УДК 616.34-089.84

THE ROLE OF LOCAL CHANGES OF SOME BIOCHEMICAL PROCESSES IN THE DEVELOPMENT OF INTESTINAL SUTURES INSUFFICIENCY

РОЛЬ ЛОКАЛЬНИХ ЗМІН ДЕЯКИХ БІОХІМІЧНИХ ПРОЦЕСІВ У РОЗВИТКУ НЕСПРОМОЖНОСТІ КИШКОВИХ ШВІВ

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The research has been done to study local changes of indices of lipid peroxidation, fibrinolysis and proteolysis in the intestinal tissues on the model of sutures insufficiency. It has been established that excessive activation of enzymatic fibrinolysis and collagen degradation in the intestinal walls was combined with disbalance of the pro- and antioxidant systems. Such combination of rising of destructive processes with the depletion of protective systems may contribute in a disturbance of regeneration of the connection area with the onset of sutures insufficiency.

Key words: intestinal sutures insufficiency, lipid peroxidation, fibrinolytic activity, proteolysis.

Дослідження проведене з метою вивчення місцевих змін показників перекисного окислення ліпідів, фібринолізу та протеолізу у тканинах кишок на експериментальній моделі кишкових швів. Встановлено, що надмірна активація ферментативного фібринолізу та деградація колагену у кишковій стінці поєднується з дисбалансом про- та антиоксидантних систем. Таке поєднання зростання деструктивних процесів з виснаженням систем захисту може сприяти порушенню регенерації ділянки з‘єднання з виникненням її неспроможності.

Ключові слова: неспроможність кишкових швів, перекисне окислення ліпідів, фібриноліз, протеоліз.

Introduction. For practitioners surgeons intestinal sutures insufficiency (ISI) is one of the most unpleasant and serious complications after operations on the hollow digestive organs. The frequency of the onset of ISI depends of type of surgery according urgency, region of digestive tract and technical conditions of intervention, it

is variable with range 2,3-32 % [3, 8, 9]. In this pathology the mortality rate is up to 50%, but when it becomes a leading cause of the onset of extended postoperative peritonitis, fatality rate rise up to 90% [1, 10]. It's important that unsatisfactory results of treatment of this polyethiological complication are largely associated with an insufficient study of all its pathogenetic aspects [14].

It is known that the normal healing of intestinal anastomosis is characterized by the specific reparative stages, depends on different conditions, most significant: a blood supply, a bacterial contamination and an adequate load of suture line [6, 7, 13]. In some of the early stages an important role in implementation of damaging influence of surgical trauma is an activation of lipid peroxidation in the sutured tissues, triggering factor of last one is almost always an local hypoxia [2]. The primary biological leak-resistance of sutures on the hollow digestive organs is provided by the formation of fibrin on the serous membranes at the place of their connection [4]. Furthermore, the tissue fibrin network is a matrix for fibroblasts that stimulates their growth and synthesis of the collagenous fibers, contributing to an optimal healing of the sutured area. Speed of regeneration depends on the processes of formation and destruction of the connective tissue controlled by the activity of the proteolytic, fibrinolytic, pro- and antioxidant systems [11]. Bibliographic analysis shown the most studied patterns of intestinal mucosal regeneration, on the contrary the data about reparative peculiarities of other structures of the intestinal wall, such as submucosal, muscular and serous layers, are not enough [12]. Individual papers are partially devoted to a study of the some biochemical processes in the tissue of the hollow organs of digestion [14]. Based of these data it is impossible to make convincing conclusions about of the essence of the changes that occur in site of connected tissues. A state of the problem of peculiarities of accumulation of lipid peroxidation products compared on activities of antioxidant, fibrinolytic and proteolytic system in intestinal tissues directly in the region of applied sutures in case of their incompetence remains obscure and require furtner research [5].

Purpose of the research: to experimentally study changes of indices of products of lipid peroxidation, activities of fibrinolytic, proteolytic and antioxidant systems in the tissues of intestine in the region of applied sutures under conditions of the development of their insufficiency.

Material and methods of the investigation. 72 albino nonlinear male rats, weighting 180±20g, were selected for the experiments. All the animals underwent a resection of the cupula of the cecum with suturing the intestinal foramen by means of interrupted stitches (polyamide 5-0). ISI was modelled by way of excessive mobilization of the area of junction and a rare application of stitches in the animals of the experimental group. In 12, 24, 48 and 72 hours following a surgical interference an euthanasia of the animals was performed under ether anesthesia and the samples of the intestinal tissue in the region of sutures were taken for an analysis. The indices of products of lipid peroxidation: diene conjugates (DC), malonic aldehyde (MA) and activity of the antioxidant enzymes: superoxide scavenger (SOS), catalase (Ct) and glutathione peroxidase (GPO) were researched with the aid of an assay kit "Simko Ltd" (Ukraine). The indices of fibrinolitic activity: total (TFA), nonenzymatic (NFA), enzymatic (EFA) and proteolytic activity by the lysis of: azoalbumin (AA), azocollagen (ACg), azocasein (ACs) have been researched according to Kukharchuk's procedure (1996). The Statistical processing of the investigation results was performed on PC by means of the application "Primer of biostatistics (Primer of Biostatistics, 4th Edi-

tion, S.A.Glantz, McGraw-Hill). Data from the groups were compared using Mann-Whitney‘s t-test. To reject the null hypothesis the significance level was used equal to p<0,05. The experiments were carried out with the observance of the requirements of the European convention as to the protection of vertebrate animals that are used for experimental and other scientific purposes (Strasbourg, 1986).

Results of the research are presented on the table.

Table

Indices of lipid peroxidation products and activities of antioxidant, fibrinolytic and proteolytic systems in the tissues of the rat intestine in sutures line‘s area

Indices	Intact	12 hours		24 hours		48 hours		72 hours	
		C	E	C	E	C	E	C	E
Diene conjugates (nmole/mg of protein)	-	0,333± 0,017	0,470± 0,021	0,385± 0,037	0,675± 0,018 ***	0,131± 0,015	0,793± 0,012 ***	0,223± 0,023	0,589± 0,007 ***
Malonic aldehyde (nmole/mg of protein)	-	0,154± 0,024	0,594± 0,057 ***	0,331± 0,046	0,461± 0,021	0,286± 0,006	1,211± 0,089 ***	0,545± 0,074	1,578± 0,110 ***
Superoxide scavenger (Units/mg of protein/min)	-	0,627± 0,041	0,237± 0,018 ***	0,962± 0,089	0,469± 0,025 **	0,572± 0,070	0,476± 0,024	1,130± 0,118	0,270± 0,031 ***
Catalase (mmole H2O2/ min/mg of protein)	-	20,734 ± 1,31	0,309± 0,041 ***	29,82± 2,091	0,316± 0,057 ***	23,941 ± 1,16	0,554± 0,034 ***	29,966 ± 2,030	0,429± 0,045 ***
Glutathione peroxidase (Gsh/mg of protein/min)	-	0,076± 0,018	0,013± 0,003 *	0,186± 0,030	0,009± 0,0003 **	0,230± 0,037	0,010± 0,0003 ***	0,207± 0,035	0,010± 0,0007 ***
Total fibrinolytic activity (E440/h × g)	40,48± 1,56	55,80± 1,48	82,60± 1,024 ***	43,04± 1,99	86,64± 1,12 ***	48,76± 1,97	80,32± 1,12 ***	45,52± 2,19	83,44± 1,34 ***
Nonenzymatic fibrinolytic activity (E440/h × g)	21,20± 1,079	28,80± 1,29	44,36± 0,995 ***	22,32± 1,64	45,04± 1,072 ***	24,40± 1,035	40,16± 0,54 ***	21,96± 1,19	40,40± 0,95 ***
Enzymatic fibrinolytic activity (E440/h × g)	19,28± 0,64	27,00± 0,43	38,24± 0,508 ***	20,72± 0,49	41,60± 0,32 ***	24,36± 0,94	40,16± 0,58 ***	23,56± 1,007	43,04± 0,57 ***
Azoalbumin lysis (E440/h × g)	43,80± 1,27	56,80± 1,19	77,76± 1,33	74,40± 1,73 ***	101,80 ± 1,24	83,52± 0,86 ***	114,04 ± 1,47	80,08± 0,98 ***	124,96 ± 1,84 ***

Indices	Intact	12 hours		24 hours		48 hours		72 hours	
Azocollagen lysis (E440/h × g)	14,68± 0,92	17,40± 1,296	31,52± 1,602 ***	18,04± 1,62	55,92± 1,602 ***	32,84± 1,48	48,24± 1,68 **	23,36± 1,36	46,88± 0,91 ***
Azocasein lysis (E440/h × g)	56,78± 1,45	81,84± 1,54	106,64 ± 1,401 ***	67,00± 1,84	120,00 ± 1,77 ***	103,56 ± 1,39	116,64 ± 1,97**	90,20± 1,45	111,84 ± 1,19 ***

Notes: C – control; E – experiment
** - P<0,05; ** - P<0,01; *** - P<0,001 – statistical reliable distinctions.*

According to the obtained data in the animals from the experimental group in comparison with the control one a reliable rising of the level of DC was detected since 24 h. of the observation, while the more increased indices of MA have been observed since 12 h. after operation. The indices of activity of all the antioxidant enzymes (SOS, Ct, GPO) were reliably lower in the animals with ISI as compared the animals without this one throughout the entire period of observation. The parameters of the fibrinolytic and proteolytic activity under study were reliably higher in the animals of the experimental group as compared with the control one.

When analysing the obtained findings it has been established that the inhibition of activity of the studied antioxidant enzymes in tissues of intestine of experimental group was more pronounced for SOS and Ct indices, beginning from the earliest periods of the observation (p<0,001). Along with a 4-fold increase of MA content in tissues, it can be evidence of a more pronounced inflammatory reaction in the connection area in animals with ISI as compared with control group.

In this time the steady activation of tissues proteolysis take place in the animals with unfavorable postoperative period. So, in 12-24 h. following the operation a reliably higher activity of lysis of AA, ACs and ACg was detected in the animals of the experimental group (p<0,001). It's testify about increase of proteolytic modification of the low- and high-molecular proteins. In particular, the activity of ACg lysis in the animals of the trial series exceeded twice the control findings which indicates a deeper degradation of collagen molecules in investigated tissues. Increased proteolytic activity are also contributes to the intensified lysis of fibrin in the junction area at the expense of a direct enzymatic action [2]. At this period of observation in the animals with IIS there occurs a proved rise of TFA, both at the expense of NFA and EFA (p<0,001).

As it is generally known, an activation of the nonenzymatic fibrinolysis is a counterbalance of a stress reaction [11]. The formation of the adrenaline-heparin-antithrombin III complex, activating plasminogen, contributing to its transformation into plasmin and splitting of fibrin, underlies it. However, such an impetuous and pronounced activation of fibrinolysis in the region of the connection may bring about a disturbance of the primary biological leak resistance of the suture line, infecting the thread canal and a penetration of microorganisms out of the intestinal lumen on their surface. The formation of loose adhesions with the participation of infiltrated hyperemic tissues of the omentum, the loops of the small intestine and the adjacent loops of the large intestine constituted visual manifestations of primary biological leakage of a junction zone in all the animals of the experimental group during this period.

During a later period (48-72 h.) we observed a tendency to rise of the indices of

tissue proteolysis, especially indices of ACg lysis, which were one and a half time higher than data of the control group. The long increased degradation of collagen molecules in tissues of the junction zone on the conditions of insufficient blood supply may be one of the mechanisms of disturbance of regeneration of sutured tissues [5]. An elevation of the tissue fibrinolytic activity was detected in the animals with IIS, largely at the expense of EFA which exceeded twice the control data. Such an excessive activation of the tissues fibrinolysis at the expense of lysis of the fibrin matrix may cause a disturbance of the fixation of fibroblasts in the tissues of the connection area and its regeneration [2, 4].

At this period we defined a great accumulation of final products of lipid peroxidation in the animals of the experimental group ($p<0,001$). So, concentrations of DC and MA were higher in 3-6 times in latter as compared with the control ones. The indices of activity of majority from the investigated antioxidant enzymes were 10 times less in the animals with ISI. Such disbalance of the pro- and antioxidant systems may be one of the mechanisms of implementation of the damaging effect of active oxygen forms on the conditions of ischemia in the area of sutures with the ISI development [2]. Thus, numerous hemorrhages and solitary films of fibrin in the region of the connection with separate defects and interintestinal abscesses were revealed within the specified period in the trial series against a background of a considerable amount of serous-fibrinous exudate in the abdominal cavity.

Conclusions. In case of intestinal sutures insufficiency an accumulation of lipid peroxidation products and specific differently directed changes of activities of antioxidant system and fibrinolytic-proteolytic activity are observed in the tissues of the junction area. In the early terms (12-24 h.) an increased level of proteolytic and fibrinolytic activity may be one of the mechanisms of disturbance of the primary (biological) leak-resistance of the suture line. At a later stage (24-72 h.) excessive activation of enzymatic fibrinolysis and collagen degradation in a combination with disbalance of the pro- and antioxidant systems may contribute in a disturbance of regeneration of the connection region with the onset of sutures insufficiency. On the basis of the last one, we consider it expedient to study correlations between the biochemical changes and degree of microbial contamination of the region of the interintestinal connection at the conditions of sutures insufficiency for the further research.

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