Susceptibility in vitro of Helicobacter pylori to cetylpyridinium chloride

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Abstract

The antimicrobial agent cetylpyridinium chloride (CPC) which is used in therapy of oro-pharyngeal infections and for antiseptic treatment of the oral cavity is active against different bacterial species. Determination of the minimal inhibitory concentration (MIC) using the agar dilution technique revealed that the gastric pathogen Helicobacter pylori in vitro is highly susceptible to CPC as indicated by an MIC of 10 μM (3.4 μg ml⁻¹) which was significantly lower than the MIC of CPC against other bacterial species, which were analyzed in comparison to H. pylori. Bacteria of the genus Campylobacter, various Streptococcus spp., Staphylococcus aureus and Escherichia coli showed higher MICs ranging from 100 μM to 2 mM. In summary, this finding renders CPC-containing drugs candidates possibly useful for eradication or for the prevention of transmission of the gastric pathogen. © 1999 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

The human gastric pathogen Helicobacter pylori is the causative agent of chronic gastritis and peptic ulceration, and infection is associated with the development of gastric cancer and MALT lymphoma. The indications for eradication therapy of this pathogen have markedly expanded in recent years [1]. The current eradication therapy includes combinations of proton pump inhibitors with nitroimidazoles (tinidazole or metronidazole), macrolides, tetracycline or amoxicillin [2]. The increasing number of patients who receive sufficient, or in the case of partly resistant strains, suboptimal antibiotic therapy, might be responsible for the spread of antibiotic resistance which is rising in the H. pylori population. Antimicrobial resistance reduces the efficacy of triple or quadruple therapies, and especially the treatment of metronidazole-resistant strains has been shown to favor development of secondary clarithromycin resistances [3]. The fact that even the different modes of action of the antibiotics in use did not prevent the development of resistance indicates that it will be worth while in the future to have additional antimicrobial substances which attack other cellular target structures.

The cationic detergent cetylpyridinium chloride (CPC) [4], which exhibits strong antibiotic activity...
against various microorganisms [5,6], is used in the therapy of oro-pharyngeal infections and of periodontal diseases [7]. As a component of various commercial mouth washes it was shown to be active in the oral cavity. CPC destroys the integrity of cellular membranes, which is a mode of action different from that of the antibiotics mentioned above. The detergent could therefore represent a possible candidate for eradication therapy or for disinfection of the oral cavity, thus preventing reinfection and oral transmission of \textit{H. pylori}. Because CPC is a component of drugs long used in the therapy of tonsillitis [4,15], it is possible that treatment with CPC could interfere with the \textit{H. pylori} colonization of the oral cavity or of the stomach.

The aim of this study was to evaluate whether CPC exerts antimicrobial activity against \textit{H. pylori} and to determine whether the minimal inhibitory concentration (MIC) could be of relevance for a possible interaction of CPC-containing drugs with \textit{H. pylori} colonization.

2. Materials and methods

2.1. Bacterial strains

Fourteen clinical isolates and five reference strains of \textit{H. pylori} were investigated by CPC susceptibility testing. Clinical isolates of \textit{Campylobacter jejuni}, \textit{C. coli}, \textit{Streptococcus mutans}, \textit{S. sanguis}, \textit{S. intermedius}, \textit{S. pyogenes}, \textit{Staphylococcus aureus}, and the laboratory strain HB101 of \textit{Escherichia coli} [12] were analyzed in comparison to \textit{H. pylori}.

2.2. Susceptibility testing

The CPC chemical was purchased from Sigma (C5460). The MIC of CPC was determined by the agar dilution technique using the blood agars Isosensitest [9] or HHP [10], and the less complex medium BBC [11]).

For the MIC determinations, bacteria grown for 48 h on HHP blood agar were resuspended in phosphate buffered saline and diluted to an optical density (at 600 nm) of 0.4. For inoculation, aliquots of 1 µl were applied as dots (5 mm diameter) on the test agars (HHP, Isosensitest, and BBC, see above) which contained CPC at increasing concentrations (0.1 µM to 10 mM). The inoculation was performed with an AM80 automate (Denley-Tech Ltd.; Dynatech AG). The plates were then incubated at 37°C in a microaerobic atmosphere (5% O$_2$, 10% CO$_2$, 85% N$_2$) and MIC values were determined by monitoring bacterial growth after 2 days and after 5 days.

The MIC was defined as the concentration of CPC which inhibited bacterial growth completely as indicated by the absence of any visible cell layer formation in the inoculation zone on the agar. The experiments were performed in triplicate and the MIC values determined were identical.

3. Results

3.1. Antimicrobial activity of CPC against \textit{H. pylori} strains

In order to determine whether the HHP blood agar and the BBC agar are suited for susceptibility testing of \textit{H. pylori}, the strains NCTC 11638, ATCC 43504, G27, P1, and 2012 were grown on both media without CPC supplementation. The formation of a confluent cell layer after 2 days of incubation indicated that both types of media seemed to be useful for determination of the MIC for CPC by the agar dilution technique.

Supplementation of the media with CPC inhibited growth of the \textit{H. pylori} reference strains as indicated by the lack of any cell layer formation in the presence of CPC at a concentration of 10 µM (3.4 mg l$^{-1}$; Fig. 1). At a lower CPC concentration of 1 µM (0.34 mg l$^{-1}$), the MICs varied in a strain-dependent manner: strains NCTC 11638 and ATCC 43504 did not grow whereas strains P1, G27, and 2012 formed a confluent cell layer (Fig. 1).

In order to investigate the antimicrobial activity of CPC against clinical isolates of \textit{H. pylori}, the MIC of CPC was determined for 14 strains isolated from gastric biopsy specimens. The susceptibility testing
was performed on Isosensitest agar and on HHP blood agar. Both media are known to be well suited for susceptibility testing of primary isolates of \textit{H. pylori} which are not adapted to growth under laboratory conditions [9]. Growth of all clinical isolates was completely inhibited by a CPC concentration of 10 μM (3.4 mg l\(^{-1}\)) but the formation of a cell layer in the presence of CPC at 1 μM (0.34 mg l\(^{-1}\)) demonstrated that the strain-dependent polymorphism observed for the reference strains (see above) seems not to be representative for the \textit{H. pylori} population (Fig. 2).

Concentrations of CPC below 1 μM (0.34 mg l\(^{-1}\)) did not exert any inhibitory effect on the \textit{H. pylori} reference strains and clinical isolates tested (Fig. 2). The MICs determined on HHP, Isosensitest, and BBC agar were identical, indicating that these media are well suited for susceptibility testing of \textit{H. pylori} and that the differing formulation does not interfere with the antimicrobial activity of CPC.

### 3.2. Antimicrobial activity of CPC against other bacterial species

Clinical isolates of other bacterial species, which were analyzed in comparison to \textit{H. pylori} on HHP and BBC agar, exhibited higher resistance levels to CPC (Fig. 1). For \textit{S. aureus} and various species of \textit{Streptococcus} (\textit{mutans}, \textit{sanguis}, \textit{intermedius}, \textit{pyogenes}) the MICs were 100 μM (34 mg l\(^{-1}\)), 200 μM (68 mg l\(^{-1}\)), and 400 μM (136 mg l\(^{-1}\)) were determined for clinical isolates of other bacterial species.

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### 4. Discussion

Susceptibility testing in \textit{H. pylori} is hampered by the fact that any standardization for this fastidious, microaerophilic organism is lacking [13]. Furthermore, the test conditions are particularly sensitive to the medium composition and the inoculum size [14]. Finally, the prognostic impact of the MICs determined in vitro for the probability of eradication success remains uncertain, due to the particular pharmacokinetics in the gastric environment. This is especially true for metronidazole, where the clinically reliable breakpoint is still awaiting definition.

\textit{H. pylori} reference strains and clinical isolates tested in the present study showed variable susceptibility levels to CPC in a rather narrow concentration range (between 0.001 and 0.01 mM), but all strains were inhibited by a concentration of 0.01 mM (3.4 mg l\(^{-1}\)). This concentration is much lower than any concentration of CPC to be expected in

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**Fig. 1.** Susceptibility in vitro of \textit{H. pylori} reference strains in comparison to various other bacterial species. The bars represent the MIC values of CPC determined for each strain in triplicate using the dilution technique on HHP blood agar and on BBC agar. The MIC values determined on BBC agar and on HHP blood agar were identical. The MICs of CPC against \textit{H. pylori} reference strains were 1 μM (0.34 mg l\(^{-1}\)) and 10 μM (3.4 mg l\(^{-1}\)). MICs of 100 μM (34 mg l\(^{-1}\)), 200 μM (68 mg l\(^{-1}\)), and 400 μM (136 mg l\(^{-1}\)) were determined for clinical isolates of other bacterial species.

**Fig. 2.** Antimicrobial activity of CPC against clinical isolates and reference strains of \textit{H. pylori}. The bars represent the number of strains that were able to form a dense cell layer in the presence of CPC at concentrations of 0.1 μM (0.034 mg l\(^{-1}\)), 1 μM (0.34 mg l\(^{-1}\)), and 10 μM (3.4 mg l\(^{-1}\)), as indicated on the x axis. MIC values determined on HHP agar and on Isosensitest agar were identical.
saliva, when CPC-containing drugs are applied locally. Such drugs used in the local antisepitic therapy of pharyngeal infections, as well as hexetidine solutions used for prevention of periodontal diseases, contain CPC at concentrations of 1–3 mg per sucking tablet or 0.5–1 mg ml⁻¹ (500–1000 mg l⁻¹) [15]. Because the MICs of CPC against *H. pylori* observed in this study were much lower than the CPC levels expected in saliva, it can be suggested that the CPC concentrations in the drugs could interfere with the *H. pylori* colonization of the oral cavity or even the stomach.

Recently, it has been shown that during therapy with omeprazole/amoxicillin *H. pylori* was detected in the saliva of 85% of *H. pylori*-positive patients by PCR. All of the patients who received eradication therapy had detectable *H. pylori* in their saliva or dental plaque on day 42 after treatment [16]. Therefore it could be of use to supplement systemic antimicrobial eradication regimes with local application of microbiocidal substances in the oral cavity. In addition, the low MICs of CPC against *H. pylori* observed in this study make CPC-containing drugs possibly useful candidates for preventing reinfection after *H. pylori* eradication as well as for interfering with the oral-oral route of transmission of the pathogen. So far, further clinical studies concerning the in vivo effects of CPC as a supplementary therapy in *H. pylori* eradication trials seem to be justified.

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References


