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The role of vitamin E and NO-synthase system in cytoprotective mechanisms of gastric mucosa

Rola witaminy E i systemy syntazy NO w mechanizmach cytoprotekcji śluzówki żołądka

In the cytoprotective processes of gastric mucosa (GM), numerous factors are involved including vitamin E (Vit E) and nitric oxide (NO). Small doses of Vit E are known to possess antioxidant properties and exert a protective effect in GM. In the research of recent years, it was stated that in ulcerogenic stress-induced damage to GM, Vit E reduced lipoperoxidation processes and ulcer index, but cytoprotective effect of Vit E was not associated with acid and saliva secretion and increased the content of PGE2 [1, 6, 8] In physiological conditions, NO is synthesized in the stomach by eNOS, while in the inflammatory processes and cancerogenesis, NO concentration increases sharply due to the activation of iNOS. NO synthesized by eNOS and iNOS acts diversely on the processes of cytoprotection in ulcer lesions of GM. Thus, injections of a nonselective blocker of eNOS and iNOS – L-NNA considerably aggravated the degree of stomach lesions whereas selective blockage of iNOS displayed its protective effect [9]. Injection of L-arginin at the background of ulcerogenic lesions of the stomach, diminished the activity of lipoperoxidation processes and resulted in manifested gastroprotection [7, 11].

The objective of the research was to estimate cytoprotective processes in GM under a combined effect of Vit E with a substrate for NO synthases – L-arginine or under the blockage of iNOS with L-canavalin.

MATERIAL AND METHODS

The investigations were carried out on 22 white rats, according to the international regulations for work with the use of laboratory animals. Ulcerogenic lesions of GM were modeled with adrenaline injected intraperitoneally in the dose of 2 mg/kg. Vit E (intramuscular dose of 150 mg/kg), L-canavalin (in the dose of 100 mg/kg), a blocker of inducible NO-synthase, and L-arginine (in the dose of 300 mg/kg), the substrate for NO synthases, were injected 30 min. prior to the modeling of ulcerogenic lesions.

The research comprised six series: in the 1st series, there was determined ulcerogenic effect of adrenaline; in the 2nd – effect of Vit E at the background of stress-induced damage to the stomach, in the 3rd – action of the blocker of inducible NO-synthase L-canavalin at the background of adrenaline effect; in the 4th – action of L-arginine at the background of adrenaline effect; in the 5th – a combined

action of Vit E and L-canavaline; and in the 6th – a combined action of Vit E and L-arginine. Changes of lipoperoxidation processes were investigated on the basis of the assessments of MDA, the activity of the antioxidant protection enzymes (SOD and catalase), and the content of nitric oxide (NO_2), measured with the use of Griess reagent [5]. Results of the investigation were processed by the method of variation statistics with the deternmination of Student's t-criteria.

RESULTS

The obtained findings showed that adrenaline impact induces the development of characteristic structure-hemorrhagic lesions of GM in the form of separate ulcerative defects, erosions, micro- and macroscopic hemorrhages; besides, there were observed increases of the concentration of products of thiobarbituric acid (PTBA) from 238.99±25.46 mcmol/g × tis up to 306.83±16.0 mcmol/g tis (by 28%, $p < 0.05$), and of nitric oxide concentration – from 14.55±1.31 up to 23.5±2.46 mcmol/l (by 62%), the activity of SOD enhanced from 20.57±7.54 to 32.14±4.74 MO×10^{-2} (by 56%), and that of catalase diminished from 0.45±0.09 down to 0.298±0.062 mcmol H2O2/g × hr (by 34%), as compared to the data in the intact animals.

Injection of Vit E at the background of ulcerogenic action of adrenaline resulted in a decrease of the area of structure-hemorrhagic lesions, a slight decrease of MDA content (by 9%), and insignificant changes of NO content. At the same time, SOD activity reduced by 34% as compared to the effect exerted by adrenaline, and catalase activity failed to show any considerable alterations.

Development of ulcerative lesions of GM is known to be accompanied not only by the increase of iNOS expression and intensified biosynthesis of nitric oxide, but also by the neutrophilic infiltrations to GM, enhanced activity of myeloperoxidase, and increased levels of anti-inflammatory cytokines – IL-1β, necrosis factor of tumor-α [2, 9].

To evaluate the role of Vit E and NO-synthase system, we conducted investigations of iNOS blockage with the selective blocker L-canavalin under its combined action with Vit E, at the background of the ulcerogenic effect produced by adrenaline. Under a combined blockage of iNOS and injection of Vit E, MDA content decreased sharply – by 41% ($p<0.05$) and the content of nitric oxide – by 37% ($p<0.05$) versus the data due to the effect of Vit E and adrenaline. At the same time, the activity of SOD was somewhat enhanced – by 12% and catalase activity reduced by 12%. It has to be mentioned that due to the blockage of iNOS at the background of adrenaline impact, MDA content decreased by 38% ($p<0.05$) and a combined action of Vit E and L-canovalin induced a decrease of MDA concentration by 46%, which is evidence of the predominant action of NO, synthesized by iNOS, in the activation of lipoperoxidation processes, and the antioxidant effect of Vit E was less pronounced. The combined action of Vit E and L-canavalin displayed a unidirected antioxidant action realized by different mechanisms.

Thus, in combined blockage of iNOS and injection of Vit E we observed a reduced activity of lipoperoxidation processes and a decreased content of nitric oxide whereas the activity of antioxidant protection enzymes changed insignificantly. The combined action of L-arginine and Vit E at the background of adrenaline impact resulted in a decrease of the area of structure-hemorrhagic affections, of MDA content – by 14%, and nitric oxide content – by 48%, as compared to the data obtained in the combined action of Vit E at the background of adrenaline effect. SOD activity got intensified by 50% ($p<0.05$) and catalase activity reduced by 22%. The solitary action of L-arginine at the background of ulcerogenic impact of adrenaline caused a decrease in the contents of MDA by 48%, and NO – by 13%, SOD activity diminished by 77% ($p<0.05$) and that of catalase – by 55% ($p<0.05$). Thus, antioxidant action of Vit E simultaneous with L-arginine, on the one hand, results in

a decrease of NO content, and on the other hand, in the enhancement of SOD activity, which can be a cause of the enhancement of gastroprotective processes.

CONCLUSIONS

Stress-induced damage to GM is accompanied by the increase of MDA content, enhancement of lipid oxidation, activation of mitochondrial SOD, by the reduction of peroxidase activity, and a decrease of glutathione content due to the generation of OH with the involvement of iron ions [3]. Injection of Vit E in a single dose of 150 mg/kg induced insignificant cytoprotective effects and a slight reduction of lipoperoxidation processes. The increase of NO content is also worth mentioning.

Nitric oxide in the mucous membrane participates in the processes of both cytoprotection and ulcerogenic changes. It has to be noted that gastroprotective action of the injected L-arginine and, therefore, NO in stress-induced damage to GM were associated with the maintenance of mucus secretion, enhancement of eNOS and iNOS activity, with the increase in the content of nitrates/ nitrites in GM, and maintenance of the level of blood supply [10].

Vit E, under the blockage of iNOS or injected precursor of NO-synthases – L-arginine, displays, a modulatory antioxidant action. Under a simultaneous blockage of iNOS and injection of Vit E, gastroprotection got intensified, lipoperoxidation processes reduced, with the effect of iNOS blockage being predominant. Injection of L-arginine and Vit E also results in the enhancement of gastroprotection which is realized by a decrease of NO content and the enhancement of SOD activity.

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SUMMARY

In the experiments on rats with modeled stress-induced damage to gastric mucosa by means of adrenaline (2mg/kg), we studied the effect of Vit E in the condition of iNOS blockage and injection of L-arginine. Under the blockage of iNOS or injection of L-arginine, precursor of NO-synthases, Vit E was observed to exert a modulatory antioxidant effect. Blockage of iNOS with a simultaneous injection of Vit E results in the enhancement of gastroprotection and a reduction of lipoperoxidation processes with a predominant role of iNOS blockage. Injection of L-arginine and Vit E also induces enhancement of gastroprotective processes realized by a decrease of NO content and intensified activity of SOD.

STRESZCZENIE

W eksperymencie na szczurach z modelowanym indukowanym stresem uszkodzeniem śluzówki żołądka przy użyciu adrenaliny (2 mg/kg) oceniano działanie witaminy E w warunkach blokady iNOS i po podaniu L-argininy. Po blokadzie iNOS lub wstrzyknięciu L-argininy, prekursora syntazy NO, witamina E modulowała efekt antyoksydacyjny. Blokada iNOS z jednoczesnym wstrzyknięciem witaminy E dawała w efekcie wzrost gastroprotekcji i redukcję procesów lipoperoksydacji z przeważającą rolą blokowania iNOS. Podawanie L-argininy i witaminy E również indukowało wzrost procesów gastroprotekcji poprzez spadek zawartości NO i intensyfikowania aktywności SOD.