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INTERRELATION OF ENDOThelial NITRic OXIDE SYNTHASE ACTIVITY in TISSUES oF the STOMACH AND MAGNesium BALANCE in the PERIOD oF EroSIVE-ULCERATIVE ACID-INDUCED LEsIoN DEVELOPMENT in Rats with DIFFerent RESISTANCE to STRESS

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ВЗАИМОСВЯЗЬ АКТИВНОСТИ ЭНДОТЕЛИАЛЬНОЙ НИТРОКСИДСИНТАЗЫ В ТКАНЯХ ЖЕЛУДКА И МАГНИЕВОГО БАЛАНСА В ПЕРИОД ФОРМИРОВАНИЯ ЭРОЗИВНО-ЯЗВЕННОГО ДЕФЕКТА АЦЕТАТНОЙ ПРИРОДЫ У КРЫС С РАЗНОЙ УСТОЙЧИВОСТЬЮ К СТРЕССУ

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Acetate stomach ulcer was experimentally modeled in stress resistant and stress nonresistant rats for immunohistochemical identifying a specific number and expression intensity of eNOS-positive cells and determination of the magnesium level in biological media. The content of intra-erythrocyte magnesium in reaction with titanium yellow was reduced in rats with different resistance to stress in low eNOS activity in the gastric mucosa, in the muscular layer in stress nonresistant animals and almost complete absence of antigen-positive cells in the submucosa. A positive correlation was found between the level of intra-erythrocyte magnesium and the specific number of eNOS-positive cells in the gastric mucosa in both groups of animals and also between expression intensity in the submucosa of stress resistant rats. A relationship was established between the magnesium content, specific number and expression intensity of eNOS in stress nonresistant rats in the stomach submucosa.
В эксперименте на стрессоустойчивых и стрессонезависимых крысах моделировалась ацетатная язва желудка с оценкой иммуногистохимическим методом удельного числа и интенсивности экспрессии eNOS-позитивных клеток и уровня магния в биологических средах. По окончании эксперимента уровень внутриэпителиальной мембраны в реакции с титановым желтым снизился у крыс с разной устойчивостью к стрессу на фоне низкой активности eNOS в слизистой оболочке желудка, у стрессонезависимых животных — и в мышечной оболочке при практически полном отсутствии eNOS в подслизистом слое. Установлена прямая корреляционная связь между содержанием магния у крыс в обеих группах и удельным числом eNOS-позитивных клеток в слизистой оболочке желудка. У стрессонезависимых крыс определена зависимость между содержанием магния, удельным числом и интенсивностью экспрессии eNOS в подслизистом слое.

Ключевые слова: эндотелиальная нитроглайдсинтаза, магний, ацетатная язва, стрессоустойчивые, стрессонезависимые крысы

Helicobacter pylori-induced ulcers of the gastrointestinal tract (GIT) presents chronic inflammation. The ulcer models highly resemble acid-induced ulcers in terms of development. It has been revealed that an infiltrative-destructive inflammation area primarily induced by acetic acid is infected once more with Helicobacter pylori in experimental animals [4, 10].

The aim of the study was to determine resistance in gastric tissues by identifying a specific number and intensity of endothelial nitric oxide synthase (eNOS, NOS3) in gastric tissues damaged by ulceration and the role of magnesium, on the one hand, as metabolic activator and on the other hand, as calcium (Ca++), antagonists that activates eNOS remains insufficiently studied.

The aim of the study was to determine resistance in gastric tissues by identifying a specific number and intensity of endothelial nitric oxide synthase expression in the mucous membrane, submucosa and muscular layer of the stomach and impact on indicators of its activity of changes in the level of intra-erythrocyte magnesium in stress resistant and stress nonresistant rats with experimental acute ulcer.

Material and Methods. Experiments were performed on white Wistar rats of both sexes on a standard diet. All experimental animals were divided by the «open field test» into stress resistant and stress nonresistant groups.

Experimental record and withdrawal of animals from the experimental studies were performed in accordance with the principles of bioethics set forth in the «The International Guiding Principles for Biomedical Research Involving Animals» (1985) and the order of the Ministry of Health of the Russian Federation No 708n dated August 23, 2010 «On approval of rules for laboratory practice».

The 3 series of experiments were performed in each group. The first series involved intact animals (4 rats each). In the second control series (4 paraffin-embedded rats), modeling of acetic ulcer without damage of the gastric mucosa by acetic acid was simulated in rats under Nembutal anesthesia (40 mg/kg of body weight). In the third series (4 rats each) — acetic ulcer was modeled in the prepyloric region by the method of Okabe S. [9].

Blood was taken from the subclavian vein (Vs) before starting the experiment and in 7 days after completing, urine samples were collected in over a period of 24 hours.

Stomach tissues in all experimental animals were taken from the area of ulcerative defect for immunohistochemical examination with a preliminary visual assessment of ulcerative defect square.

Monoclonal antibodies to eNOS («Novocastra», Great Britain), explored for work with paraffin sections, were used to identify endothelial nitric oxide synthase.

The reaction was evaluated by a semi-quantitative method, taking into account the intensity of staining and a specific number of antigen-positive cells in the tissues of the pyloric region of the stomach. The immunohistochemical reaction was assessed as negative — «0» (no reaction), weak-positive «1» (weak colored cells), moderately positive — «2» (cells of mean color intensity) and strongly positive or overexpression — «3» (cells of high color intensity) [6].

Mg²⁺ content in the blood plasma and daily urine volume were determined by reagents of the set of «Lahe-ma» company. Concentration of Mg²⁺ in the erythrocyte mass of the blood was determined according to Kamysnikov V. S. [5].

The mean values under normal distribution were expressed as M±m, statistical significance of differences was assessed according to Student’s t-test; if distribution differed from normal — as the median (Me), 25/75 percentile, significance of differences was assessed using the Wilcoxon test in the level of evidence Q <0.05. Results of the study were processed by a statistical variation method using standard Data Analysis Tools of Microsoft Excel.

Results and Discussion. A defect of the gastric mucosa, usually in a form of crater with a granulation shaft, hyperemia, hemorrhages, developed in stress resistant and stress nonresistant animals after 7 days of modeling of acetic ulcer. The area of ulceration in stress resistant rats was 38.72±4.09 mm², in stress nonresistant ones it was 68.7±9.44 mm² (Table 1).

The results of the study in rats with different resistance to stress showed that a significant number of eNOS-positive cells with low expression intensity was detected in the gastric mucosa in stress resistant animals at the initial state, while a trace number of antigen-positive cells was detected in the submucosa, also with trace expression intensity. A specific number of cells positive to eNOS was greater in the muscular layer than in the submucosa, but less than in the mucous membrane with minimal expression intensity.

A moderate number of eNOS-positive cells had average expression intensity in stress nonresistant rats in the mucous membrane. A specific number of cells expressing endothelial nitric oxide synthase was significantly higher with minimal intensity of staining in the submucosa compared to stress resistant animals. But both a specific number and intensity of eNOS expression had a trace character in the muscular layer.
Specific number and expression intensity of eNOS-positive cells in stress resistant and stress nonresistant rats with acetate stomach ulcer (Me [25 and 75 percentile])

<table>
<thead>
<tr>
<th>Stomach tissue</th>
<th>Index</th>
<th>Initial state</th>
<th>Control series</th>
<th>Acetate stomach ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress resistance</td>
<td>Expression intensity</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Submucosa</td>
<td></td>
<td>1</td>
<td>[0; 0]</td>
<td>0</td>
</tr>
<tr>
<td>Muscular layer</td>
<td></td>
<td>1</td>
<td>[1; 1]</td>
<td>0</td>
</tr>
<tr>
<td>Specific number</td>
<td></td>
<td>50</td>
<td>[17.5; 41.25]</td>
<td>0</td>
</tr>
</tbody>
</table>

The note: * – significance of the differences between stress resistant and stress nonresistant rats in different experiment series. Q<0.05; ** – stress resistant rats; *** – stress nonresistant rats.

In the control series, a number of eNOS-positive cells decreased in the gastric mucosa in stress nonresistant animals and greatly in stress resistant rats, reaching approximately equivalent values in a maximum increase of expression intensity in both groups. A specific number of antigen-positive cells was minimal with a minimal degree of staining of the submucosa in both groups of the experimental rats. The number of eNOS-positive cells significantly decreased in stress resistant rats sharply increased in stress nonresistant animals in the muscular layer in average expression intensity in both subgroups.

Modeling of control series, including laparotomy and layer-by-layer closure of the abdominal wall, can be regarded to as a stress model of moderate intensity. Analysis of the results under study shows that under such conditions which G. Selye defined as a general adaptation syndrome, a specific number of antigen-positive cells, expression intensity were approximately at the same level in the mucosa and submucosa of the stomach in both groups of the animals with different resistance to stress. Stress reduces the number of cells expressing eNOS in the gastric mucosa and submucosa and differences are observed only in the muscular layer of damaged stomach tissues: a specific number of antigen-positive cells obviously decreases in stress resistant rats, and evidently increases in moderate expression intensity in stress nonresistant animals.

Endothelial nitric oxide synthase is a calcium-dependent enzyme [2]. It is known that a functional calcium antagonist is magnesium. At the initial state the magnesium level in the erythrocyte mass of blood taken from the subclavian vein in stress-resistant rats is 25.9 % lower than in stress nonresistant rats(p<0.001). Its content in blood plasma in rats with different resistance to stress is almost identical (p>0.1), but the loss of Mg²⁺ ions with the daily urine in stress-resistant animals is higher up to 220.3 % (p<0.001) compared to stress nonresistant rats (Table 2).

The area of ulcerative defect and the magnesium level in biological media in stress resistant and stress nonresistant rats with acetate stomach ulcer (M±m)

<table>
<thead>
<tr>
<th>Index</th>
<th>Initial state</th>
<th>Control series</th>
<th>Acetate stomach ulcer</th>
<th>Initial state</th>
<th>Control series</th>
<th>Acetate stomach ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress resistant rats</td>
<td>Stress nonresistant rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area of the ulcer</td>
<td>-</td>
<td>-</td>
<td>36.7±4.09</td>
<td>-</td>
<td>-</td>
<td>68.7±9.44</td>
</tr>
<tr>
<td>Mg²⁺ in the blood plasma from Vs, mmol/L</td>
<td>0.82±0.022</td>
<td>0.93±0.019***</td>
<td>0.91±0.006</td>
<td>0.88±0.012</td>
<td>0.99±0.019</td>
<td></td>
</tr>
<tr>
<td>Mg²⁺ in the erythrocyte mass of the blood from Vs, mmol/l</td>
<td>1.42±0.101</td>
<td>1.75±0.094*</td>
<td>1.21±0.012***</td>
<td>1.91±0.074</td>
<td>2.15±0.184</td>
<td>1.48±0.175#</td>
</tr>
<tr>
<td>Mg²⁺ in the daily urine, μmol/l</td>
<td>27.00±4.50</td>
<td>18.59±0.416</td>
<td>7.15±0.19***</td>
<td>8.43±1.998</td>
<td>12.81±0.527#</td>
<td>15.11±2.522</td>
</tr>
</tbody>
</table>

The note: * in relation to the previous parameter in stress resistant rats: * – p < 0.05; ** – p < 0.01; *** – p < 0.001; in stress nonresistant rats: # – p < 0.05; ## – p < 0.01; ### – p < 0.001.

Magnesium activates more than 300 enzymes of energy formation and energy consumption [8]. The high level of magnesium probably leads not only to a decrease of the functional activity of calcium, but creates more optimized conditions for energy production and energy consumption, and participates in the stabilization of non-coding mRNAs.

In general, a higher concentration of intracellular magnesium in low stress-resistance of intact animals is associated with a lower specific number of eNOS-positive cells in both the mucosa and in the submucosa, and their almost complete absence in the muscular layer of the stomach.

In the control series of experiments there was no significant difference in the magnesium content in the erythrocyte mass of blood from Vs in stress nonresistant animals in comparison with stress resistant rats. The content of Mg²⁺ in blood plasma from Vs in stress resistant animals in relation to stress nonresistant rats is more up to 5.7 % (p<0.05). Loss of magnesium with the urine in the rats with high resistance compared to stress nonresistant rats was higher up to 31.1 % (p<0.001). In this case, the magnesium content in the urine of stress resistant animals of the control series decreased by 31.2 % (p<0.001) in comparison with the initial state, and the magnesium content increased to 52 % (p<0.05) in stress nonresistant ones.

Analysis of the obtained results shows that in the control series the leveling of the magnesium content between the groups of animals with different resistance to stress is associated with the leveling of the specific number and intensity of endothelial nitric oxide synthase expression in the mucous membrane, submucosa and expression intensity in the muscular layer.

In case of acetate ulcer the specific number of eNOS-positive cells in the mucous membrane and mus-
cicular layer increased from 7.5 [4;11.25] to 22.5 [20; 27.5], (Q<0.05) and from 7.5 [5;11.25] to 52.5 [33.75;75] (Q<0.05) in rats with high resistance to stress correspondingly (Table 1). Expression intensity was detected at the level of average values in all examined stomach tissues.

In the group of animals with low stress resistance, the specific number of antigen-reactive cells decreased from 50 [45;52.5] (Q<0.05) to 22.5 [20;26.25] (Q<0.05) in the muscular layer of the stomach; it remained unchanged in the mucous membrane (12.5 [8.75;15] (Q<0.05) and 12.5 [10;15] (Q<0.05), while it reduced to trace quantities in the submucosa. At the same time, antigen expression intensity in the mucous membrane and muscular layer was minimal and trace in the submucosa (Figure).

A comparative analysis of the results shows that the submucosa contributes less to the regulation of vasodilatation in rats of both subgroups, both in norm and in pathology.

The most reactive stomach tissues, expressing endothelial nitric oxide synthase under stress of different intensity in conditions of the control series and modeling of acetate ulcers is the muscle layer, the components of which are involved in the development of ulcerative defect of acetate origin, i.e. damage of the gastric mucosa begins with contact of the serous membrane with acetic acid.

The level of magnesium in the erythrocyte mass of the blood from Vs decreased both in stress resistant rats by 31.1 % (p<0.001) and in stress nonresistant ones — by 31.1 % (p<0.05) when acetate ulcer had developed (Table 2). However, there was no significant difference between the groups.

At the same time, the concentration of Mg2+ considerably decreased in the daily urine in stress-resistant animals by 61.5 % (p<0.001); nonresistant rats only showed a tendency to increase of the loss of Mg2+ with the urine (p>0.1), leading to a significant reduction of its content in the erythrocyte mass of the blood.

Comparative analysis shows a lower level of eNOS in the gastric tissues in stress nonresistant rats compared to stress resistant rats. At the same time, a positive correlation relationship (r=0.37; r=0.70) is revealed between the content of intracellular intra-erythrocyte magnesium in both groups of rats with different resistance to stress and the specific number of antigen-positive cells in the gastric mucosa. Than greater the magnesium content is in the erythrocyte mass of the blood, than higher the specific number of eNOS-positive cells is in the mucous membrane.

Stress resistant rats also show a moderate positive correlation between the concentration of magnesium, the number and intensity of expression of the studied enzyme (r=0.52, r=0.57) in the submucosa of the stomach. But a negative moderate relationship between the above mentioned indices is revealed in the submucosa of stress nonresistant animals: r=-0.54; r=-0.54. Apparently, the trace number and trace intensity of the enzyme expression are functionally compensated by higher magnesium content. In other words, insignificant activity of nitric oxide synthase and nitric oxide is compensated by another vasodilator — magnesium.

Conclusions. Thus, based on the studies carried out, it can be concluded that 1. In case of acetate ulcer, the level of intra-erythrocyte magnesium is reduced in rats with different resistance to stress in low eNOS activity in the gastric mucosa; in the muscular layer in stress nonresistant animals and almost complete absence of antigen-positive cells in the submucosa. 2. Also a positive correlation was found between the content of intra-erythrocyte magnesium and the specific number of eNOS-positive cells in the gastric mucosa in both groups with different resistance to stress and also between expression intensity in the submucosa of stress nonresistant animals. A negative relationship was established between the magnesium level, specific number and expression intensity of antigen-positive cells to endothelial nitric oxide synthase in stress nonresistant rats in the stomach submucosa.
The effect of a low-intensity magnetic field combined with polarized light on the microvasculature links in periodontal tissues during orthopaedic treatment by fixed dentures in patients with partial secondary adentia and periodontal diseases were determined using an experiment on laboratory animals. The experiment was made on selected 107 random-bred rats. The animals were divided into three groups: control group 1, control group 2, and the experimental group. A dynamic analysis of morphological changes in the tissues of pathologically changed periodontium in three groups of animals demonstrated that, as compared to other animals, the rats from the experimental group, following experimental magneto phototherapy, had early elimination of inflammation (1 hour after termination of the impact) in all gingival components and perifocal structures due to vaporization of inflammatory cells, activation of vessels (plethora and a noticeable increase in number), while regeneration and full epithelialization of the gingival pocket were completed 2 days after termination of magneto phototherapy.

Keywords: partial secondary adentia, periodontal diseases, magneto phototherapy, animal studies

Представлены результаты экспериментального исследования по изучению влияния низкоинтенсивного магнитного поля в сочетании с поляризованным светом на звенья микроциркуляторного русла в тканях периodontа на 107 рандомбрэдных крысах. Динамический анализ морфологических изменений в тканях патологически измененного периодонта в трех группах животных показал, что по сравнению с остальными животными...