Effect of resveratrol in *Helicobacter pylori* clinical isolates studied by different methods

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Helicobacter pylori is associated with digestive diseases and it is considered a risk factor in the development of gastric cancer. Although several antibiotics and antacids have been used for treatment, there is a failure rate of up to 20%. The *in vitro* activity of natural substances, as phenolic compounds, against *H. pylori* was reported in some previous studies. Wine is a natural product containing polyphenolic compounds from 1.06 to 1.8 g/L in red wine and 0.16 to 0.30 g/L in white wine.

The aim of this study was to compare the effect of resveratrol containing compounds in *H. pylori* clinical isolates by different methods.

Methods: Pure resveratrol (RPu) and two different resveratrol compounds (R1 and R2) were studied in *H. pylori* clinical isolates. Disc diffusion and minimum inhibitory concentration (MIC) were used following standard recommendations. A propidium iodine method previously described, was studied to know the effect over membrane permeability. The activity of R1 and R2 by killing curves using standard procedures were also studied.

Results: The inhibition zones against *H. pylori* clinical isolates by disc diffusion was 15 mm for RPu, 15.7 mm for R1 and 17.9 mm for R2. RPu showed the lowest minimum inhibitory concentration using broth microdilution (8 and 32 mg/L). The less active compound was R1 with MIC=128mg/L in all the strain tested. When studied with propidium iodine, the three compounds produced membrane damage, being the effect of RPu the highest in 3 of the 5 strains tested, whilst R1 showed the highest in the other two.

Table 1 Emitted fluorescence by different strains treated with the phenolic compounds and with propidium iodine. A higher fluorescent level is related with higher membrane damage.

Strain tested	R1	R2	RPu
3177553	$21x10^3$	$2.4x10^3$	$0.3x10^3$
3166912	$2.5x10^3$	$9.9x10^3$	$12x10^3$
3167664	$1.9x10^3$	$3.7x10^3$	$138x10^3$
3175144	$1.9x10^3$	$3.7x10^3$	$138x10^3$
HP146128	$18x10^3$	$4x10^3$	$15x10^3$

R1 and R2 showed bactericidal activity against one of the *H. pylori* strains tested by killing curves and R1 showed bactericidal effect in another strain.

Conclusion: The different resveratrol compounds showed potent effect against *H. pylori* clinical isolates. R2 showed the highest effect by disc diffusion, probably due to a synergic effect with some unknown compounds able to diffuse in the agar. However, RPu showed the highest activity by microdilution test and by membrane damage studied with iodine propidium, due, probably, to the highest concentration of resveratrol.

Keywords: *Helicobacter pylori*; resveratrol

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Effect of Surface Charge on the Behaviour of Antimicrobial Peptide GL13K with Model Membranes

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GL13 peptides are novel thirteen-residue peptides that are derived from the sequence in the human salivary parotid secretory protein (PSP) that is believed to have antimicrobial properties [1]. GL13K is a small, cationic peptide with a net charge of +5 at physiological pH that has strong activity against Gram-negative and biofilm-forming bacteria while exhibiting low hemolytic and cytotoxic activity [2].

Model membrane studies have shown that GL13K likely attacks via the carpet mechanism by folding into ß-sheets when exposed to anionic membranes [3]. It removes lipids from the outer leaflet by forming lipid-peptide aggregates, causing the formation of transient, poorly-defined pores that produce slow, graded leakage. On the other hand, GL13K does not appear to interact with membranes in the absence of anionic lipids. In this study, we probe the impact of charge dilution and membrane rigidity on the mechanism of interaction using model membranes of 1,2-dioleoylphosphatidylglycerol (DOPG) and cholesterol at various mixture ratios. Specifically, the addition of cholesterol is utilized to investigate the effect of charge dilution and the subsequent effect on the electrostatic interactions between the membranes and GL13K peptides. Results from liposome (CD and carboxyfluorescein release assays) and monolayers studies (isotherms and PM-IRRAS) will be presented.

Keywords: antimicrobial peptide; model membranes; membrane disruption

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