailey 1985, Sedlock et al 1984).

No loss of antiseptic efficacy in presence of interfering substances

The antiseptic effects of four commercial antiseptics and hydrogen peroxide 3 % were compared using the test organisms **S. aureus**, **E. faecium**, **P. aeruginosa**, and **E. coli** in the quantitative suspension test system without protein load as well as in presence of different combinations of interfering substances (i.e. blood, protein, mucin) with contact times up to 10 min. Against all tested organisms, the preparation containing octenidine reached log reductions of > 6.0 within 30 s in absence of interfering substances.
In the presence of interfering substances a log reduction of > 5 was observed after 1 min of contact time, indicating the pronounced antiseptic efficacy of octenidine against relevant pathogens in vitro in combination with interfering substances relevant in wounds or on mucous membranes. The antiseptic efficacies of chlorhexidine, PVP-iodine and octenidine were comparable independent of a protein load, while antiseptics containing cetylpyridinium chloride, and even more hydrogen peroxide, were much less effective in vitro (Pitten et al 2003).

Table 6: Comparison of the antimicrobial effects of a preparation of 0.1 % octenidine and 2 % 2-phenoxyethanol against PVP-iodine (10 %) in the quantitative suspension in vitro

Interfering substance	P. aeruginosa		E.coli		S. aureus		Enterococcus faecium	
	Octenidine	PVP-I	Octenidine	PVP-I	Octenidine	PVP-I	Octenidine	PVP-I
none	+	+	+	+	+	+	+	+
sheep blood, 10 %	+	+	+	+	+	+	+	+
bovine albumine, 10 %	+	+	+	+	+	-	+	-
mucin, 1 %	+	+	+	+	+	+	+	-
combination[A]	+	+	+	+	+	-	+	+

All assays were performed using a contact time of 1 min;
[A]: sheep blood 4.5 %, bovine albumin 4.5 %, mucin 1 %
+: mean log reduction: > 5.0;
-: mean log reduction: ≤ 5.0
(modified according to Pitten et al 2003)

4.2 Residual effect

Octenidine long lasting antimicrobial effect on skin

Investigating the efficacy of two commercially available, alcohol based antiseptic solutions (one containing octenidine) in decontaminating the skin insertion site of central venous catheters (CVC), the octenidine/propanol preparation was more effective than alcohol alone in reducing microflora of the skin at the CVC insertion site over a 24 hr period (Dettenkofer et al 2002).

The long-lasting antiseptic effect of octenidine (0.1 %) has been investigated in n = 62 consecutive patients needing central venous catheterisation. 135 CVCs were inserted during the study period (2,462 catheter days). Prior to dressing changes (at least once weekly) octenidine was used for antisepsis of the skin surrounding the insertion site (at least one minute contact time).

At baseline and in weekly intervals prior to disinfection with octenidine, skin cultures from the skin surrounding the catheter entry site were taken. Patients stayed catheterised for a mean duration of 19.1 days with mean catheter-days of 2.5. Fifty-seven of 433 quantitative skin cultures performed showed growth of microorganisms. Fig. 1 shows the distribution of positive cultures and the mean colony skin counts during placement of the CVCs. The application of octenidine resulted in a substantial decline in bacterial density of the skin at the catheter insertion site over time. After two weeks most cultures became negative (Tietz et al 2005).

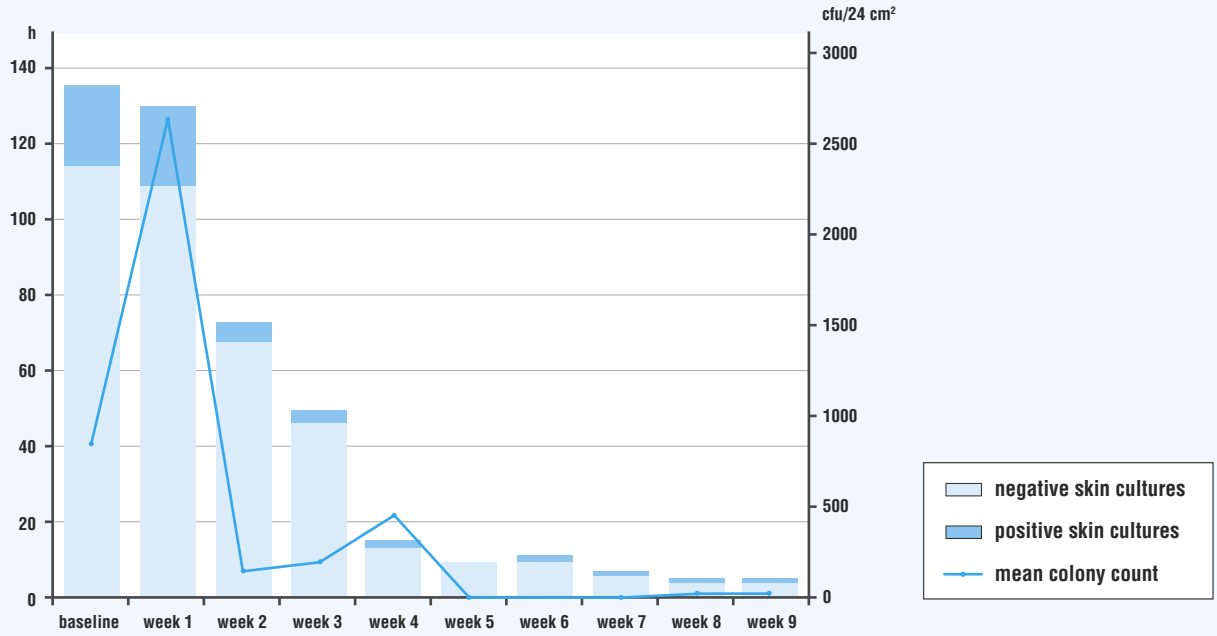


Fig. 1 Distribution of the positive skin cultures and mean colony counts over time. Baseline cultures were performed prior to preparation of the insertion site with povidone-iodine, and week 1 cultures were performed immediately prior to the first application of octenidine. CFU = colony-forming units

In conclusion, the repeated application of octenidine (0.1 %) on skin surrounding catheter insertion sites resulted in a sustaining decrease of bacterial skin colonization.

4.3 Resistance

The ability of bacteria, especially methicillin-resistant **Staphylococcus aureus** (MRSA), to develop resistance to antimicrobials, in particular antibiotics, is well documented. Although not as common, resistance to biocides such as antiseptics has also been reported. It has been shown that MRSA strains with multi-drug resistant pumps coded by the qacA gene exhibit increased restistance also to cationic compounds (e.g. chlorhexidine). Exposing different MRSA strains to octenidine in quantitative suspension tests did not decrease any of the strains in their susceptibility to octenidine as shown by their MIC values and log reduction factors.
Therefore, octenidine is not affected by a common mechanism to gain resistance to cationic compounds (Goroncy-Bermes 1999). An octenidine based antiseptic did not show any difference in its effectiveness to methicillin-sensitive **S. aureus** (MSSA) or MRSA strains in suspension tests (Goroncy-Bermes 1998).

Recently a total of 76 MRSA and 24 MSSA were tested (Al-Doori et al 2006). The 76 MRSA strains included representatives of the five major international epidemic MRSA clones (Enright 2002). The MIC of octenidine was tested on Muller Hinton Agar according to NCCLS methodology (NCCLS M7-A4). The MIC (Table 7) of octenidine for all isolates (MRSA and MSSA) was in the range of 2 – 4 mg/L and gave MIC_{50} and MIC_{90} of 4 mg/L.
The MSSA (MIC_{50} = 2 mg/L) appeared to be more susceptible than the MRSA (MIC_{50} = 4 mg/L).

Table 7: The MIC of octenidine dihydrochloride for all isolates (MRSA and MSSA)

Isolates	No. Tested	MIC Range	* MIC_{50}	** MIC_{90}
MRSA	76	2 – 4 mg/L	4 mg/L	4 mg/L
MSSA	24	2 – 4 mg/L	2 mg/L	4 mg/L
All	100	2 – 4 mg/L	4 mg/L	4 mg/L

* MIC_{50} = the concentration at which 50 % of the collection of isolates tested are inhibited.
** MIC_{90} = the concentration at which 90 % of the collection of isolates tested are inhibited.

The isolates were also tested for development of resistance/reduced susceptibility using a modification of the methods of Gradel et al (2005) and Langsrud et al (2003).
The MIC and bactericidal activity (based on EN1040) of octenidine for the "parent" and "adapted" isolates were tested. Serial dilutions and plating were performed by the Drop Plating method of Isenberg (Isenberg 1992). To ensure purity parent and adapted isolates were compared by a PCR based typing method (16S – 23S rRNA spacer length polymorphism). The parent isolates of all five clones tested in the adaptation studies had a MIC of 4µg/ml to octenidine. Following continuous exposure to increasing low level concentrations of octenidine over a three month period some differences were observed between clones in their ability to grow at increasing concentrations. The highest octenidine concentration allowing growth in broth was 8 µg/ml for strain CC5 and CC45.
Although growth occurred at concentrations higher than the MIC of the "parent" strain the MIC of the adapted isolates was identical to that of the parent (4 µg/ml). The data indicates that, under these experimental conditions, the five epidemic MRSA clones tested failed to acquire resistance/reduced susceptibility following continuous exposure to low level concentrations of octenidine.

5 Toxicology of octenidine dihydrochloride

5.1 Toxicological profile

Toxicology

The toxicity of octenidine has been investigated for single and repeated dosing after peroral, topical, intraperitoneal and intravenous application in different species.

Systemic toxicity in high oral doses only – Large safety margin

After single peroral administration, octenidine showed toxic effects with LD_{50} levels at rather high doses of 800 mg/kg b. w. (rats) and 933 mg/kg b. w. (mice), respectively, indicating a large safety margin of octenidine as a topical antimicrobial substance. No overt species differences were apparent. Studies investigating the toxicity of Octenisept® (0.1 % octenidine and 2 % Phenoxyethanol) or both active substances after repeated peroral or topical application showed that toxic signs were observed at high doses only, without overt species differences.

The results summarised in Table 8 below, also show that no signs of toxicity were observed after topical application.

Table 8 a: Single-dose toxicity of octenidine dihydrochloride (Oc)

Substance	Species	Route	Toxicity (LD_{50}, mg/kg b. w.)	Reference
Single Dose				
Oc	Mice	oral	None	Internal data
Oc	Rat	oral	800	Internal data
Oc	Rabbit	oral	> 800	Internal data
Oc	Rat	i. v.	10.0	Internal data

Table 8 b: Repeat-dose toxicity of octenidine dihydrochloride (Oc)

Repeat dose			NOAEL (mg/kg b. w.)	
Oc	Rat	14 d, o.d., oral	250	Internal data
Oc	Rat	5 wks., o.d., oral	≥ 5 ml	Internal data
Oc	Rabbit	topical*	≥ 2 %	Internal data
Oc	Beagle dog	1 month, topical, twice a day	≥ 0.1 %	Internal data
Oc	Beagle dog	30 d, oral cavity, four times a day	≥ 1.1 %	Internal data
Oc	Beagle dog	5 wks., o.d., oral	< 1.0	Internal data
Oc	Rabbit	6 months, topical, o.d. (2.5 ml)	≥ 0.5%	Internal data
Oc	Mice	13 wks, o.d., oral	< 0.5	Internal data
Oc	Mice	13 wks, diet	≥ 32	Internal data
Oc	Rat	12 months, oral	< 2	Internal data
Oc	Beagle dog	12 months, oral	< 2	Internal data

LD_{50}: dose killing 50 % of treated animals; NOAEL: highest tested dose with no observed adverse effect

* : duration of treatment not mentioned; % : tested as diluted solution (m/m); o.d. : once daily

No mutagenic, genotoxic or carcinogenic potential – No embryonic toxicity

Octenidine did not show any mutagenic or genotoxic potential in several test systems in vitro or in vivo (internal data) and did not have carcinogenic effects when applied orally or dermally (internal data).
Moreover, studies on reproductive and developmental toxicity in rats and rabbits (internal data) revealed that even at doses far higher than those administered in octenidine-containing formulation negative effects on reproduction and embryonic development did not occur.

No local toxicity

In a battery of in vivo studies on local tolerance no irritant effects on skin, the vagina, or the eyes were observed.
Octenidine had no sensitising potential (Table 9).

Table 9: Local tolerance studies in vivo of octenidine dihydrochloride

Test	Species (n)	Concentration	Major findings	Reference
Photosensitation	Guinea pig (5 male, 5 female/group)	(1) 0.05 ml of 2 % solution (6 % isopropanol), (2) 2 % solution (water), (3) vehicle	One grade-1 oedema with (1)	Internal data
Patch-test (Bühler)	Guinea pig (52)	2 % octenidine, vehicle control (occluded)	No sensitation, no irritation	Internal data

5.2 Cytotoxicity and tissue compatibility

Although several in vitro studies have suggested that antiseptic agents are cytotoxic to fibroblasts and other cell cultures (Kramer et al 1993), in vivo studies with octenidine and octenidine-containing preparations have failed to demonstrate an adverse effect to wound healing.
In comparison to Ringer`s solution there was no statistical difference in healing of artificial wounds on piglets after daily treatment with an octenidine-containing antiseptic up to 28 days (Kramer et al 2004). Histologically, no significant differences could be verified at any time between the two groups.
The tolerance of octenidine in the vaginal area was shown by electromicroscopy (Spitzbart 1994) in an ex vivo model with vital vaginal mucosa. Whilst PVP iodine leads to massive damage of mucosa within 5 minutes of use, when using an octenidine-based product merely an astringent effect on the superficial cells exists, whereby the intermediate layer remains unaffected.

In order to fill the gap between its cytotoxicity in vitro and its tolerance in the preclinical and clinical setting, further experiments were conducted with octenidine (Müller and Kramer, in preparation).
Combinations of octenidine with fibroblasts, bovine serum albumin (BSA), chondroitin sulphate (CS), lecithin, cholesterol and cardiolipin, were investigated for their microbiocidal effects on **E. coli** and **S. aureus** and their in vitro cytotoxicity for fibroblasts within 30 min contact time. Cardiolipin abolished the microbiocidal activity of octenidine completely, CS resulted in diminished microbiocidal activity. All other combination partners were ineffective. The combinations of octenidine and the partners mentioned above were associated with a decrease in cytotoxicity.

The most profound effect was seen in combination with fibroblasts. After binding to fibroblasts the cytotoxicity was completely abolished though retaining full antimicrobial efficacy.
A reduction factor (RF) > 5 was achieved at concentrations > 0.001 % octenidine within 1 min. This was not influenced by presence of proteins. If a biocompatibility index (BI) is introduced taking into account both the results of the in-vitro cytotoxicity, i.e. the concentration at which 50 % of the cells are damaged, and the microbiocidal activity, i.e. the concentration at which the baseline burden of the test microorganisms is reduced by at least 5-log steps, octenidine showed the most favourable relation between efficacy and cytotoxicity (BI = 1.51). For $AgNO_3$ and Ag-SD no BI value was calculable since no reduction in the baseline bacterial burden was observed. Colloidal silver has a BI value of 0.13, chlorhexidine 0.73, PVP-iodine (based on iodine) 0.95 and polihexanide 1.33. Therefore, on the basis of the optimal microbiocidal concentration, octenidine has the lowest cytotoxic side effect, which is weakened still further in the presence of proteins or lipids.

These results offer a possible explanation for the discrepancy between the relatively high cytotoxicity in vitro and the compatibility of octenidine on mucous membranes and wounds reported under practical applications.
Due to its specific capability to adhere and form complexes with chemical cell components and its high antimicrobial efficacy, octenidine may be considered as a unique topical antimicrobial compound exerting its activity through a non-cytotoxic complex on the site of action.

5.3 Systemic availability

In several studies the systemic bioavailability of octenidine has been investigated.

Systemic availability: octenidine: no

As shown in animal studies with 14C-labelled material, octenidine is not absorbed via the gastrointestinal tract or via skin or mucous membranes. Orally administered radioactively labelled octenidine was absorbed via the mucous membranes of the gastrointestinal tract in mice, rats and dogs only in very small amounts (0 – 6 %).
In mice it was found that topically applied amounts of octenidine were not absorbed during a 24-hour contact time under an occlusive dressing.
The results show that octenidine was not detected in the blood stream as it has not been absorbed after peroral or topical administration. The results are summarised in Tab. 10.

Table 10: Systemic availability of octenidine

Substance	Species	Route	Major findings	Reference
Oc*	Mice	oral	not detectable in serum, faecal elimination	internal data
Oc*	Rats	oral	total tissue radioactivity: < 0.05 %	internal data
Oc*	Rats	oral		internal data
Oc*	Beagle dog	oral	not detectable in serum, faecal elimination	internal data
Oc*	Mice	dermal	no systemic radioactivity detectable	internal data

Oc*: administration of 14C-octenidine dihydrochloride

On the basis of an established placental perfusion model (ex vivo model), passage of octenidine across the placenta was ruled out (Weissenbacher et al 1997).

6.1 Wound infection control

Better wound granulation than Ringer solution
– No impairment of wound healing

In a prospective, controlled, randomised double blind study including 47 patients the antimicrobial efficacy and local tolerance of an 0.1 % octenidine containing aquous solution was compared with Ringer solution when applied to infected chronic ulcers. The preparation was applied daily to the wound for a duration of 4 weeks. Wound healing was evaluated in terms of visual signs of infection (i.e. redness, oedema/swelling, lymphangitis), granulation, fibrinous coating and planimetric measuring of the wound area. The application of the octenidine solution significantly increased granulation ($p = 0.014$ PP-analysis, 0.034 ITT-analysis; one-sided tests). Adverse reactions were recorded rarely in both groups. In conclusion, used for locally infected chronic wounds octenidine quickly reduced signs of infection and thus resulted in an increase of granulation in comparison to Ringer solution. Octenidine did not impair the wound healing process compared to Ringer solution (Vanscheidt et al 2005).

Under the clinical conditions of this trial, the in vitro cytotoxicity of the test solution did not adversely affect the clinical outcome.

6.2 Care of CVC insertion sites

In a double blind, randomised, controlled trial the efficacy and tolerability of two commercially available, alcohol-based antiseptic solutions for preparation and care of central venous catheter (CVC) insertion sites were investigated (Dettenkofer et al 2006). One solution contained octenidine. The trial was conducted in the haematology units of university hospitals, and in one surgical unit. Adult patients with a non-tunneled CVC were enrolled prospectively after informed consent and randomly assigned to different skin disinfection regimens at the insertion site: (a) 0.1 % octenidine with 30 % 1-propanol and 45 % 2-propanol, (b) 74 % ethanol with 10 % 2-propanol.

Treatments were compared with respect to (i) skin colonisation within the first 10 days after CVC insertion; (ii) positivity of the catheter tip (> 15 CFU) and (iii) occurrence of CVC related sepsis. Quantitative skin cultures were obtained from the insertion site at regular intervals, and cultures of the CVC tip on removal were done with the roll plate technique. CVC-related bloodstream-infection (BSI) was defined according to criteria set up by the Centres for Disease Control and Prevention (CDC).

Four hundred patients with inserted CVC were enrolled during 5/2002 and 4/2005. Both groups showed no difference in patient characteristics. Skin colonization at the CVC insertion site during the first 10 days showed a highly significant reduction (Group A vs. B: 0.21; CI95: 0.11 – 0.4, $P < 0.0001$; analysis of 365 patients due to missing values). Positivity of the catheter tip was significantly lower for Group A (7.9 %) vs. B (17.8 %): odds ratio = 0.40 (CI95: 0.20 – 0.81, $P = 0.011$; analysis of 322 patients). There were fewer laboratory-confirmed CVC-related BSIs in Group A (n = 8) vs. B (n = 16). However, this reduction showed only a trend to statistical significance (OR = 0.44; CI95: 0.18 – 1.09). Side effects (i.e., burning) showed no relevant difference between the groups.

6.3 Decontamination of MRSA colonised patients

Due to its high efficacy against **S. aureus** strains (MSSA, MRSA) octenidine has turned out to be a promising agent for the decontamination of patients and health care workers colonised with MRSA on different sites of the body (e.g. axilla, groin, wounds).
Rohr et al (2003) investigated the efficacy of a MRSA multisite carriage decolonization by intranasal application of mupirocin combined with an octenidine bodywash in 32 hospitalised carriers over a period of five days. The overall decolonization rates for all sites (i.e. nose, forehead, neck, axilla, groin) was 64 % seven to nine days after the procedure. The authors concluded that nasal mupirocin combined with octenidine whole-body wash is effective in eradicating MRSA from patients with variable site colonization.

In another clinical trial on MRSA eradication, 28 patients were washed for a period of five days with a 1 : 1 diluted preparation based on octenidine. At the same time the Vestibulum nasii was treated with mupirocin. Elimination of the MRSA was achieved in 75 % of the cases (Sloot et al 1999).

In a monocentric clinical trial 45 hospitalised carriers were treated by intranasal application of mupirocin combined with an octenidine bodywash (Octenisan®). After the first wash cycle over 5 days MRSA could no longer be detected in 31 out of 45 patients (69 %). 8 of the 14 patients who remained MRSA-positive after the first cycle underwent a second cycle. Here, the eradication rate was 50 % (4 out of 8 patients). Overall 35 out of 45 patients (78 %) were completely MRSA eradicated (Lemmen 2005).

6.4 Treatment of vaginal infections

In a phase III clinical trial the efficacy and local tolerance of an octenidine-based product (Octenisept®) for the therapy of bacterial vaginal infections (bacterial colpitis, bacterial fluor vaginalis, bacterial vaginosis) was compared with an antiseptic standard therapy of PVP-iodine (Betaisodona®). Carried out in 29 gynaecologist's practices, a total of 308 patients with a bacterial vaginal infection was enrolled in the study and randomly assigned to the two therapy groups. During the initial diagnosis and after seven days of therapy a visual and microscopical diagnosis by the gynaecologist were carried out.

With 75 % the improvement of symptoms tended to be higher for patients using Octenisept® compared to patients using PVP-iodine (65 %, t = 1,89). Particularly, the recovery of lactobacilli was significantly increased (46 % vs. 29 %, t = 2,76). Due to application problems and the withdrawal from therapy compliance was better when using Octenisept®. In both groups no changes in cytomorphology or proliferation of the vaginal epithelium were observed. The local tolerance of both antiseptics was good.

Taking into account its efficacy, local tolerance and compliance on the one hand and the emergence of antibiotic-resistant bacteria and higher incidence of side effects by antibiotic use on the other hand, Octenisept® offers a new promising approach to the local treatment of bacterial vaginal infections (Friese et al 2000).

In many cases not only bacteria are responsible for vaginal infections but also fungi may contribute to the signs of infection. Therefore, the aim of another prospective, multicenter, randomised, case-control study was to investigate the efficacy of Octenisept® with proven antifungal effects in patients with acute symptomatic vaginal candidosis, in comparison to a specific topical antifungal agent (clotrimazole), particularly in respect to non-**Candida albicans** yeasts (Friese et al 2003). From 29 gynecological practices a total of 491 patients, who had new clinical vaginal mycosis not treated with antifungal agents in the last 12 months, were included in the study.
The diagnosis in each case was confirmed by microscopy and positive culture. The majority of the vaginal mycosis were infections with **C. albicans** (72 %). In 28 % of patients a non-**Candida** species (mainly **C. glabrata**) contributed to the infection. Except for vaginal discharge, the success of treatment was between 71 % and 91 % for both clinical and subjective parameters. The relatively high proportion of **C. glabrata** isolates in this study tended to be more successfully treated by Octenisept® (to 72 %) than by the administration of clotrimazole (59 %). Although the clotrimazole treatment resulted in an overall higher curing rate, the success of treatment with Octenisept® was between 70 % and 90 % described for topical antifungal agents. Both the good efficacy of the topical antiseptic and the increased prevalence of non-**Candida albicans** species causing vaginal infections mean that the use of an antiseptic may be considered a suitable alternative therapeutic concept to an appropriate topical antifungal agent in the treatment of acute vaginal candidosis and infections caused by a mixed population of bacteria and fungi.

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