16. Непараметрическая статистика и подгонка распределения [Електронний ресурс]. – Режим доступу : http://www.statsoft.ru/home/textbook/modules/stnonpar.html.

17. Патент 62419, Україна, МПК (2011.01), A01N63/00. Спосіб культивування поза організмом ооцит-кумулюсних комплексів (ОКК) за температури, осцилюючої з одногодинним періодом / Корчан Н.О., Денисюк П.В.; заявник і патентоутримувач Інститут свинарства ім. О.В. Квасницького НААН; опубл. 25.08.2011, Бюл. № 16.

18. Патент 68013, Україна, МПК (2012.01), C12N 5/00, A61M 1/00. Застосування примусової біоритмічної осциляції pH середовища культивування поза організмом як способу, призначеного для збільшення міри розростання ооцит-кумулюсних комплексів (ОКК) / Корчан Н.О., Денисюк П.В.; заявник і патентоутримувач Інститут свинарства і агропромислового виробництва НААН. – № u 2011 10439; заявл. 29.08.2011; опубл. 12.03.2012, Бюл. № 5.

19. Патент RU № 2070387, Россия, A01K 41/00. Способ инкубации яиц сельскохозяйственной птицы / Фандеев Е.И. и др.; заявители и патентообладатели Фандеев Е.И. и др. – № 94028901/15; заявл. 03.08.1994; опубл. 20.12.1996.

20. Патент RU № 2107434, Россия, A01K 41/00. Способ инкубации яиц сельскохозяйственной птицы и устройство для его осуществления / Фандеев Е.И. и др.; заявители и патентообладатели Фандеев Е.И. и др. – № 94035822/13; заявл. 27.09.1994; опубл. 27.03.1998.

21. Abeydeera L.R. Development and viability of pig oocytes matured in protein free medium containing epidermal growth factor // Theriogenology. – 2000. – Vol. 54. – P. 787–797.

22. Beek J., Bijttebier J., Maes D. et al. The importance of cumulus expansion for monospermic fertilization of porcine cumulus-oocyte complexes // Reprod. Fertil. Dev. – 2011. – V. 23, N. 1. – P. 202.

23. Booth F.W., Chakravarthy M.V., Spangenburg E.E. Exercise and gene expression: physiological regulation of the human genome through physical activity // J. Physiol. (Lond). – 2002. – Vol. 543, Is. 2. – P. 399-411.

24. Brauch A., Keisler D.H. Effect of follicular fluid supplementation on in vitro maturation of porcine oocytes [Електронний ресурс] // Undergraduate Research and Creative Achievements Forum (MU) : collection. – 2007 [163]. – Режим доступу : http://hdl.handle.net/10355/1572.

25. Brinster R.L. A method for in vitro cultivation of mouse ova from two-cell to blastocyst // Exp. Cell Res. – 1963. – Vol. 32. – P. 205-208.

26. Dolmetsch R.E., Xu K., Lewis R.S.. Calcium oscillations increase the efficiency and specificity of gene expression // Nature. – 1998. – Vol. 392. – P. 933–936.

27. Mizobe Y., Yoshida M., Miyoshi K. Enhancement of cytoplasmic maturation of in vitro-matured pig oocytes by mechanical vibration // J. Reprod. Dev. – 2010. – Vol. 56, N. 2. – P. 285-290.

28. Ozil J. The parthenogenetic development of rabbit oocytes after repetitive pulsatile electrical stimulation // Development. – 1990. – Vol. 109. – P. 117-127.

29. Swann K., Lai F.A. A novel signaling mechanism for generating $Ca^2(+)$ oscillations at fertilization in mammals // BioEssays. – 1997. – V. 19. – P. 371-378.

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РОЗВИТОК ООЦИТ-КУМУЛЮСНИХ КОМПЛЕКСІВ СВИНІ ЗА ПОСТІЙНИХ І ОСЦИЛЮЮЧИХ ТЕМПЕРАТУРИ Й *РН*

Установлено, що заміна постійних температури й pH культивування на осцилюючі достовірно не зменшує приріст діаметра ооцит-кумулюсних комплексів (ОКК) у результаті їх дозрівання в культурі in vitro протягом доби. Підвищення вмісту фолікулярної рідини від 10 % до 20 % у середовищі їх дозрівання – NCSU, не впливає на величину приросту діаметра ОКК.

Ключові слова: ооцит-кумулюсних комплексів, фолікулярна рідина.

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РАЗВИТИЕ ООЦИТ-КУМУЛЮСНЫХ КОМПЛЕКСОВ СВИНЬИ ПРИ ПОСТОЯННЫХ И ОСЦЕЛИРУЮЩИХ ТЕМПЕРАТУРЫ И *РН*

Выявлено, что замена постоянных температуры и pH культивирования на осцилирующие достоверно не уменьшает прирост диаметра ооцит-кумулюсных комплексов (ОКК) в результате их дозревания в культуре in vitro на протяжение суток. Повышение содержания фоликулярной жидкости от 10% к 20% в среде их дозревания – NCSU, не влияет на величину прироста диаметра ОКК.

Ключевые слова: ооцит-кумулюсный комплекс, фолликулярная жидкость.

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EVIDENCE OF OXIDATIVE STRESS DEVELOPMENT IN PANCREATIC CELLS OF RATS WITH CHRONICALLY SUPPRESSED GASTRIC ACID SECRETION

Long-term hypochlorhydria is sometimes associated with mild pancreatitis development. Up to date, there is no clear evidence on mechanisms involved in pancreatic damage upon these conditions. The aim of study was to estimate the intensity of free-radical processes in rat pancreatic cells upon experimental hypochlorhydria. The increased hydrogen peroxide content (1,8 times), Nos2 gene mRNA level (2,9 times), total NO-synthase (4 times), thioredoxin reductase (1,6 times) and mitochondrial superoxide dismutase (1,5 times) activities, as well as decreased content of total (1,3 times), protein-bound (1,3 times) and nonprotein (1,4 times) SH-groups in rat pancreas were established. Thus, there is evidence of oxidative stress development in rat pancreatic cells upon long-term suppression of gastric acid secretion, suggesting the involvement of disturbed redox balance in pathophysiologic mechanisms of mild pancreatitis development upon these conditions.

Key words: oxidative stress, hypochlorhydria, dysbiosis, pancreas.

Introduction. Hypochlorhydria is defined as a state of low hydrochloric acid content in gastric juice with complex etiology: it develops as a consequence of pharmacologic suppression of gastric acid secretion, as a natural process during aging of digestive tract, as a complication upon some disease (atrophic gastritis, autoimmune disordrs, *H. Pylori* infection, achylia etc.) and in rare cases it has genetic causes [13].

In contrast to hyperacidic states, low stomach acidity is substantially harder to diagnose due to less prominent symptoms. At the same time, 10-15% of adult population and up to 50% people of retirement age in developed countries has hypochlorhydria [30]. Loss of gastric juice bactericidal properties is accompanied by bacterial overgrowth in different regions of gastrointestinal tract (GIT). Formation of dysbiotic bacterial biofilms in duodenum leads to initiation of endogenous inflammation that can involve associated organs, such as pancreas [3-4].

Although development of pancreatic disorders upon hypochlorhydria isn't unambiguous, there is some evidence of mild acute pancreatitis development (AP) upon long-term use of gastric H^+/K^+-ATPase inhibitors, such as omeprazole

(one of the most common causes of hypochlorhydria) [6, 27, 31]. At the same time, data on exact pathophysiologic and biochemical mechanisms of initiation and propagation of AP upon long-term hypochlorhydria are absent.

The imbalance between pro- and antioxidant factors with significant intensification of free-radical processes in pancreatic cells upon different experimental models of AP was shown during last two decades [15]. Oxidative stress (OS) is known to be a non-specific biochemical mechanism of cellular damage upon different pathological states [26]. Thus, determination of pro-/antioxidant balance indices can be used as a markers of pancreatic damage, and may help in elucidation of involved pathologic mechanisms in pancreatitis-like signs development upon long-term hypochlorhydria.

The **aim** of current study was to estimate the intensity of free-radical processes and state of antioxidant systems in rat pancreatic cells upon long-term suppression of gastric acid secretion by proton-pump inhibitor omeprazole.

Materials and methods. All experiments were performed on white non-strain male rats with initial weight around 180-200 g, which were kept on standard diet. International recommendations on performance of medical and biological investigations with the use of animals according to "European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes" were followed.

All animals were divided into two groups. Rats injected abdominally with 0,2 ml of physiological solution and 0,5 ml of water were used as a control (first group). Hypochlorhydria (second group) was modeled by abdominal injection of omeprazole (14 mg/kg once a day) during 28 days [37].

Total nitric oxide synthase (NOS) activity was estimated by method [24]. In brief, this assay is based on determination of nitric oxide aerobic oxidation products. The content of hydrogen peroxide (H_2O_2) stable products was measured by sorbitol/xylenol orange assay [21]. Thioredoxin reductase (TRR) activity was determined by rate of NADPH-dependent reduction of Ellman's reagent with use of sodium aurothiomalate as TRR inhibitor [10]. The content of total and nonprotein SH-groups was estimated with Ellman's reagent in whole homogenates and protein-free fraction, respectively [7]. Superoxide dismutase (SOD) activity was measured in mitochondrial (corresponds to Mn-SOD activity) and cytosolic (corresponds to Cu,Zn-SOD activity) fractions of pancreatic cells by competition with nitroblue tetrazolium for electrons generated in NADH / phenazine methosulfate mixture [38]. Total protein concentration in whole homogenates and separate fractions was determined by Lowry assay [16].

RNA was isolated following Chomczynski et al. [5]; cDNA was synthesized in 20 µl of reaction mix upon such conditions: 70°C – 5 min, further 37°C – 5 min, 42°C – 1 hour. Amplification of DNA fragments was carried out upon such temperature conditions: initiative denaturation 94ºC – 4 min; further 35 cycles: DNA denaturation 94ºC – 45 s; hybridization of primers 52ºC – 45 s for *Nos2* (440 b.p.), and 49ºC – 40 s for *Actb* (521 b.p.) (internal control of reaction); chain extension 72ºC – 1 min 15 s for *Nos2* and 1 min for *Actb*. Further fill-in of PCR products was performed upon 72ºC for 5 min. Such primer sequences were used in reactions – for *Nos2*: forward GTGTTCCACCAGGAGATGTTG, and reverse – CTCCTGCCCACTGACTTCGTC; for *Actb*: forward – TGGGACGATATGGAGAAGAT, and reverse – ATTGCCGATAGTGATGACCT. Separation of PCR products was performed electrophoretically with following semiquantitative analysis of amplicons expression based on densitometry the ImageJ 1.45s program was used. Indices of mRNA expression were calculated for each sample following Konturek et al. [14].

Statistical processing of experimental data was performed with analysis of varience. Probability of difference between control and test measurements was assessed with Student's t-test. The difference between compared data was treated as probable if $p < 0,05$. All calculations and graph plotting were carried out in "OriginLab Origin 8.6" and "Microsoft Excel 2003" programs.

Results and discussion. To establish the state of pro-/antioxidant balance in rat pancreas upon long-term experimental hypochlorhydria such indices were assessed:

1) Prooxidants: hydrogen peroxide content, total NOS activity, expression of *Nos2* gene.

2) Antioxidants: SOD activity (mitochondrial isoform), total TRR activity, content of total, protein-bound and nonprotein SH-groups.

Hydrogen peroxide is a type of reactive oxygen species, which is produced upon two-electron reduction of oxygen or as a consequence of superoxide radical dismutation. Biological effects of H_2O_2 are concentration-dependent: in low concentrations H_2O_2 acts as a signal molecule, while in high experiments – as a cytotoxic oxidant [12].

It was established, that the level of H_2O_2 stable products in rat pancreas upon long-term hypochlorhydria was 1,8 times higher in comparison with the control. Observed increase in hydrogen peroxide level indicates the intensification of free-radical processes in rat pancreas and general depletion of cellular antiperoxide ability. The source of H_2O_2 production upon these conditions, probably, is a dismutation of superoxide radicals upon action of SOD and high XO activity. The increase in superoxide anion level and XO activity in pancreatic cells upon hypochlorhydria was shown in our recent study [34], thus confirming the probable source of hydrogen peroxide synthesis in these conditions. Hydrogen peroxide is known not only for its possibility to directly oxidize biomolecules, but also for its ability to split into highly toxic kind of ROS – hydroxyl radical ($\cdot OH$), which can rapidly oxidize lipids and proteins, and also damage cellular genome.

NOS is a group of NADPH-, FAD-, FMN- and BH_4-dependent oxidoreductases that catalyze conversion of L-arginine and O_2 to citrulline and nitrogen oxide (NO) [8]. There are 3 isoforms of NOS: endothelial, neuronal and inducible (iNOS). Expression of constitutive NOS isoforms was shown in pancreatic acinar cells of rodents [15] in normal circumstances, and there is also data on low basal level of iNOS expression in rat acini [29]. Depletion of constitutive isoforms expression with simultaneous elevation of iNOS level was established upon different experimental models of AP [1, 15].

It was established, that total pancreatic NOS activity in rats treated only with omeprazole for 28 days was 4 times higher in comparison with the control. Observed elevation of total NOS activity may reflect the activation of both constitutive and inducible isoforms of NOS, but according to a literature data activation of inducible NOS isoform is more probable and justified in stress conditions [8, 15]. To resolve this issue the level of iNOS gene (*Nos2*) expression in rat pancreatic tissue was estimated.

Table 1. **Indices of prooxidant factors in rat pancreas upon long-term suppression of gastric acid secretion (M ± m, n = 10)**

Parameter \ Group	Control	Omeprazole
Total NO-synthase activity, nmol × min^{-1} × mg of $protein^{-1}$	2,41 ± 0,23	9,73 ± 0,95*
Hydrogen peroxide content, mmol × gram of $tissue^{-1}$	2,61 ± 0,28	4,82 ± 0,45*

Remarks: * – $p < 0,05$ in relation with control.

According to Vaquero et al., there is low level of *Nos2* gene expression in pancreatic acinar cells in rats [29]. Moreover, it's known that different NOS isoforms are expressed in vascular endothelium and nerves spanning the pancreas [15]. Significant increase in *Nos2* gene expression upon different models of experimental AP was shown recently [1, 15, 19].

The investigation of *Nos2* gene expression level showed that in the control the mRNA level of this gene was 0,326 ± 0,034 (Fig. 1). In animals with long-term hypochlorhydria, the level of *Nos2* mRNA was 2,9 times higher than control values.

The observed existence of iNOS mRNA in pancreatic cells of control rats is in accord with data of Vaquero et al. [29]. Significantly increased level of *Nos2* gene expression in rat pancreas upon long-term suppression of gastric acid secretion was established in our study. According to papers of different authors, high level of iNOS expression was observed upon arginine-, taurocholate and caerulein-induced AP models [1, 15, 19]. The expression of iNOS was located mainly in vascular endothelial and muscle cells of pancreas in these experiments [15]. Unfortunately, the conditions of our study don't allow us to fix the exact place of iNOS expression, since the contamination of analyzed pancreatic tissue with fragments of vessels is possible. However, the increased level of *Nos2* mRNA can be responsible for earlier indicated high total NOS activity (table 1).

Enormous level of nitric oxide (synthesized by iNOS) in pancreatic cells favors nitration and nitrosilation of cellular proteins and DNA, that can lead to structural and functional damage of the pancreas [35]. Moreover, it can be suggested the generation of toxic peroxynitrite ($ONOO^-$) – condensation product of nitric oxide and superoxide anion ($\cdot O_2^-$) – upon these conditions, since the increase of $\cdot O_2^-$ level was shown recently [33-34]. Peroxynitrite is known to induce damage of wide spectra of biomolecules, such as oxidation of NH- and SH-groups in proteins, peroxidation of membrane lipids and one-chain brakes in DNA [15].

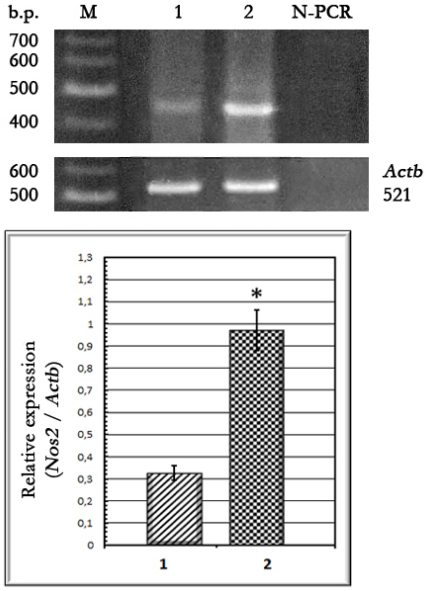

Fig. 1. Level of *Nos2* gene mRNA in rat pancreas upon long-term gastric hypochlorhydria

Remark: M – molecular mass marker; 1 – control; 2 – omeprazole; N-PCR – negative PCR control; * – $p < 0,05$ in relation with control

All aerobic organisms have evolved complex antioxidant systems (AOS) aimed on counteraction to free-radical processes and maintaining cellular redox homeostasis. AOS is a dynamic compound formation including both high-molecular enzymes and low-molecular non-enzymatic substances of different chemical nature [36]. Disturbance of AOS function in rat pancreas upon AP and inflammation was observed in a whole series of investigations [15, 23, 28]

Enzymes from SOD family constitute the first line of antioxidant cellular defense. SOD activity is crucial for effective inactivation of superoxide radicals and, thus, for whole control of free-radical processes in the cell. There are 3 isoforms of SOD in mammalian tissues: Cu,Zn-SOD (cytosolic), Mn-SOD (mitochondrial) and EC-SOD (extracellular) [18]. Regulation of *sod* genes expression, as well as enzymatic activity and compartmentalization of SOD isoforms is important for maintaining of stable ROS concentration in cells.

It was established that SOD activity in mitochondrial fraction (corresponds to Mn-SOD activity) of rat pancreas upon long-term gastric hypochlorhydria was 1,5 times higher in comparison to the control (table 2).

Table 2. **Indices of antioxidant system in rat pancreas upon long-term suppression of gastric acid secretion (M ± m, n = 10)**

Parameter / Group	Control	Omeprazole
Superoxide dismutase activity in mitochondrial fraction, units × min^{-1} × mg of $protein^{-1}$	0,194 ± 0,018	0,298 ± 0,027*
Thioredoxin reductase activity, μmol × min^{-1} × mg of $protein^{-1}$	5,512 ± 0,491	8,643 ± 0,780*
Total SH-groups content, mmol × mg of $protein^{-1}$	0,337 ± 0,032	0,255 ± 0,024*
Protein-bound SH-groups content, mmol × mg of $protein^{-1}$	0,229 ± 0,021	0,176 ± 0,016*
Nonpreotein SH-groups content. mmol × mg of $protein^{-1}$	0,108 ± 0,010	0,079 ± 0,008*

Remarks: * – $p < 0,05$ in relation with control

Thus, activation of second SOD isoform upon long-term suppression of gastric acid secretion were observed. Activation of Mn-SOD may be a consequence of increased superoxide radicals generation by mitochondrial electron-transporting chain, since $\cdot O_2^-$ is a specific substrate of SOD. On the other hand, transcription factors NF-κB and AP-1 stimulating *sod2* gene (encoding mitochondrial isoform of SOD) expression are usually upregulated as a result of proinflammatory cytokine stimulation and upon oxidative stress [15, 18].

Mammalian TRR is a key enzyme in so called "thioredoxin AOS", which also includes NADPH and thioredoxin [2]. Main function of this system is reduction of disulfide bonds in proteins and maintaining protein-bound SH-groups in reduced state. There 2 isoforms of TRR in mammalian cells – cytosolic/nuclear and mitochondrial.

Determination of TRR activity in rat pancreatic tissue indicated that total TRR activity upon long-term suppresiion of gastric acid secretion was 1,6 times higher in comparison with the control. On the basis of these data it may be suggested that increased TRR activity leads to high level of reduced thioredoxin in rat pancreatic cells upon long-term hypochlorhydria. Thioredoxin is a main cellular denitrosilating and disulfide reducing agent, thus increased thioredoxin level may be a compensatory reaction to enhanced NO generation and oxidation of protein-bound SH-groups in pancreatic cells in these conditions. This idea is supported by indicated above elevation of total NOS activity, as well as by shown earlier increased level of NO upon these conditions [33]. In order to estimate cellular thiols state the content of total, protein-bound and nonprotein SH-groups in rat pancreatic homogenates was determined.

It was established that protein-bound SH-groups level in control rat pancreatic homogenates was more than 2 times higher than nonprotein SH-groups content (table 2); these data is in accord with general distribution of SH-groups in tissues [25]. In pancreatic homogenates of rats with long-term hypochlorhydria the content of total, protein-bound and nonprotein SH-groups was, respectively, in 1,3; 1,3 and 1,4 times higher in relation to the control.

Observed decreased nonprotein SH-groups content is in accord with our earlier obtained data on reduced glutathione depletion in rat pancreas upon these conditions [32], since reduced glutathione is a main non-protein thiol in pancreatic cells [36]. Thus, decreased level of total, protein-bound and nonprotein SH-groups in rat pancreas upon long-term hypochlorhydria indicates general shift of redox-balance in pancreatic cells towards activation of oxidative processes and oxidative stress development.

On the basis of these and earlier obtained results [32-34] the following biochemical mechanism of oxidative stress development in rat pancreas upon long-term hypochlorhydria can be suggested. Long-term administration of omeprazole leads to dysbiosis development in duodenum – the nearest to pancreas part of GIT. Secretory or cellular components of dysbiotic microbiota and (or) stimulation by proinflammatory cytokines (caused by dysbiotic bacteria) near the pancreas lead to development of inflammatory cycle in pancreatic tissue [17]. Pancreatic cells are known to express some types of Toll-like receptors (TLR), which are sensitive to bacterial cell wall components [20]. Upon bacterial colonization of duodenum or pancreatic ducts, activation of TLR and inflammation initiation seems to be probable. At that, release of proinflammatory cytokines, such as TNF-α, IL-1, IL-8 and PAF with simultaneous IL-10 depletion may occur [9]. These cytokines stimulate expression of cell adhesion molecules (CAM) on the surface of neutrophils and endotheliocytes leading to neutrophils migration from blood vessels into pancreatic tissue [22]. Activation of neutrophils leads to further increase in proinflammatory cytokines production, CAM expression, vascular permeability and ROS generation in acinar cells [9, 17]. Cytokine release and high CAM expression favor even greater leukocyte migration into acini – thus develops inflammatory cycle [9]. As a result of this cycle, powerful leukocytic infiltration into pancreatic tissue develops with further ROS generation and intensive OS [15]. Elevation of ROS and RNS content, lipid and protein oxidation products level, as well as depletion of AOS enzymes in rat pancreatic cells upon long-term hypochlorhydria were observed in our recent researches, thus (together with results of this study) confirming OS development upon these conditions.

Conclusions. The elevation of prooxidant factors (hydrogen peroxide content, total NOS activity, expression of *Nos2* gene) and depletion of many antioxidants (total, protein-bound and nonprotein SH-groups content, TRR activity) in rat pancreas upon long-term experimental hypochlorhydria were established in this study. Obtained results undoubtedly indicate oxidative stress development in pancreatic cells of rats upon these conditions suggesting the important role of cellular redox homeostasis disequilibrium in pancreatitis-like signs development. Elucidation of initiating events underlying observed results requires further investigations.

References

1. Al-Mufti R.A., Williamson R.C., Mathie R.T. Increased nitric oxide activity in a rat model of acute pancreatitis // Gut. – 1998. – Vol. 43. – P. 564-570.

2. Cai W., Zhang L., Song Y., et al. Small molecule inhibitors of mammalian thioredoxin reductase // Free Rad, Biol, Med. – 2012. – Vol. 52. – P. 257-262.

3. Canani R., Terrin G. Gastric acidity inhibitors and the risk of intestinal infections // Curr. Opin. Gastroenterol. – 2010. – Vol. 26, № 1. – P. 31-35.

4. Chey W., Spiegel B. Proton pump inhibitors, irritable bowel syndrome, and small intestinal bacterial overgrowth: coincidence or Newtons third law revisited? // Clin. Gastroenterol. Hepatol. – 2010. – Vol. 8, № 6. – P. 480-482.

5. Chomczynski P., Sacchi N. Single-step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction // Anal. Biochem. – 1987. – Vol. 162. – P. 156-159.

6. Eland I., Huerta Alvarez C., Stricker B., Garcia Rodriguez L. The risk of acute pancreatitis associated with acid-suppressing drugs // Br. J. Clin. Pharmacol. – 2000. – Vol. 49. – P. 473-478.

7. Ellman G. Tissue sulfhydryl groups // Arch. Biochem. Biophys. – 1959. – Vol. 82, №1. – P. 70-77.

8. Forstermann U., Sessa W.C. Nitric oxide synthases: regulation and function // Eur. Heart. J. – 2012. – Vol. 33 (7). – P. 829-837.

9. Frossard J.L., Pastor C.M. Experimental acute pancreatitis: new insights into the pathophysiology // Front. Biosci. – 2002. – Vol. 7. – P. 275-287.

10. Hill K.E., McCollum G.W., Burk R.E. Determination of thioredoxin reductase activity in rat liver supernatant // Anal. Biochem. – Vol. 253. – P. 123-125.

11. Holmgren A., Lu J. Thioredoxin and thioredoxin reductase: current research with special reference to human disease // Biochem. Biophys. Res. Commun. – 2010. – Vol. 396. – P. 120-124.

12. Kanta J. The role of hydrogen peroxide and other reactive oxygen species in wound healing // Acta Medica (Hradec Kralove). – 2011. – Vol. 54 (3). – P. 97-101.

13. Kitchen J. Hypochlorhydria: a review – Part 1 // Townsend Let. Doct. Pat. – 2001. – P. 56-60.

14. Konturek P.C., Brzozowski T., Pierzchalski P. et. al. Activation of genes for spasmolytic peptide, transforming growth factor alpha and for cyclooxygenase (COX)-1 and COX-2 during gastric adaptation to aspirin damage in rats // Aliment. Pharmacol. Ther. – 1998. – Vol. 12. – P. 767-777.

15. Leung P.S., Chan Y.C. Role of oxidative stress in pancreatic inflammation // Antioxid. Redox Signal. – 2009. – Vol. 11 (1). – P. 135-165.

16. Lowry O.H., Rosebrough N.J., Farr A.L., Randal R.I. Protein measurement with Folin phenol reagent // J. Biol. Chem. – 1951. – Vol. 193, № 1. – P. 265-275.

17. McClave S. Drivers of oxidative stress in acute pancreatitis: the role of nutrition therapy // J. Parenter. Enteral. Nutr. – 2012. – Vol. 36 (1). – P. 24-35.

18. Miao L., Clair D.K. Regulation of superoxide dismutase genes: implications in diseases // Free Radic. Biol. Med. – 2009. – Vol. 47 (4). – P. 344-356.

19. Motoo Y., Su S.B., Xie M.J., et al. Effect of herbal medicine keishito (TJ-45) and its components on rat pancreatic acinar cell injuries in vivo and in vitro // Pancreatology. – 2001. – Vol. 1. – P. 102–109.

20. Nakamura T., Ito T., Oono T., et al. Bacterial DNA promotes proliferation of rat pancreatic stellate cells through TLR-9: potential mechanisms for bacterially induced fibrosis // Pancreas. – 2011. – Vol. 40 (6). – P. 823-831.

21. Nourooz-Zadeh J., Tajaddini-Sarmadi J., Wolff S.P. Measurement of plasma hydroperoxide concentrations by the ferrous oxidation – Xylenol Orange assay // Anal. Biochem. – 1994. – Vol. 220. – P. 403-409.

22. Pham C.T. Neutrophil serine proteases: specific regulators of inflammation // Nat. Rev. Immunol. – 2006. – Vol. 6 (7). – P. 541-550.

23. Rau B., Poch B., Gansauge F., et al. Pathophysiologic role of oxygen free radicals in acute pancreatitis: initiating event or mediator of tissue damage? // Ann. Surg. – Vol. 231 (3). – P. 352-360.

24. Salter M., Knowles R.G., Moncada S. Widespread tissue distribution, species and changes in activity of Ca^{2+}-depend and Ca^{2+}-independent nitric oxide syntases // FEBS Lett. – 1991. – Vol. 291, № 1. – P. 145-149.

25. Sedlak J., Lindsay R. Estimation of total, protein-bound and nonprotein sulfhydryl droups in tissue with Ellman's reagent // Anal. Biochem. – 1968. – Vil. 25 (1). – P. 192-205.

26. Storz G., Imlay J. Oxidative stress // Curr. Opin. Microbiol. – 1999. – Vol. 2. – P. 188-194.

27. Sundstrom A., Blomgren K., Alfredson L., Wiholm B. Acid-suppressing drugs and gastroesophageal reflux disease as risk factors for acute pancreatitis – results from a Swedish Case-Control Study // Pharmacoepidemiol. Drug Saf. – 2006. – Vol. 15, № 3. – P. 141-149.

28. Tsai K., Wang S.S., Chen T.S., et al. Oxidative stress: an important phenomenon with pathogenetic significance in the progression of acute pancreatitis // Gut. – 1998. – Vol. 42 (6). – P. 850-855.

29. Vaquero E., Gukovsky I., Zaninovic V., Gukovskaya N., Pandol S. Localized pancreatic NF-κB activation and inflammatory response in taurocholate-induced pancreatitis // Am. J. Physiol. Gastrointest. Liver Physiol. – 2001. – Vol. 280. – P. 1197-1208.

30. Wright J. Why stomach acid is good for you: natural relief from heartburn, reflux and GERD. – M. Evans & Company, 2001. – 285 p.

31. Youssef S.S., Iskandar S.B., Scruggs J., Roy T.M. Acute pancreatitis associated with omeprazole // Int. J. Clin. Pharmacol. Ther. – 2005. – Vol. 43 (12). – P. 558-561.

32. Дворщенко К., Вакал С., Гайда Л., Остапченко Л. Антиоксидантні ефекти мультипробіотику Симбітер у підшлунковій залозі щурів за умов гіпоацидного стану // Вісник Київського національного університету імені Тараса Шевченка. – №61. – 2012. – С. 42-45.

33. Дворщенко К., Вакал С., Драницина А., Остапченко Л. Вільнорадикальні процеси у підшлунковій залозі за умов тривалої гіпоацидності та при дії мультипробіотика Симбітер // Вісник КНУ імені Тараса Шевченко. Серія: біологія. – 2013. – № 63. – С. 26-28.

34. Дворщенко К.О., Вакал С.Є., Драницина А.С., Сенін С.А., Остапченко Л.І. Стрес-респонсивні системи підшлункової залози щурів в умовах тривалої шлункової гіпохлоргідрії та за введення мультипробіотика "Симбітер®" // Український біохімічний журнал. – 2013. – Т. 85, № 2. – С. 68-77.

35. Лазебник Л.Б., Дроздов В.Н., Барышников Е.Н. Роль оксида азота (NO) в патогенезе некоторых заболеваний органов пищеварения // Эксперим. и клин. гастроэнтерол. – 2005. – № 2. – С. 4-11.

36. Харченко В.В. Природні антиоксиданти та печінка // Сучасна гастроентерологія. – 2006. – № 38. – С. 79-85.

37. Цирюк О.І., Берегова Т.В. Вплив омепразол-викликаної гіпергастринемії на базальну шлункову секрецію у щурів // Вісник проблем біології і медицини. – 2007. – Вип. 3. – С. 38-43.

38. Чевари С., Чаба И., Секей Й. Роль супероксиддисмутазы в окислительных процессах клетки и метод определения ее в биологических материалах // Лаб. дело. – 1985. – № 11. – С. 678-681.

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РОЗВИТОК ОКИСНОГО СТРЕСУ В КЛІТИНАХ ПІДШЛУНКОВОЇ ЗАЛОЗИ ЩУРІВ ЗА УМОВ ХРОНІЧНОГО ПРИГНІЧЕННЯ СЕКРЕЦІЇ ШЛУНКОВОГО СОКУ

Тривала гіпохлоргідрія інколи супроводжується розвитком помірного панкреатиту. На сьогодні точно невідомі механізми, що залучені до пошкодження підшлункової залози за цих умов. Метою дослідження було оцінити інтенсивність вільнорадикальних процесів у клітинах підшлункової залози щурів при експериментальній гіпохлоргідрії. Було встановлено підвищення вмісту пероксиду водню (у 1,8 рази), мРНК гена Nos2 (у 2,9 р.), загальної NO-синтазної (у 4 р.), тіоредоксинредуктазної (у 1,6 р.) та мітохондріальної супероксиддисмутазної (у 1,5 р.) активностей, а також зниження вмісту загальних (у 1,3 р.), білок-зв'язаних (у 1,3 р.) та небілкових (у 1,4 р.) SH-груп у підшлунковій залозі щурів. Отже, встановлено факт розвитку окисного стресу у клітинах підшлункової залози щурів за умов тривалої гіпохлоргідрії, що вказує на залучення порушення про-/антиоксидантного балансу до патофізіологічних механізмів розвитку помірного панкреатиту за даних умов.

Ключові слова: окисний стрес, гіпохлоргідрія, дисбіоз, підшлункова залоза.

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РАЗВИТИЕ ОКИСЛИТЕЛЬНОГО СТРЕССА В КЛЕТКАХ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ КРЫС В УСЛОВИЯХ ХРОНИЧЕСКОГО УГНЕТЕНИЯ СЕКРЕЦИИ ЖЕЛУДОЧНОГО СОКА

Длительная гипохлоргидрия иногда сопровождается развитием умеренного панкреатита. На сегодняшний день точно не известны механизмы задействованные в повреждение поджелудочной железы в этих условиях. Целью исследования было оценить интенсивность свободно-радикальных процессов в клеток поджелудочной железы крыс при экспериментальной гипохлоргидрии. Было установлено повышение содержания пероксида водорода (в 1,8 раз), мРНК гена Nos2 (в 2,9 р.), общей NO-синтазной (в 4 р.), тиоредоксинредуктазной (в 1,6 р.) и митохондриальной супероксиддисмутазной (в 1,5 р.) активностей, а также снижение содержания общих (в 1,3 р.), белок-связанных (в 1,3 р.) и небелковых (в 1,4 р.) SH-групп в поджелудочной железе крыс. Таким образом, установлен факт развития окислительного стресса в клетках поджелудочной железы крыс в условиях длительной гипохлоргидрии, что указывает на вовлечение нарушения про-/антиоксидантного баланса в патофизиологические механизмы развития умеренного панкреатита в данных условиях.

Ключевые слова: окислительный стресс, гипохлоргидрия, дисбиоз, поджелудочная железа.