In vitro activity of citropin 1.1 alone and in combination with clinically used antimicrobial agents against Rhodococcus equi

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Objectives: The aim of this study was to investigate the in vitro activity of citropin 1.1, an antimicrobial peptide derived from the Australian tree frog Litoria citropa, alone and in combination with ampicillin, ceftriaxone, doxycycline, netilmicin, ciprofloxacin, rifampicin, linezolid, vancomycin, clarithromycin and imipenem against 12 nosocomial isolates of Rhodococcus equi.

Methods: Antimicrobial activity of citropin 1.1 was measured by MIC, MBC, time–kill studies and chequer-board titration method.

Results: All isolates were inhibited at concentrations of citropin 1.1 between 2 and 8 mg/L. Combination studies demonstrated synergy only when the peptide was combined with clarithromycin, doxycycline and rifampicin.

Conclusions: Our findings show that citropin 1.1 is active against R. equi and that its activity could be enhanced when it is combined with hydrophobic antibiotics.

Keywords: Gram-positive cocci, antimicrobial peptides, synergy, antibiotics

Introduction

Rhodococcus equi, an aerobic, facultative intracellular, Gram-positive, acid-fast coccobacillus, is a well-known pathogen in domestic animals, especially horses, which causes suppurative bronchopneumonia with a high mortality rate in young foals.1,2 R. equi has also emerged as a significant opportunistic pathogen in immunocompromised hosts, especially those infected with the HIV. Clinical reports have shown that, despite antibiotic therapy, frequent relapses occur during the course of the disease.1,2 Although combined therapy with erythromycin and rifampicin has dramatically improved the survival rate in foals, this treatment regimen is not without problems for the recently reported emergence of rifampicin resistance in R. equi.1,3

In the last few years many positively charged polypeptides have been isolated from a wide range of animal, plant and bacterial species.4 The dual hydrophobic and hydrophilic nature of these molecules is important for their initial interaction with the bacterial membrane, which may allow entry of several substrates inside the cell.5

The citropins are antimicrobial peptides isolated from the dorsal glands of Australian tree frogs. They possess a broad spectrum of biological activity and some are specific to certain pathogens. Citropin 1.1 is a small peptide (16 residues) produced by both the dorsal and submental glands of the Australian green tree frog Litoria citropa. It has a significant antimicrobial, anticancer and nitric oxide synthetase activities.6

The aim of the present study was to evaluate the in vitro activity of citropin 1.1 and its bactericidal effect for several R. equi strains, as well as to investigate its in vitro interaction with 10 clinically used antibiotics.

Materials and methods

Organisms

Twelve nosocomial isolates of R. equi were tested. The isolates were obtained from distinct patients from Central Italy with unrelated sources of infection and admitted to the Hospital Umberto I, Ancona Italy, from January 1990 to December 2003. The strains were identified...
Rhodococcus equi susceptibility to citropin 1.1

Results and discussion

All isolates were inhibited by citropin 1.1 at concentrations between 2 and 8 mg/L. Interestingly, it showed MBC ranges of 2–16 mg/L. Comparative evaluation of other antimicrobial agents did not show important differences in their activity. In fact, all antimicrobial agents were highly active against at least 90% of R. equi isolates, with exhibited MICs less or similar to those of citropin 1.1. However, most of the control agents showed MBCs much higher than their MICs. The results are summarized in Table 1. Killing by citropin 1.1 was shown to be very rapid: its activity on bacterial isolates was complete after 20 min exposure period at a concentration of 2 × MIC.

In interaction studies synergy was observed only for combinations between citropin 1.1 and rifampicin, doxycycline or clarithromycin. For each of the six strains FIC indices were <0.5. Actually, median values of 0.385, 0.385 and 0.312 were observed by testing citropin 1.1 combined with rifampicin, doxycycline and clarithromycin, respectively. In contrast, the other experiments with the other antibiotics gave values between 1.167 and 1.833.

Polymorphonuclear cells and monocytes/macrophages represent the first defence line against invading microorganisms, but although R. equi can be susceptible to neutrophil-mediated killing, it is able to resist innate macrophage defences and establishes residence within the intracellular environment of that phagocyte.1–3 The ability to replicate within the macrophage is associated with virulence, and correlates in animals with the possession of a large plasmid and expression of the plasmid-encoded surface-expressed lipoprotein. Despite the good in vitro activities of traditional antibiotics, therapy is often partially effective and relapses occur during the course of the disease.4,5 For this reason, new strategies based on novel molecular targeting agents are warranted to further improve the long-term outcome of R. equi infection treatment. It is well known that the major killing mechanism of the neutrophils is based on the cationic peptides associated with azurophilic granules of these dedicated antimicrobial phagocytes.4,5 Accordingly, it may be useful to investigate the therapeutic strategy of combining clinically used antibiotics with peptides against bacteria able to infect macrophages.

**Table 1.** MICs and MBCs of citropin 1.1 and other clinically used antibiotics for 12 R. equi clinical isolates

<table>
<thead>
<tr>
<th>Agent</th>
<th>MIC (mg/L)</th>
<th>MBC (mg/L)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>range</td>
<td>median</td>
</tr>
<tr>
<td>Citropin 1.1</td>
<td>2–8</td>
<td>4.00</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.25–8</td>
<td>4.00</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>1–16</td>
<td>1.50</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.25–2</td>
<td>0.50</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.50–2</td>
<td>1.00</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.25–4</td>
<td>1.00</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.25–2</td>
<td>0.37</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.50–2</td>
<td>0.50</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>0.50–2</td>
<td>1.00</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.50–8</td>
<td>0.75</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.25–2</td>
<td>0.50</td>
</tr>
</tbody>
</table>
Our data demonstrate that citropin 1.1 is active against \emph{R. equi} and show a rapid bactericidal effect. These findings confirm the activity of naturally derived peptides against this organism, as already described by our group.\textsuperscript{10} Combination studies showed that it exhibited positive interaction with hydrophobic antibiotics. Previous reports demonstrated that polycationic peptides present properties of synergy with lipophilic agents such as rifampicin, macrolides, fusidic acid and novobiocin. It is a complex mechanism that probably involves the peptide-induced entrance of large lipophilic molecules into the cell. The cationic peptides, by triggering the activity of bacterial murein hydrolases, may cause degradation of the peptidoglycan and have direct membrane-permeabilizing activity with maximal entry of hydrophobic compounds. Several cationic antimicrobial peptides are known to interact with bacterial membranes, making the outer protective shield more permeable.\textsuperscript{4,5} It is possible that the synergic interaction is a result of a combined effect of increased access to the intracellular target for clarithromycin, doxycycline or rifampicin, and secondary effects of the peptides themselves. A hypothesis including increased uptake and accessibility to the target, combined with drugs acting on a common pathway, can explain the observed synergy for hydrophobic antibiotics. In fact, other mechanisms may be involved in this interaction: it has been demonstrated that cationic peptides by disintegrating the biological membranes yield to uncoupling of the oxidative respiration.\textsuperscript{4}

In conclusion, our data suggest that citropin 1.1, alone or in combination with conventional antibiotics, may be a promising agent for the management of \emph{R. equi} infections.

**Acknowledgements**

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**References**


