ФУНКЦИОНАЛЬНАЯ И МЕТАБОЛИЧЕСКАЯ АКТИВНОСТЬ НЕЙТРОФИЛЬНЫХ ГРАНУЛОЦИТОВ КРОВИ У ДЕТЕЙ С ЭРОЗИВНО-ЯЗВЕННЫМ ПОРАЖЕНИЕМ ЖЕЛУДКА И 12-ПЕРСТНОЙ КИШКИ С ВЫЯВЛЕННОЙ ИНФЕКЦИЕЙ *HELICOBACTER PYLORI*

Литвинова И.С., Коленчукова О.А.

Научно-исследовательский институт медицинских проблем Севера – обособленное подразделение ФГБНУ «Федеральный исследовательский центр "Красноярский научный центр Сибирского отделения Российской академии наук"», г. Красноярск, Россия

Резюме. Одним из ведущих этиопатогенетических факторов формирования язвенной болезни желудка и двенадцатиперстной кишки у детей и взрослых в настоящее время рассматривается бактерия *Helicobacter pylori*. Несмотря на множество исследований в данной области, механизмы фагоцитарной активности в ответ на воздействие *Helicobacter pylori* до конца не ясны. Целью работы являлось получение результатов по функциональной и метаболической активности нейтрофильных гранулоцитов крови у детей с *Helicobacter pylori*-ассоциированным эрозивно-язвенным поражением желудка и двенадцатиперстной кишки. Объектом исследования являются нейтрофильные гранулоциты, выделенные из крови больных и контрольной группы. Образцы были взяты у 46 лиц с *Helicobacter pylori*-ассоциированным эрозивно-язвенным поражением желудка и 12-перстной кишки в возрасте от 11 до 18 лет и контрольную группу, которую составляли 55 практически здоровых лиц, у которых было исключено данное заболевание в аналогичном возрастном диапазоне. Проведен сравнительный анализ функциональной активности клеток с помощью хемилюминесцентного анализа и метаболической активности биолюминесцентным методом. В качестве активатора хемилюминесценции использовался люминол. Измерение функциональной активности фагоцитов основывалось на определении базовой активности (спонтанная реакция) и резервных возможностей клеток при воздействии на них неспецифическим индуктором зимозаном. Наблюдается пониженный индекс активации нейтрофилов больных относительно контрольной группы, что может характеризовать пониженные метаболические резервы клеток. В нейтрофильных гранулоцитах происходит снижение Г6ФДГ-фермента, который запускает гликолиз по пентозофосфатному пути и способствует восстановлению никотинамидаденин-динуклеотидфосфата (NADP) до NADPH, который необходим для образования восстановленного глутатиона, связывающего окислители. При его недостаточности происходит снижение энергетических запасов клеток. В нейтрофильных гранулоцитах крови у детей с эрозивно-язвенным поражением желудка и 12-перстной кишки с выявленной инфекцией *H. pylori* наблюдается понижение метаболических резервов, что связано с ингибированием метаболических процессов в клетках.

Ключевые слова: Helicobacter pylori, гастрит, язва, нейтрофильные гранулоциты, функциональная активность, метаболизм, ферменты

Адрес для переписки:

*Коленчукова Оксана Александровна
Научно-исследовательский институт медицинских проблем Севера
660022, Россия, г. Красноярск,
ул. Партизана Железняка, 3г.
Тел.: 8 (923) 573-00-98.
E-mail: i.s.litvinova93@gmail.com*

Address for correspondence:

*Oksana A. Kolenchukova
Research Institute of Medical Problems of the North
3g Partizan Zheleznyak St
Krasnoyarsk
660022 Russian Federation
Phone: +7 (923) 573-00-98.
E-mail: i.s.litvinova93@gmail.com*

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FUNCTIONAL AND METABOLIC ACTIVITY OF BLOOD NEUTROPHILIC GRANULOCYTES IN CHILDREN WITH EROSIVE AND ULCERATIVE LESIONS OF THE STOMACH AND DUODENUM WITH DETECTED *HELICOBACTER PYLORI* INFECTION

Litvinova I.S., Kolenchukova O.A.

Research Institute of Medical Problems of the North, Krasnoyarsk Science Center, Siberian Branch, Russian Academy of Sciences, Krasnoyarsk, Russian Federation

Abstract. The *Helicobacter pylori* bacterium is currently considered one of the leading etiopathogenetic factors in the formation of gastric and duodenal ulcer in children and adults. Despite many studies in this area, the mechanisms of phagocytic activity in response to exposure to *Helicobacter pylori* are not completely clear. The aim of the work was to obtain results on the functional and metabolic activity of blood neutrophilic granulocytes in children with *Helicobacter pylori*-associated erosive and ulcerative lesions of the stomach and duodenum. The object of the study are neutrophilic granulocytes isolated from the blood of patients and the control group. Samples were taken from 46 persons with *Helicobacter pylori*-associated erosive – ulcerative lesions of the stomach and duodenum aged 11 to 18 years and the control group, which consisted of 55 practically healthy persons who had this disease excluded in the same age range. A comparative analysis of the functional activity of cells using chemiluminescent analysis and metabolic activity by the bioluminescent method was carried out. Luminol was used as a chemiluminescence activator. The measurement of the functional activity of phagocytes was based on the determination of the base activity (spontaneous reaction) and the reserve capacity of the cells when they were exposed to the nonspecific inducer zymosan. There is a reduced activation index of neutrophils in patients relative to the control group, which may characterize reduced metabolic reserves of cells. In neutrophilic granulocytes, there is a decrease in G6PDG, an enzyme that triggers glycolysis along the pentose phosphate pathway and contributes to the reduction of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH, which is necessary for the formation of reduced glutathione that binds oxidants. With its insufficiency, a decrease in the energy reserves of cells occurs. In neutrophilic blood granulocytes in children with erosive and ulcerative lesions of the stomach and duodenum with *H. pylori* infection, a decrease in metabolic reserves is observed, which is associated with inhibition of metabolic processes in cells

Keywords: Helicobacter pylori, gastritis, ulcer, neutrophilic granulocytes, functional activity, metabolism, enzymes

Introduction

One of the leading etiopathogenetic factors in the formation of peptic ulcer of the stomach and duodenum in children and adults is currently considered the bacterium *Helicobacter pylori*. The severity of the clinical course of helicobacteriosis largely depends on the degree of pathogenicity of the pathogen strains. Despite many studies in this area, the mechanisms of phagocytic activity in response to exposure to *H. pylori* are not completely clear [1, 2, 5].

Materials and methods

The objects of the study were neutrophilic granulocytes isolated from venous blood in 101 people: 46 people with *H. pylori*-associated erosive and ulcerative lesions of the stomach and duodenum aged 11 to 18 years and the control group, which consisted of 55 practically healthy individuals of the same age range, and who hadn't had the disease.

The indicators of the following oxidoreductases were determined: glucose-6-phosphate dehydrogenase (G6PDH), glycerol-3-phosphate dehydrogenase (G3PDH), malic enzyme (NADPMDH), NAD^+ and NADH-dependent reaction of lactate dehydrogenase (NALDDH and NADHLDH), NAD^+ and NADH-dependent reaction of malate dehydrogenase (NADMDH and NADHMDG), $NADP^+$ and NADPH-dependent reaction of glutamate dehydrogenase (NADPGDH and NADPHNGDG), NAD^+ and NADH-dependent reaction of glutamate dehydrogenase (NADHDH and NADHMDG), NAD^+ and NADP-dependent isocitrate dehydrogenases (NADICDH) and NADFITSDG). Enzyme activity is expressed in enzymatic units (1 E = 1 μmol/min) per mg of protein. The study was carried out on the NAD(P)H:FMN oxidoreductase-luciferase enzymatic system.

One of the most sensitive methods for assessing the formation of ROS (reactive oxygen species) is

chemiluminescence analysis. Luminol is used as a chemiluminescence activator. The determination of the functional activity of phagocytes was based on the determination of the base activity (spontaneous reaction) and the reserve capacity of the cells when exposed to the nonspecific inductor zymosan. Bioluminescent and chemiluminescent analyzes were carried out on a BLM-3607 chemiluminescent analyzer (Russia).

Results and discussion

In the study of chemiluminescence in patients' neutrophil fraction with the inducer luminol, a decrease in the time to reach the peak is observed, but an increase in intensity and an area under the curve relative to the control. The activation index is more than 2 times lower in the patients' group (Figure 1).

Metabolic enzymes of neutrophilic granulocytes, which are indicators of intracellular metabolism, were also studied. They take an active part in bioenergetic processes. Also, they participate in the directed coordination of conjugated metabolic flows, largely determine the adaptive changes in cellular metabolism. Thus, there was a decrease in G6PDH, NADHLDH, NADFICDH, NADICDH, NADLDH, NADMDH, NADFMDH, NADDMDH, NADPHNG, NADNGDH with an increase in the activity of G3PDH and NADHDG (Figure 2).

It was found that in the luminol-dependent process, an increase in the area under the curve and the maximum intensity of the luminescence of neutrophils in the blood of patients relative to control neutrophils determines an increased level of production of reactive oxygen species, which in general may reflect the cytotoxic activity of neutrophilic granulocytes in the focus of inflammation. However, there is a reduced activation index of neutrophils in patients relative to the control group, which may characterize reduced metabolic reserves of cells [4].

Thus, in neutrophilic granulocytes, there is a decrease in G6PDG, an enzyme that triggers glycolysis along the pentose phosphate pathway and contributes to the reduction of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH, which is necessary for the formation of reduced glutathione that binds oxidants. With its insufficiency, there is a decrease in the energy reserves of cells [1]. Also, in the group of patients, the LDH enzyme, one of the key enzymes of glycolysis, is reduced. At the same time, the enzymatic reaction, which can compensate for the outflow of substrates from glycolysis, is catalyzed by G3PD, the activity of which is increased in neutrophils in the group of patients [3]. There is also an increase in NADHDH activity in patients, which indicates substrate stimulation of the Krebs cycle by products of amino acid metabolism reactions. One of the metabolic systems maintaining the hydrogen gradient is the malate-aspartate shunt, the key reaction of which is carried out by NADHMDH. In blood neutrophils in the group of patients, its activity is reduced relative to the parameters of the control group.

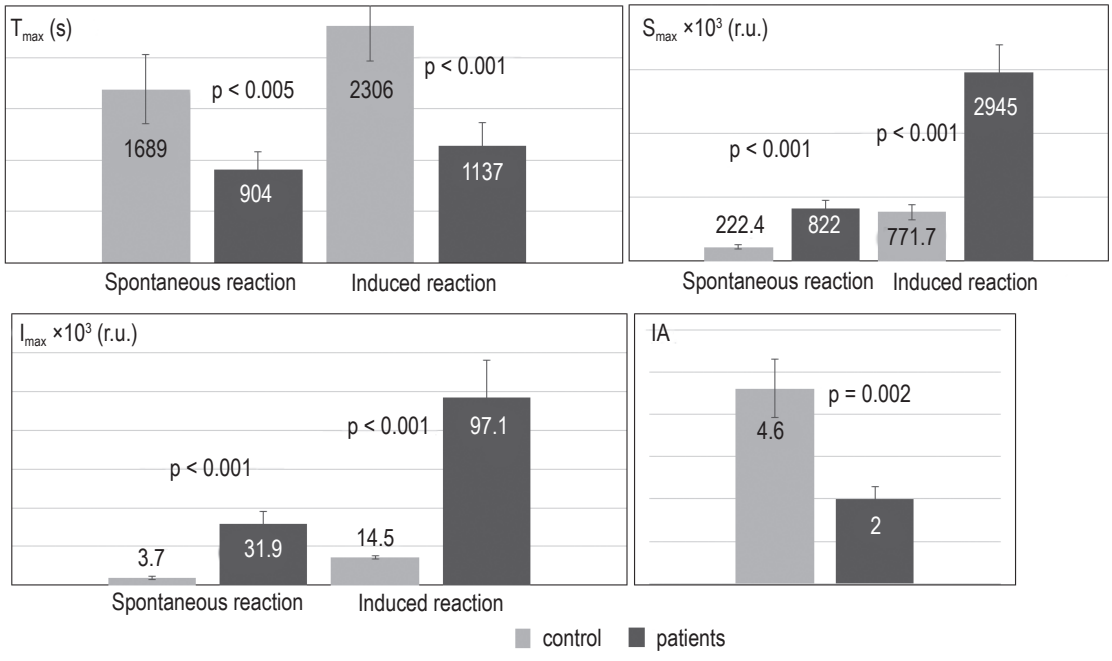

Figure 1. Luminol-dependent chemiluminescence of the patients' neutrophilic granulocytes and the control group
Note. T_{max}, time to reach the reaction peak; I_{max}, maximum luminescence intensity; S_{max}, area under the curve; IA, activation index.

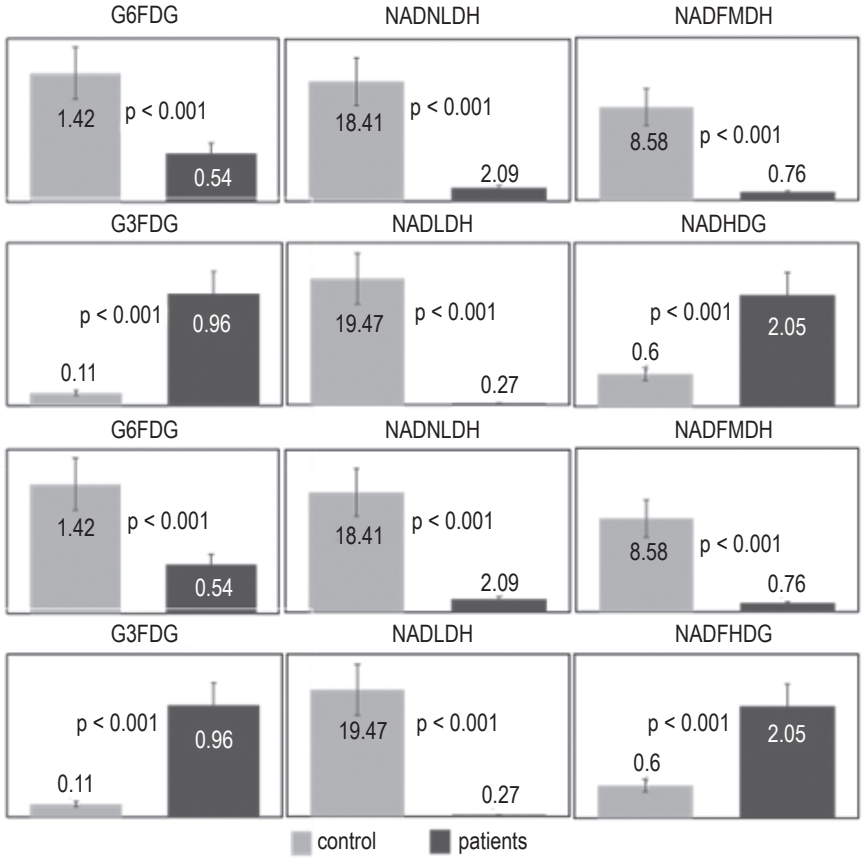

Figure 2. Bioluminescent activity of NAD(P)H-dependent enzymes in the patients' neutrophilic granulocytes of patients and the control group

Conclusion

In neutrophilic blood granulocytes in children with erosive and ulcerative lesions of the stomach and duodenum with *H. pylori* infection, a decrease in metabolic reserves is observed, which is associated with inhibition of metabolic processes in cells.

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Авторы:

Литвинова И.С. *– аспирант Научно-исследовательского института медицинских проблем Севера ФИЦ КНЦ СО РАН, г. Красноярск, Россия*

Коленчукова О.А. *– д.б.н., доцент, ведущий научный сотрудник Научно-исследовательского института медицинских проблем Севера ФИЦ КНЦ СО РАН, г. Красноярск, Россия*

Authors:

Litvinova I.S., *Postgraduate Student, Research Institute of Medical Problems of the North, Krasnoyarsk, Russian Federation*

Kolenchukova O.A., *PhD, MD (Biology), Associate Professor, Leading Research Associate, Research Institute of Medical Problems of the North, Krasnoyarsk, Russian Federation*

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