EVALUATION ACTIVITIES OF CHITOSAN ASCORBATE AGAINST RODS OF *HELICOBACTER PYLORI* ISOLATED FROM GINGIVAL POCKETS AND ATHEROSCLEROTIC PLAQUES

Anna Kędzia[1], Barbara Kochańska[2*], Alina Gębska[1], Maria Wierzbowska[1], Andrzej Kufel[3]

[1] *Department of Oral Microbiology, Medical University of Gdańsk, Do Studzienki str. 38, 80-227 Gdańsk, Poland*

[2] *Department of Conservative Dentistry, Medical University of Gdańsk, E. Orzeszkowej str. 18, 80-208 Gdańsk, Poland*
e-mail: bkochan@gumed.edu.pl

[3] *Department of Vascular Surgery, Swissmed Hospital, Wileńska str. 44, 80-215 Gdańsk, Poland*

ABSTRACT

The aim of the study was determine activity of chitosan ascorbate against 17 strains of Helicobacter pylori. The strains were isolated from gingival pockets (7 strains), atherosclerotic plaques from carotid and femoral (10 strains). Chitosan was obtained from krill chitin and deacetylation degree was equal 60%. The ratio of ascorbic to chitosan was equal 1. The susceptibility of rods was determined by means of plate dilution technique in Brucella agar with 5% sheep's blood. The inoculum contained 10^5 CFU/spot. Incubation was performed in anaerobic jars with Campy Pak (BBL) for 48 hrs. The MIC was interpreted as the lowest concentration of chitosan ascorbate inhibiting the growth of bacteria. The results indicated, that chitosan ascorbate at the lowest concentrations was active against 35% of the strains. The Helicobacter pylori rods isolated from gingival pockets were the highest susceptible than isolated from atherosclerotic plaques.

Key wards: *susceptibility, chitosan ascorbate, atherosclerotic plaques, gingival pockets, Helicobacter pylori*

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1. Introduction

In 1983, spiral-shaped organisms were isolated from a human gastric biopsy specimen and were named *Campylobacter pyloridis* [**1**-**3**]. In 1989 *Campylobacter pylori* was renamed *Helicobacter pylori.* The *Helicobacter pylori* (*H. pylori*) is Gram-negative rods. The bacteria have a spiral shape in young cultures, but can assume coccoid forms in older cultures. The rods are highly motile (3-5 polar flagella) and produces an abundance of urease [**2**-**4**]. These bacteria are catalase- and oxidase- positive, but do not ferment or oxidise carbohydrates [**2**, **3**, **5**]. They can metabolize amino acids. The growth of rods is in microaerophilic conditions, with decreased oxygen and increased carbon dioxide [**2**, **5**]. *H. pylori* rods are protected from phagocytosis and intracellular killing by production the enzymes of catalase and super dismutase. The virulence is connected with vacuolating cytotoxin A that causes damage to host cells. The rods colonized the human gastrointestinal tract in approximately half of all adults and are associated with the development of peptic and duodenal ulcers, chronic gastritis, gastric cancer and has been suggested, with coronary heart disease, cardiovascular risk factors and atherosclerotic vascular disease [**6**-**9**]. Experimental studies have suggested that *H. pylori* can presence in atherosclerotic plaques to localize in carotid, femoral and iliac artery [**10**]. The rods has been found in the environmental of the oral cavity i.e. saliva, dental plaque and gingival pockets [**11**-**16**]. The question remains whether oral cavity is permanent reservoir of *H. pylori* and a potential source of reinfection [**11**].

The aim of the study was determine activity of chitosan ascorbate against strains of *Helicobacter pylori* isolated from various source.

2. Materials and Methods

A total of 17 strains of *Helicobacter pylori* isolated from gingival pockets (7 strains), atherosclerotic plaque from carotid (6 strains), femoral (4 strains) and 3 standards strains from following species: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were examined. Isolates were identified as *H. pylori* based on morphology, Gram staining and biochemical tests included production of catalase, oxidase and urease (API Campy, bioMerieux). Chitosan ascorbate was obtained from krill chitin and deacetylation degree was equal 60%. The ratio of ascorbic acid to chitosan was equal 1. In the date chitosan was used at the following concentrations: 2.0, 1.0, 0.5, 0.25, 0.12 and 0,06 mg/ml. The susceptibility of the rods was determined by means of the plate dilution technique in Brucella agar supplemented with 5% defibrinated sheep blood. The inoculum containing 10^5 CFU per spot were seeded upon the surface of the medium containing chitosan ascorbate or without chitosan ascorbate (growth control of tested strains). The agar plates were incubated in anaerobic jars with Campy-Pak Plus (BBL) at 37°C for 48 hrs. Minimal inhibitory concentrations (MIC) was interpreted as the lowest concentrations of chitosan ascorbate completely inhibiting the growth of the bacteria.

3. Results

The data presented in Table 1 show that chitosan ascorbate at the lowest concentrations (MIC in ranges ≤ 0.06 – 0.25 mg/ml) was active against 6 (35%) of the strains.

From amongst 17 examined strains of *H. pylori*, the rods isolated from gingival pockets were the highest susceptible than isolated from atherosclerotic plaques. The chitosan ascorbate inhibited the growth of 4 (57%) strains isolated from gingival pockets at low concentrations from ≤ 0.06 to 0.25 mg/ml. However remaining 3 strains were susceptible to high concentrations of chitosan ascorbate (MIC > 2.0 mg/ml). The strains of *H. pylori* isolated from carotid plaque were less sensitive. The growth of 2 (33%) of these strains were inhibited by chitosan at concentrations from ≤ 0.06 to 0.12 mg/ml. The growth of remaining 4 strains was inhibited by concentrations > 2.0 mg/ml. The chitosan ascorbate was the least

effective against strains of *Helicobacter pylori* isolated from femoral plaques. All strains were resistant to testing concentrations of chitosan ascorbate (MIC >2.0 mg/ml).

Table 1. Susceptibility of *Helicobacter pylori* strains isolated from gingival pockets and from atherosclerotic plaque to chitosan ascorbate

Microorganisms	Number of strains	Minimal inhibitory concentration MIC mg/ml						
		>2.0	2.0	1.0	0.5	0.25	0.12	≤0.06
Strains isolated from gingival pockets:								
Helicobacter pylori 1p	1	1						
Helicobacter pylori 2p	1					1		
Helicobacter pylori 3p	1	1						
Helicobacter pylori 4p	1					1		
Helicobacter pylori 5p	1							1
Helicobacter pylori 6p	1							1
Helicobacter pylori 7p	1	1						
Total	**7**	**3**				**2**		**2**
Strains isolated from atherosclerotic plaque in carotid:								
Helicobacter pylori 1s	1						1	
Helicobacter pylori 2s	1	1						
Helicobacter pylori 3s	1	1						
Helicobacter pylori 4s	1	1						
Helicobacter pylori 5s	1							1
Helicobacter pylori 6s	1	1						
Total	**6**	**4**					**1**	**1**
Strains isolated from atherosclerotic plaque in femoral artery:								
Helicobacter pylori 1u	1	1						
Helicobacter pylori 2u	1	1						
Helicobacter pylori 3u	1	1						
Helicobacter pylori 4u	1	1						
Total	**4**	**4**						
***Helicobacter pylori* strains Total**	**17**	**11**				**2**	**1**	**3**

In our previous study [**17**] *Helicobacter pylori* strains were isolated from pathological gingival pockets only and they were less susceptible to chitosan ascorbate. Concentrations of chitosan ascorbate in range of ≤ 0.06 – 0.5 mg/ml inhibited the growth of 30% those strains obtained from 10 patients with periodontal diseases. In our present data 57% tested strains isolated from gingival pockets was susceptible to concentrations from ≤ 0.06 to 0.25 mg/ml (Table 1).

The tested standard strains were more resistant to chitosan ascorbate (Table 2).

Table 2. Susceptibility of standards bacterial strains to chitosan ascorbate

Microorganisms	Number of strains	Minimal inhibitory concentration MIC mg/ml						
		>2.0	2.0	1.0	0.5	0.25	0.12	≤ 0.06
Staphylococcus aureus ATCC 25923	1		1					
Escherichia coli ATCC 25922	1	1						
Pseudomonas aeruginosa ATCC 27853	1	1						

4. Conclusions

The chitosan ascorbate at the lowest concentrations was active against 35% of the all tested strains. The *Helicobacter pylori* rods isolated from gingival pockets showed the highest susceptible to chitosan ascorbate than strains isolated from atherosclerotic plaques.

5. References

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