HELICOBACTER PYLORI: THOUGHTS, FACTS, HYPOTHESES

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Abstract In the article it is reported the results of laboratory researches on study of the influence of urease on the acidity of human gastric juice. Experiments in vitro show dramatic decrease in free HCl concentration in gastric juice in the presence of the enzyme urease. This fact testifies that Helicobacter pylori producing this enzyme is most likely a protection factor of gastric mucous tunic from peptic effect of HCl rather than a factor of aggression. This article is the beginning of new direction upon research of pathogenesis of gastric and duodenal ulcer disease.

Key words: Stomach ulcer, Helicobacter pylori, Acidity of gastric juice, ureasa, ammonia.

Relevance. Infective theory is the oldest theory that attempts to explain etiology and pathogeneses of ulcer disease of stomach and duodenum. As far back as 1851 H.Lebert in his experiments observed the development of ulcer in stomach of rabbits after pus introduction into their jugular vein. Supporters of this theory also considered that the cause of ulcers in stomach is bacterial invasion due to dysentery and puerperal infections in women (M.Letull, 1888), appendicitis (E.Payr, 1907), carious teeth (E. Rosenow, 1913) (1). Bacterial invasion from carious teeth can be explained somehow, but there is no any anatomical precondition contributing bacterial invasion from appendix and organs in small pelvis. In sepsis stomach affection can also take place in addition to metastases into other organs, but in this situation this lesion must be of purulent character like an abscess or phlegmon. Therefore this theory was not supported by most scientists of 20^{th} century. Owing to discovery of Helicobacter pylori by Australian scientists B.Marshall and R.Warren in 1983 infective theory got its second wind. It became reason for scientific sensation around this bacterium. This boom got stronger more than usual after awarding these scientists with Nobel Prize. Especially microbiologists and pathomorphologists, some pharmaceutical companies and their advertising agencies were extremely zealous. Scientific and popular editions were full of different articles. If they were all summarized, it would make approximately the following picture: Helicobacter pylori is a Gram-negative flagellant turbinal bacterium. It lives only in stomach. It produces the enzyme urease, which breaks up urea into ammonia and carbon dioxide. It is the only reason of ulcer disease of stomach and duodenum. And all clinicians implicitly admitted this postulate as axiom, though in the opinion of M.Woodley and A.Whelan the role of this bacterium in pathogenesis of ulcer disease is not completely cleared (2). And there is no word in defense of these unfortunate bacteria, though I am sure that in the world useful bacteria are more than pathogenic ones and these ones may be one of them. The following obvious facts point exactly to this: 1. Presence of recognized etiological and pathogenic factors of ulcer disease (stress, alimentary disorders, secretory malfunction of stomach, hormonal changes in the patient's organism and

others) (8). 2. Helicobacter pylori is detected in healthy people and even in some animals, particularly in ruminants and this fact emphasizes their physiological necessity in digestive process. 3. Frequently ulcerative process is found in duodenum, but there are no any bacteria there. 4. Despite of invasion of all mucus surface of stomach with these bacteria ulcers usually occur on lesser curvature. 5. After surgical treatment most of patients do not have recurrences of ulcer disease, although the major part of mucus surface is still invaded by Helicobacter pylori. Especially this is observed after vagotomy. Due to these circumstances it is reasonable to carry out research to prove my suggestion about probable role of Helicobacter pylori in protective process of stomach mucous tunic. And famous thesis of Doc.Shwarz from Zagreb "No acid – no ulcer" set a trend for this research.

Purpose of research. To study the influence of the enzyme urease on the acidity of gastric juice.

Materials and Methods. Gastric juice was taken in healthy people while they were undergoing preventive esophagogastrofibroscopy and in patients by aspiration with election suction pump on an empty stomach in the morning. The gastric juice of three health persons and seven patients with catarrhal-erosive gastroduodenitis was examined. The amount of gastric juice was 18-20 ml in healthy persons, and 50-70 ml in patients. As the main active factor of Helicobacter pylori is urease, which breaks out urea into ammonia and carbon dioxide, and it is also found in watermelon seeds, its suspension is prepared by grinding peeled watermelon seeds in mortar with 5 ml of distilled water (6). pH of gastric juice and suspension of watermelon seeds (urease) was tested by express method with test strip Combina 10M. The acidity of gastric juice was tested by Michaelis's method: 1% solution of phenolphthalein and 0.5% solution of dimethylamidoazobensol were added by drops into 5-10 ml of gastric juice and it was titrated with 0.1% solution of sodium hydroxide. The acidity of gastric juice was primarily detected. Then two test tubes were filled with 5-10 ml of gastric juice. Into the first test tube 5 ml suspension of 10 watermelon seeds was added, into the second tube 5 ml of distilled water was added, and they were shaken up, and titrated after being kept in room temperature for 15-20 min (7).

Results and Their Discussion. Results of laboratory tests of gastric juice are showed in Table 1 in absolute numbers.

As it can be seen from the table, in most patients free hydrochloric acid of gastric juice was 20-40 titre units (t.u.), total acidity – 35-62 t.u., the sum of free and bound HCl – 29-55 t.u., bound HCl – 12-30 t.u., and acid residue – 5-10 t.u. The results of titration of gastric juice by adding 5 ml of suspension of watermelon seeds were considerably different from the previous ones. In three cases free HCl was in the range of 1-4 t.u. In rest seven cases titration showed the absence of HCl in researched objects (0 t.u.). Total acidity of gastric juice on the contrary was insignificantly increased and became 40-76 t.u. Total result of free and bound HCl was not substantially changed, but specific weight of bound HCl was considerably increased and was 20-76 t.u. In two cases the amount of acid residue was noticeably raised (20-27 t.u.), but in rest seven cases they almost did not change. In portions of gastric juice with 5 ml distilled water in only three cases there was insignificant decrease in free

HCl titre by titration. In rest cases both free and bound HCl and total acidity did not change.

In all cases pH of gastric juice varied in the range of 5-5.5, and pH of suspension of watermelon seeds was 6.0-6.25.

Example. The patient of 39 years came with the result of esophagogastrofibroscopy from 13.11.2012: Post-ulcerative scarring of duodenal bulb. Acute catarrhal gastroduodenitis. Semitransparent colorless gastric juice with greyish tint was taken in amount of 50 ml and its pH was 5.5.

Test 1. 10 ml of gastric juice was taken into a test tube and a drop of 1% solution of phenolphthalein and 0.5% solution of dimethylamidoazobensol was added. Gastric juice got light pink color. Results of titration:

1st level (initial) - 22.0 (light pink tint)
2nd level - 24.0 (orange, salmon color)
3rd level - 25.0 (lemon)
4th level - 26.0 (stable pink with changing to crimson)

Calculation: Free HCl = 24.0 – 22.0 = 2.0 x 10 = 20
Total acidity = 26.7 – 22.0 = 4.7 x 10 = 47
Sum of free and bound HCl = 25.8 – 22. 0 = 3.8 x 10 = 38
Bound HCl = 38 – 20 = 18
Acid residue = 47 – 38 = 9

Test 2. 10 ml of gastric juice was taken into a test tube, and 5 ml of distilled water and a drop of each aforesaid solution were added. The color of mixture was light pink. Results of titration:

1st level (initial) - 33.1 (light pink)
2nd level - 35.1 (orange, salmon color)
3rd level - 35.6 (lemon)
4th level - 37.8 (stable pink with changing to crimson)

Calculation: Free HCl = 35.1 – 33.1 = 2.0 x 10 = 20
Total acidity = 37.8 – 33.1 = 4.7 x 10 = 47
Sum of free and bound HCl = 36.2 – 33.10 = 4.1 x 10 = 41
Bound HCl = 41 – 20 = 21
Acid residue = 47 – 41 = 6

Test 3. 10 ml of gastric juice was taken into a test tube, and 5 ml of aqueous suspension of 10 watermelon seeds and a drop of each solution were added. Mixture got orange coloring – salmon color. pH of mixture – 6.25. Results of titration:

1st level (initial) - 27.1 (orange, salmon color)
2nd level - 27.1 (orange, salmon color)
3rd level - 31.2 (lemon)

4th level - 32.2 (stable pink with changing to crimson)

Calculation: Free HCl = 27.1 – 27.1 = 0 x 10 = 0
Total acidity = 32.2 – 27.1 = 5.1 x 10 = 51
Sum of free and bound HCl = 31.7 – 27.1 = 4.6 x 10 = 46
Bound HCl = 46 – 0 = 46
Acid residue = 51 – 46 = 5

Next test was carried out with standard 0.1% solution of HCl to detect the direct buffer features of aqueous solution of urease. 5 ml of examined solution was taken into a flask, and we added a drop of each 1% solution of phenolphthalein and 0.5% solution of dimethylamidoazobensol. Solution got light pink coloring, which during titration changed into crimson being neither orange nor lemon. Titration of the same amount of solution by addition 5 ml of aqueous solution of watermelon seeds gave the same result, while in both cases the amount of used NaOH was equal – 100 t.u.

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Table No.1. Indices of the acidity of gastric juice in titration by Michaelis's method (in titre units)

Patients No.	Initial indices of the acidity of gastric juice (t.u.)					Indices of the acidity of gastric juice + of suspension of urease					Indices of the acidity of gastric juice + of distilled water				
	Free HCL	Total acidity	Sum of free and bound HCl	Bound HCl	Acid residue	Free HCL	Total acidity	Sum of free and bound HCl	Bound HCl	Acid residue	Free HCL	Total acidity	Sum of free and bound HCl	Bound HCl	Acid residue
1	**40**	60	55	15	5	**4**	50	***47***	***43***	3	**38**	60	55	17	5
2	**40**	80	70	30	10	**0**	55	***50***	***50***	5	**28**	75	68	40	7
3	**20**	54	44	24	10	**2**	42	***37***	***35***	5	**18**	50	44	26	6
4	**25**	56	48	23	8	**0**	54	***46***	***46***	8	**20**	56	46	26	10
5	**32**	62	52	20	10	**0**	76	***76***	***76***	0	**26**	60	52	26	8
6	**20**	40	32	12	8	**0**	43	***37***	***37***	6	**16**	38	32	16	6
7	**17**	35	29	12	6	**0**	40	***20***	***20***	20	**15**	35	28	13	7
8	**20**	47	38	18	9	**0**	51	***46***	***46***	5	**20**	47	41	21	6
9	**27**	53	44	17	9	**1**	53	***26***	***25***	27	**23**	51	44	21	7
10	**24**	50	40	16	10	**0**	60	***54***	***54***	6	**22**	48	38	16	10

Comparison of the results of laboratory studies shows significant decrease in concentration of free HCl in gastric juice up to its disappearance after addition of suspension of watermelon seeds and, on the contrary, increase in concentration of its bound fraction. As to total acidity, it does not have unidirectional changes: in four cases it somewhat lowered, and in five cases they even increased, which is an evidence of their independence from the influence of urease. The absence of reliable reduction of the acidity of gastric juice after addition of distilled water indicates the presence of neutralizing effect of suspension of watermelon seeds containing urease on free HCl, which of itself is a proof of that it protects stomach mucous tunic from acid aggression. But how does it work? The aqueous suspension of watermelon seeds is not basic; its pH is in the range of 6.0-6.25, which excludes its direct buffer influence on the acidity of gastric juice. Studied with standard solution of HCl make us think that the difficulty is in the interactions between urease and elements of gastric juice. Urease is in the class of hydrolytic enzymes of amidase group. Urea is decomposed into ammonia and carbon dioxide by urease. And urea is the main component of gastric mucus. Reaction catalyzed by urease is processed in the following way (4):

$$H_2N - CO - NH_2 + H_2O = 2NH_3 + CO_2$$

Maybe here is hidden the clue of our problem. Let's look at this more deeply. Gastric mucus is a viscous substance, the function of which is the protection of gastric walls from strong peptic action of proteolytic enzymes and HCl, and it exists in two forms: gel (insoluble mucus) and soluble in gastric juice (soluble mucus), and is mucopolysaccharide by chemical structure. Polysaccharide part of mucus molecule consists of amino sugars for 50%; protein part consists of threonine, serine, alanine and proline for 50%. Peptic bonds between these amino acids give important physical feature to mucus – ability to gel formation and gives considerable resistance to the influence of HCl and pepsin. Exactly this chemical process occurs with the participation of urease (5). Resistance of insoluble mucus to the influence of HCl and pepsin is wrongly considered as protective feature. Meanwhile everyone knows that protective feature of mucus is conditioned most likely not by mechanic barrier on the way of HCl and pepsin, but by chemical process, which is more typical for complex organisms. HCl and pepsin is used up, that is neutralized by substance that is sensitive to their effect. Such substance is soluble part of gastric mucus, which is formed by the dissociation of polymer mucus (gel) after the breakup of urea molecule by urease. And this enzyme is the main life product of these bacteria. So it can be suggested that by nature they are intended for the protection of gastric mucous tunic from aggressive effects of HCl and pepsin during starvation. In fact, during the assessment of these bacteria's features first of all we must proceed according to the principle of presumption of innocence. It is doubtful that the nature put some inaccuracy to the structure of its best creature, which does not correspond to the aim of life. It is highly probable, quite the contrary, Helicobacter pylori likely participate in digestive process intended to supply the organism with energy during starvation for account of internal resources (saliva, gastric mucus), hence organizing a kind of

wasteless production and simultaneously protection of gastric mucous tunic. Indirect proof for this can be that in the proventriculus of ruminants there are microorganisms, which produce urease that contributes the processing of urea introduced with forage.

Thus summing up the results of laboratory studies the following may be concluded:

1. In the presence of urease the concentration of free HCl in gastric juice decreases dramatically (up to zero). The amount of bound HCl increases, and total acidity does not change.

2. Neutralization of free HCl of gastric juice occurs not by the direct influence on the free fraction of HCl, but by participation of gastric mucus.

3. Helicobacter pylori producing urease are most likely protectors of gastric mucous tunic from peptic effect of HCl.

4. Urease contributes the slowdown of digestive process in stomach by neutralizing free HCL of gastric juice and development of flatulence, for prevention of which it is recommended to take urease inhibitors, for example, green tea.

5. Watermelon seeds containing urease can serve as the basis for preparation of antiulcer agents.

We have a little looked into Helikobacter pylori, and I hope that we have a bit shattered the myth about their harmfulness. But what to do with the gases formed during their life activity in the stomach? As we know, carbon dioxide is a component of atmosphere and it is proven that it is harmless. What about ammonia?

Ammonia is a toxic gas with sharp irritating smell that causes unpleasant smell during belching. Physiological threshold concentration in gastric air is 0.4 mg/m^3. Ammonia in gastric juice is in the range of 20-80 mmol/L (Smirnova GP, North national medicine university). The role of “residual” ammonia in patients with ulcer disease is thoroughly studied by Gojenko AI and Avramenko AA (3). According to their data the concentration of ammonia in gastric juice is directly proportional to the severity of inflammatory process around the ulcer and it reduces with the decline of inflammation. In the opinion of the authors ammonia causes ulcer formation and keeps its activity, although simultaneously they admit the role of ammonia in neutralization of HCl of gastric juice. Thus the question in this situation is that how may the drug reducing acidity of the gastric juice be ulcerogenic factor and why do we still treat ulcer with the drugs that decrease acidity of gastric juice? Most likely, ammonia is contrarily physiological necessary agent. And increase in its concentration in accordance with the degree of destructive lesion of gastroduodenal area is explained with the rise in mucus formation in stage of acute ulcer. Gastric mucus containing urea as its component is a supplier of ammonia. If we admit the usefulness of Helicobacter pylory for human organism, then we have to acknowledge that the presence of permissible concentration of their production – ammonia in gastric juice and gas bubble of the stomach is physiological. How can ammonia be useful? The search of the answer to this question has led to the following thoughts:

First. In medicine 10% solution of ammonia is used to excite the breathing and recover consciousness of patients in syncope. Maybe exactly this feature is used by the nature to activate respiratory center of patients with apnea. Taking into consideration that this syndrome that can sometimes be the death cause is often found

in elderly people who has functional insufficiency of cardia and gastroesophegeal reflux due to age-related atrophy of gastric mucous tunic we should acknowledge the wisdom of the nature.

Second. Liquid ammonia is used to tend the places of bee, wasp or mosquito sting. And 0.5% solution was used by surgeons of preparation of their hands for operation (Spasokukkoski-Kochergin's method). So the presence of ammonia in stomach is essential condition aimed for decontamination of the food entering the stomach.

Third. Ammonia is used in food industry for treatment of meat beyond its shelf life to give it marketable state. So it is not excluded that ammonia plays a role of preservative in long-term stay of food in the stomach.

Fourth. Ammonia is used in fridge industry to freeze (freezing agen R717). Maybe this is a feature necessary to decrease temperature in the stomach during intake of hot food, tea, coffee.

Fifth. Ammonia is lighter than air, so involved in the formation of a gas bubble stomach on fundus. I guess plays the role of a hot air balloon or parachute supports suspended from the stomach into the upright position.

Thus, ammonia in gastric juice and gastric gas with normal concentration of 4.99-9.99 mmol/L is one the necessary natural conditions that enables normal course of vital processes in human organism. Listed advantageous features of ammonia once more confirms that the presence Helicobacter pylory in gastric mucous tunic is essential form of cohabitation, distinctive symbiosis of macro and microorganism to keep natural harmony of coexistence of live organisms with multipurpose assignations. Therefore, struggle against these bacteria as antihelicobacter therapy disturbs not only this harmony and endoecology of human organism, but also damages budget of public health system and families of patients with ulcer disease.

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