Activity of synthetic peptides against *Chlamydia* spp.

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Background and Objectives

First line drugs in human chlamydial infections include tetracycline and macrolides. Despite the well known *in vitro* activity of these drugs against *Chlamydia*, there are reports of tetracycline treatment failure in humans[1] leading to chronic or persistent infections. In addition, a stable tetracycline-resistance associated with *tet*(C) genomic islands integrated into the chlamydial chromosome has been reported for *C. suis* isolates[2]. Interestingly, a tetracycline resistance expressed *in vitro* by *C. trachomatis,* following cocolture with tetracycline-resistant *C. suis* strains, has been described[3].

In recent years, the interest in the antimicrobial activity of synthetic peptides has increased. The formulation of synthetic peptides with improved solubility and absence of toxic effect against mammalian cells has become extremely attractive. The *in vitro* antimicrobial activity of synthetic peptides, such as the wasp generated peptide mastoparan, clavanins (α-helical peptides with cationic properties from the marine tunicate *Styela clava*) and *Pa*-Map (an alanine-rich peptide with hydrophobic amino acid residues from the polar fish *Pleuronectes americanus*), against some Gram-positive and Gram-negative antibiotic-resistant bacteria, has been reported[4,5,6].
Since the anti-chlamydial activity of mastoparan, clavanins and *Pa*-Map peptides has not been tested so far, in the present study we investigated the *in vitro* activity of these peptides against *Chlamydia* elementary bodies (EBs). In addition, an electron microscopy investigation was performed to study the peptide effects on the EBs.

Methods

The synthetic peptides mastoparan L (ML) and mastoparan MO (MMO), clavanin (C) and clavanin MO (CMO), *Pa*-MAP 1.5 and *Pa*-Map 1.9, were diluted two-fold from 80 to 1.25 micrograms/mL. Tests were carried out on 36 isolates belonging to eight *Chlamydia* species, namely 18 *C. trachomatis* (D, E, F, G, H, I, J, K, L2 serovars), five *C. pneumoniae*, five *C. psittaci*, two *C. abortus*, two *C. pecorum*, one *C. avium*, two *C. suis* isolates and the *C. muridarum* strain Nigg. After the incubation of *Chlamydia* EBs for 2 h at room temperature with the synthetic peptides, the mixture was inoculated onto LLC-MK2 cells. EBs untreated with peptides were used as a control. After 48-72 h of incubation at 35° C the cultures were fixed and stained for the detection of *Chlamydia* immunofluorescent inclusions. The lowest peptide concentration required to achieve more than 50% reduction of chlamydial inclusions, compared to untreated control, was determined. All tests were run in triplicate and in three repeated experiments.
The electron microscopy investigation was performed on *C. pneumoniae* EBs treated with the peptides which presented an *in vitro* anti-chlamydial activity, using a trasmission electron microscope.

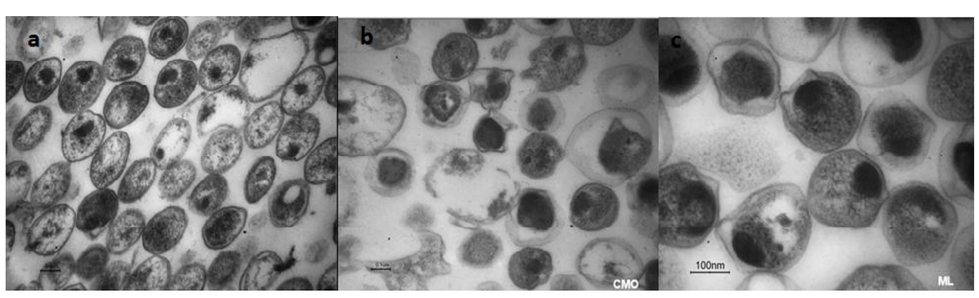

Figure 1. Transmission electron micrographs of *C. pneumoniae* EBs.
a) Untreated EBs; b) EBs treated with CMO; c) EBs treated with ML.

Results

As reported in Table 1, CMO reduced ≥50% the inclusion number of all 36 *Chlamydia* isolates at a concentration of 10 micrograms/mL; the infectivity reduction was greater than 90% at a 80 micrograms/mL concentration. ML was active against *C. trachomatis*, *C. pneumoniae*, *C. suis* and *C. muridarum* at 10 micrograms/mL and reduced ≥90% their infectivity at 80 micrograms/mL, but did not exert any inhibitory effect on the other *Chlamydia* species tested, even at 80 micrograms/mL. At the concentration of 80 micrograms/mL the peptides C, MMO, *Pa*-MAP 1.5 and *Pa*-MAP 1.9 were ineffective against all isolates. It is noteworthy that all the peptides showed, at the concentrations tested in the present study, no cytotoxic effect against LLC-MK2 uninfected cells.
The electron microscopy investigation on *Chlamydia* EBs treated with CMO or ML showed a membrane vacuolar degeneration, particularly evident in CMO-treated EBs (Figure 1).

Table 1. Activity of CMO, C, ML, MMO, *Pa*-Map 1.5 and *Pa*-Map 1.9 peptides against *Chlamydia* spp.

	Peptide concentration (µg/mL) reducing chlamydial inclusions by ≥50%					
Strains (n)	**CMO**	**C**	**ML**	**MMO**	***Pa*-Map 1.5**	***Pa*-Map 1.9**
***C. trachomatis* (18)**	10	>80	10	>80	>80	>80
***C. pneumoniae* (5)**	10	>80	10	>80	>80	>80
***C. psittaci* (5)**	10	>80	>80	>80	>80	>80
***C. suis* (2)**	10	>80	10	>80	>80	>80
***C. muridarum* (1)**	10	>80	10	>80	>80	>80
***C. pecorum* (2)**	10	>80	>80	>80	>80	>80
***C. abortus* (2)**	10	>80	>80	>80	>80	>80
***C. avium* (1)**	10	>80	>80	>80	>80	>80

Conclusions

The mechanism of action of antimicrobial peptides is primarily related to the global positive charge of most of them, leading to interaction with anionic microorganism membranes and the creation of pores, acting as detergents[4,7]. It is known that CMO interacts with bacterial lipid bi-layers causing changes in membrane morphology[8], mastoparan analogues cause bacterial membrane disruption[4] and Pa-MAP antimicrobial activity can be related to its hydrophopic interaction[6].
In the present study CMO, the most active peptide against all *Chlamydia* species tested, including the new recently identified *C. avium* species, could act at the level of *Chlamydia* membrane, thus confirming the data reported in literature about its activity on bacterial membrane. ML, active against *C. trachomatis*, *C. pneumoniae*, *C. suis*, *C. muridarum* species, could present a similar activity, too.
Further studies are needed to consider these peptides potential and promising compounds in the therapy of human and animal chlamydial infections with treatment failure, expecially if these peptides will proved active *in vivo* at the concentrations active *in vitro*, in the absence of toxic effect.

References

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