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**ORIGINAL ARTICLE**

# Antimicrobial activity of monocaprin: a monoglyceride with potential use as a denture disinfectant

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**Abstract**
Monocaprin is a 1-monoglyceride of capric acid that has antimicrobial activity against enveloped viruses, certain bacteria, and the yeast *Candida albicans.* Solutions containing monocaprin were formulated and tested *in vitro* against a number of micro-organisms, including species found in the oral cavity and common pathogenic species. The antimicrobial activity of monocaprin was tested with strains growing on a surface as well as in the planktonic phase. Micro-organisms tested were: *Streptococcus mutans*, *Candida albicans*, *Lactobacillus* sp., *Staphylococcus aureus*, *Echerichia coli*, and *Pseudomonas aeruginosa*. Two sets of dilutions were prepared for each test strain; one to be inoculated with the micro-organism growing in the planktonic phase and the other with the same strain growing on a filter paper disk. Control solutions were also prepared to find out if any of the excipients were affecting the microbicidal effect of monocaprin. Test strains growing on the filter paper surface were less sensitive to monocaprin than the same strain growing in its planktonic phase. *C. albicans* was the micro-organism that was most sensitive to monocaprin, but *S. mutans* also showed appreciable sensitivity. The indication that monocaprin may have potential as a topical agent against *Candida* was tested in an open study of denture disinfection in 32 patients attending a geriatric daycare centre. A significant, but short-term, reduction in counts of *Candida* on the fitting surface of full dentures was observed.

**Key Words:** *Antimicrobial activity, Candida albicans, monocaprin, oral flora*

## Introduction

The antimicrobial effect of a variety of lipids has been extensively studied in recent years. A number of medium-chain free fatty acids and their corresponding 1-monoglycerides have been found to inactivate enveloped viruses and various bacteria, both Gram-negative and Gram-positive [1–4], and the yeast *Candida albicans* [5]. These lipids are commonly found in natural products, for example milk, and can therefore be assumed to be non-toxic to mucosa, at least at low concentrations. In nature, these compounds are considered to be potent inhibitory factors against infection by human pathogens or parasites [6]. The mechanism by which these lipids kill bacteria is not known, but electronmicroscopic studies indicate that they disrupt cell membranes [3]. Monocaprin (Figure 1) is one of the most effective antimicrobial monoglycerides. Antiviral activity has been demonstrated *in vitro* against enveloped viruses such as herpes simplex virus (HSV), respiratory syncytial virus, visna virus and human immunodeficiency virus *in vitro* [1,6–8] Monocaprin has also been found to possess bactericidal activity *in vitro* against Gram-positive bacteria such as *Staphylococcus aureus* and Group B *streptococci* as well as certain Gram-negative bacteria such as *Helicobacter* sp. [3,4].

Previous work with hydrophilic gels containing monocaprin in a concentration of 5 mg/ml have shown more than 100,000-fold inactivation of HSV types 1 and 2 [7,8] upon contact for 1 min. It has been suggested that pharmaceutical formulations containing monocaprin could be used for prevention and treatment of infections caused by viruses or bacteria. A preliminary clinical study [9] found no irritation, ulceration, inflammation, or other adverse effects when monocaprin was applied to the oral mucosa in either of two preparations: a hydrogel or a solution.

Micro-organisms in the mouth are known to grow in biofilms, notably in dental plaque and in the

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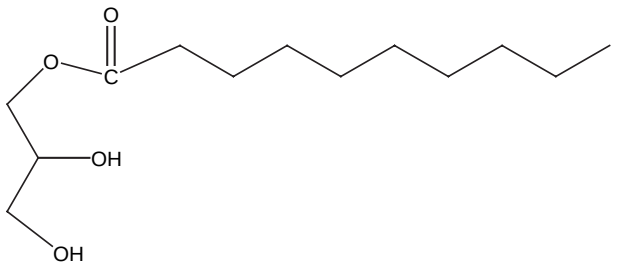


Figure 1. Monocaprin (1-monoglyceride of capric acid).

microbial deposits that form on dentures [10]. Although these organisms are generally regarded as commensal in nature, in certain circumstances they can be associated with disease in the oral cavity [10]. For example *Streptococcus mutans*, which is usually only a minor constituent in the complex flora of dental plaque, is associated with dental caries. Some members of the subgingival plaque flora have been implicated in periodontal disease and the presence of *C. albicans* on dentures is associated with denture stomatitis.

Sensitivity testing of micro-organisms in liquid cultures is usually performed using inocula of bacteria growing in a planktonic phase. This is unlike the biofilm state in which oral organisms exist in the mouth, and there is some evidence to suggest that organisms growing in a biofilm are less sensitive to the action of antimicrobial agents than the same organisms grown in a planktonic state [10–12]. The present investigation is an extrapolation of an earlier study in this laboratory [13] that had shown that oral streptococci growing in pure culture on a filter paper disk formed a film that could be visualized in the electron microscope. Bacteria growing on the surface of filter paper disks were found to be less sensitive to penicillin [13] than their counterparts growing in the planktonic phase. The clinical investigation of the activity of monocaprin against yeasts in the mouths of patients with denture stomatitis is also an extrapolation of previous work from this laboratory which has attempted to reduce the severity of denture stomatitis by disinfection of yeast colonization [14,15].

The aim of this study was 2-fold; firstly, to assess the antimicrobial activity of monocaprin *in vitro* and, secondly, to assess the anticandidal activity of monocaprin in a small group of patients with denture stomatitis.

## Material and methods

### *Materials*

Monocaprin was obtained from Danisco Ingredients, Denmark. Propylene glycol, glycofurol 75, sodium carboxymethylcellulose, and polysorbate 20 and 40 were purchased from the Sigma Chemical Co. (St. Louis, Mo., USA). Methyl- and propylparahydroxybenzoate were purchased from NMD, Norway.

### *Preparation of solutions*

In preparing the solutions, monocaprin and the methyl and propylparahydroxybenzoat were dissolved in propylene glycol or glycofurol 75. When they were fully dissolved, the solubilizing agent, Tween 20 or 40, was added. After adding water, the pH was adjusted to 7.0. Control solutions were also prepared to determine if any other component of the formulation was affecting the microbicidal effect. This was tested in a pilot study of only one strain for each species (Table II). The solutions as formulated and tested are presented in Table I. Doubling dilutions of monocaprin solutions were made in Brain-Heart-Infusion broth (Difco) in test tubes. Two sets of dilutions were prepared for each test strain, one to be inoculated with the micro-organism growing in the planktonic phase and the other with the same strain growing on a filter paper disk.

### *Micro-organisms and sensitivity testing*

Test strains of oral micro-organisms (*S. mutans*, *C. albicans*, *Lactobacillus* sp., 8–10 strains of each species) were obtained from routine clinical isolates in the microbiology laboratory of the Faculty of Odontology. Isolates of the other test strains: *S. aureus*, *Echerichia coli*, *Pseudomonas aeruginosa*, were obtained from the microbiology laboratory of the University Hospital in Reykjavík.

Micro-organisms were grown for 24 h in Brain-Heart-Infusion broth (Difco) and aliquots of 10 µl ($10^4$–$10^5$ microorganisms) were used to inoculate each dilution of monocaprin (approximately $10^6$ organisms per inoculum). This constituted the planktonic-culture sensitivity test. The same culture of each test strain was used to inoculate sterile disks prepared from absorbent filter paper (Whatmann No. 1, obtained from W. & R. Balston, UK) that had been arranged on blood agar plates. These disks were incubated overnight at 37°C and were then

Table I. Combinations of monocaprin, solvent, and solubilizing agent

| Solution | Solvent (%) | Solubilizing agent (%) | Monocaprin (mg/ml) |
|---|---|---|---|
| 1 | PG (5) | Tween 40 (0.4) | 10 |
| 1S | PG (5) | Tween 40 (0.4) | 0 |
| 2 | PG (5) | Tween 20 (1) | 5 |
| 2S | PG (5) | Tween 20 (1) | 0 |
| 3 | GF (30) | Tween 20 (1) | 5 |
| 3S | GF (30) | Tween 20 (1) | 0 |

The solvents are either propylene glycol (PG) or glycofurol 75 (GF) and the solubilizing agents are either Tween 20 or 40.

removed with sterile tweezers. One disk was used to inoculate one dilution tube of monocaprin. All the tests were incubated aerobically at 37°C for 24 h, except for streptococci, which were incubated in $CO_2$ for 48 h.

After incubation, the cultures were examined visually for the presence of turbidity in the broth, and the minimum inhibitory concentration (MIC) of monocaprin was recorded as the lowest concentration of monocaprin that clearly inhibited growth of the test strain. Samples, 10 µl, of each non-turbid culture and the first turbid culture were used to inoculate blood agar plates that were then incubated and examined after 24 h in order to determine the minimum bactericidal concentration (MBC) of monocaprin for each test strain by streaking from each dilution onto blood agar.

*Clinical study*

Necessary permissions were obtained from the relevant authorities in Iceland (National Bioethics Committee, ref. no. 04-013, National University Hospital). Subjects were 32 patients who were either attending the daycare center of the geriatric unit of the National University Hospital in Reykjavík or were patients in the short-stay hospital clinic. All gave their informed consent. The oral mucosa was examined at the beginning and at the end of the study period, and inflammation resembling denture stomatitis was recorded by the same clinician (WPH). All patients volunteered, but with the agreement of the medical staff of the unit. No subjects were therefore excluded. Impression cultures [14] were taken from the palatal and lingual mucosa and from the fitting surface of the denture using sterile foam pads in accordance with a previously tested method [14] and counts of *Candida* sp. were determined by incubating the impression pads on BBL™ CHROMagar™ Candida® (BD, Franklin Lakes, N.J., USA) and counting colonies under magnification. Colony identification was in accordance with the manufacturer's description, that is *C. albicans* appeared as smooth, green colonies with a slight green halo in the agar and *C. tropicalis* appeared as smooth, blue to blue-grey colonies with a dark brown to purple halo in the agar.

Subjects were asked to soak their dentures in 0.5% monocaprin solution daily for 2 h on each occasion for 4 consecutive days. Repeat impression samples were collected for yeast culture at the end of the period of disinfection and, where possible, 4 weeks later in order to assess the long-term effect of monocaprin disinfection.

Yeast colony counts $>40$ cfu/cm$^2$ were regarded as indicating infection as determined by previous studies [14,15]

## Results

*Laboratory study*

As can be seen from the results for MIC and MBC (Table II), monocaprin showed moderate activity against most test bacteria including *S. aureus* and *P. aeruginosa* as well as the yeast *C. albicans*. Solution 1 was the least microbicidal solution formulated not showing any activity against *S. mutans*, *P. aeruginosa*, or *Lactobacillus.* It showed activity against *E. coli* in 2.5 mg/ml concentrations (for both planktonic and surface-growing phases) and against *C. albicans* and *S. aureus* in 0.156 mg/ml concentrations in planktonic phase, and in 2.5 mg/ml against *C. albicans* in the surface-growing phase and none against *S. aureus* in the surface-growing phase (data not shown in Table II). Solution 3 was clearly more active than solution 2, but the solvent and solubilizing agents also had an inhibitory effect on the test organisms, although always considerably less than was seen when the solution contained monocaprin (Table II). In all instances, test strains growing on a surface were less sensitive to monocaprin than the same strain growing in its planktonic phase. *S. aureus* and *C. albicans* were the micro-organisms that were most sensitive to monocaprin, but *S. mutans* also showed appreciable sensitivity. Table II gives the results of a pilot study where the solutions were only tested against one strain of each micro-organism in order to determine the possible effect of solvent on the MIC. It was observed from this study that solution 2 had appreciable activity against the micro-organisms tested, although the solvent had clearly no effect. In the subsequent study, this solution alone was tested against several test strains of each species and the results are given in Table III. These results show that the solution is clearly active against all the micro-organisms tested except for *P. aeruginosa*, which shows resistance even in the planktonic phase. *S. aureus*, *C. albicans*, and *S. mutans* were sensitive in the planktonic phase to monocaprin solution 2 even though the range of activity was fairly wide. None of the micro-organisms tested showed sensitivity when the strains were tested in the biofilm, surface-growing state.

*Clinical study*

Only 4/32 subjects (12.5%) had a completely healthy oral mucosa and 21/32 subjects (66%) complained of xerostomia. Microbiological evidence of oral candidosis was found in 26/32 subjects (83%) who had $>60$ cfu of yeasts/cm$^2$. Ten subjects (31%) had poor denture hygiene. *C. albicans* was the most frequently isolated yeast followed by *C. tropicalis.* Mixed yeast cultures were seen commonly when the overall count of yeasts was high ($>40$ cfu/cm$^2$, 48%) but less commonly when the total yeast count was ($<40$ cfu/cm$^2$, 17%). Disinfection with monocaprin

Table II. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (expressed as the dilution of the original solution and as mg/ml, see Table I) of various test solutions of monocaprin with inocula in planktonic and biofilm form

| Organism in planktonic phase (P) or biofilm (B) culture | Greatest dilution of monocaprin solutions 2 and 3 (see Table I) that just inhibited growth of (MIC) – or just killed – (MBC) the inoculum (Concentrations expressed in mg/ml; S = solvent control without monocaprin) | | | |
|---|---|---|---|---|
| | 2 | 2S | 3 | 3S |
| | MIC (MBC) | MIC (MBC) | MIC (MBC) | MIC (MBC) |
| *S. mutans*, P | 32 (32)<br>0.156 (0.156) | <2 (<2) | 32 (32)<br>0.156 (0.156) | 4 (4) |
| *S. mutans*, B | 8 (2)<br>0.625 (2.5) | <2 (<2) | 8 (4)<br>0.625 (1.25) | 4 (2) |
| *S. aureus*, P | 64 (<2)<br>0.078 | <2 (<2) | 64 (<2)<br>0.078 | <2 (<2) |
| *S. aureus*, B | <2 (<2) | <2 (<2) | <2 (<2) | <2 (<2) |
| *P. aeruginosa*, P | 8 (<2)<br>0.625 | <2 (<2) | 16 (4)<br>0.313 (1.25) | 4 (<2) |
| *P. aeruginosa*, B | <2 (<2) | 2 (<2) | 8 (<2)<br>0.625 | 4 (<2) |
| *E. coli*, P | 16 (<2)<br>0.313 | <2 (<2) | 16 (2)<br>0.3125 (2.5) | 4 (2) |
| *E. coli*, B | <2 (<2) | <2 (<2) | 16 (4)<br>0.3125 (1.25) | <2 (2) |
| *Lactobacillus*, P | 16 (16)<br>0.313 (0.313) | <2 (<2) | 16 (8)<br>0.313 (0.625) | 2 (2) |
| *Lactobacillus*, B | <2 (<2) | <2 (<2) | 16 (8)<br>0.313 (0.625) | 4 (<2) |
| *C. albicans*, P | 32 (8)<br>0.156 (0.625) | <2 (<2) | 64 32<br>0.078 (0.156) | <2 (2) |
| *C. albicans*, B | <2 (<2) | <2 (<2) | 32 4<br>0.156 (1.25) | 8 (<2) |

on 4 consecutive days reduced yeast numbers significantly on the fitting surface of the denture ($t = 2.247$; $p < 0.05$) and tongue ($t = 2.083$; $p < 0.05$), but not on the palatal mucosa ($t = 1.428$; $p > 0.1$) (Figure 2). Disinfection was more effective when the dentures were clean ($t = 2.118$; $p < 0.05$) as judged by one observer (Í.A.) at the beginning of the study.

Only 17 subjects returned for a third sampling 1 month after the monocaprin study had ended. Yeast counts from these subjects were similar to pretreatment values (Figure 2).

## Discussion

Previous studies on the antimicrobial effect of monocaprin have demonstrated potentially useful activity against enveloped viruses, especially against the herpes simplex virus [7]. Antibacterial activity of monocaprin has been demonstrated particularly against Lancefield group B-beta-haemolytic streptococci [3].

This may be useful clinically, where eradication of Group B streptococci from the female genital tract can often be a problem; infection with this organism is particularly serious in pregnant women [16].

With antibiotic resistance becoming increasingly recognized, especially in hospital environments, there is considerable need for an effective antimicrobial agent that is structurally so different from traditional antibiotics that it is less likely to lead to resistance. This is particularly true for control of biofilms that are often composed of normal flora but may harbour resistant organisms that can rapidly become a clinical problem, for example in immunocompromised individuals [17].

In this study it was decided to test the effect of solutions of monocaprin on strains of oral microorganisms growing on a surface as well as the conventional inoculum of planktonic-growing bacteria. It is known that oral organisms normally grow on surfaces in biofilms and that in this phase the organism usually shows reduced sensitivity to antimicrobial agents [10]. In fact, relatively few studies show this reduced sensitivity with oral organisms [12,18]. Although biofilms are usually formed by the interaction of a number of organisms acting together, the present study was a simplification in order to make a comparison between two physical states of inoculum on the outcome of the sensitivity test. An earlier study from this laboratory [13] has demonstrated that organisms grown on filter paper disks exactly as used in the present study do indeed form a film on the disk. Furthermore, that study demonstrated reduced sensitivity of oral streptococci

Table III. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (expressed as μg/ml, see Table I) of test solution 2 of monocaprin with inocula in planktonic phase and in the form of a simple monoculture biofilm

| Organism and culture in planktonic phase (P) or biofilm (B) | MIC | MBC |
|---|---|---|
| | μg/ml | μg/ml |
| *S. mutans*, P $n=10$ | 156–625 | 156–2500 |
| *S. mutans*, B $n=10$ | >2500 | >2500 |
| *Lactobacillus*, P $n=8$ | 156– >2500 | 156–2500 |
| *Lactobacillus*, B $n=8$ | >2500 | >2500 |
| *C. albicans*, P $n=8$ | 313–625 | 625–2500 |
| *C. albicans*, B $n=8$ | >2500 | >2500 |
| *S. aureus*, P $n=9$ | 313– >2500 | 625– >2500 |
| *S. aureus*, B $n=9$ | 2500– >2500 | >2500 |
| *E. coli*, P $n=10$ | 1250– >2500 | >2500 |
| *E. coli*, B $n=10$ | 1250– >2500 | 1250– >2500 |
| *P. aeruginosa*, P $n=10$ | >2500 | >2500 |
| *P. aeruginosa*, B $n=10$ | >2500 | >2500 |

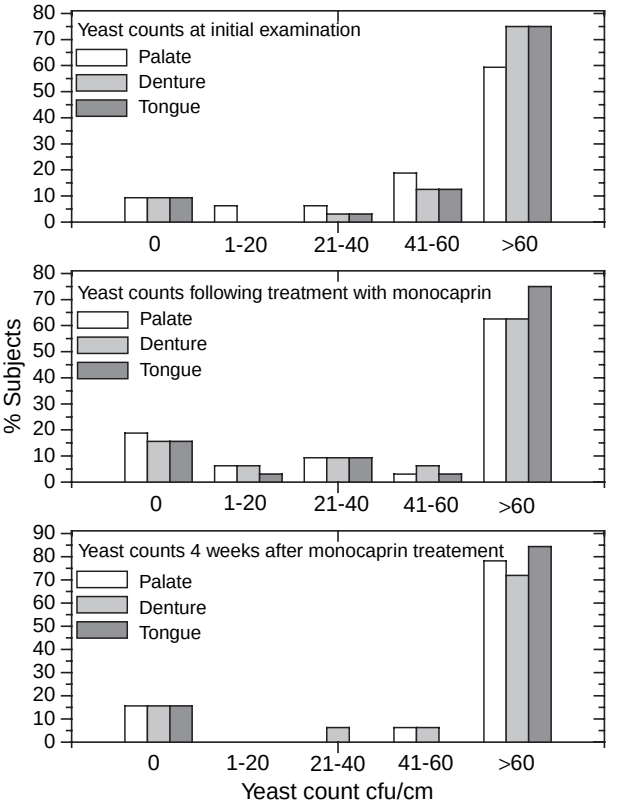


Figure 2. Colony counts of yeasts isolated from palate, denture, and tongue of patients at initial examination, following treatment, and 4 weeks after monocaprin treatment.

to penicillin, a phenomenon that was termed penicillin tolerance/persistence [13].

Monocaprin appears to have only a limited spectrum of activity against surface-growing cultures of common organisms tested in the present study, but is more active against the same bacterial strains in the planktonic phase (Table II). The results of the present study indicate that monocaprin may have potential as a broad-spectrum antimicrobial agent for topical use and for inhibiting biofilm accumulation on mucosal surfaces and on equipment and devices, such as catheters, that are difficult to disinfect once in use.

This study has also demonstrated that monocaprin is potentially useful against the yeast *C. albicans.* Infection with this organism is common in the mouth and is often difficult to eradicate when in the form of denture stomatitis, because the palatal mucosa is re-infected from the denture acrylic to which the yeasts adhere in a biofilm. While the clinical study showed a significant reduction in counts of yeasts following monocaprin treatment, the effect was short-lived. A more suitable vehicle for retaining monocaprin on the denture fitting-surface would presumably be helpful, but this was beyond the scope of this initial clinical proof of concept study. The small clinical study thus aimed only to determine if monocaprin could reduce numbers of yeasts on dentures. The method relied heavily on the ability of the patients themselves to follow the protocol. Perhaps the results would have been different had monocaprin been applied professionally, but this was not a practical possibility. Nevertheless, the results suggest that even home use of monocaprin could be expected to have a reasonable effect in reducing yeast counts on the dentures and the underlying palatal mucosa. Consequently, monocaprin merits further study as a potential denture-disinfecting agent.

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