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S. I. Riaby

Bukovinian State Medical University
(Chernivtsi)

SOME PATHOGENIC ASPECTS OF INTESTINAL SUTURES INSUFFICIENCY

Key words: *intestinal sutures insufficiency, proteolysis, fibrinolysis, lipid peroxidation.*

Abstract. *The changes of indices of proteolysis, fibrinolysis and lipid peroxidation in the sutured tissues of bowel were researched on the model of intestinal sutures insufficiency. It was established that the disturbances of primary biological leak resistance (12-24 hours) caused by excessive activation of tissues fibrinolysis, and the disturbances of regeneration of sutured tissues (24-72 hours) induced by prompt and continued activation of tissues proteolysis and lipid peroxidation associated with depletion of antioxidants, are a basis of progressing intestinal sutures insufficiency.*

Introduction

Intestinal sutures insufficiency (ISI) is one of the gravest complications after operations on the hollow digestive organs. The frequency of the onset of ISI is variable with range 2,3 – 32 % and mortality rate in case of this one is up to 50% [3, 8]. The unsatisfactory results of treatment of this polyethiological complication are largely associated with an insufficient study of its pathogenetic aspects [4].

It is known [5, 6] that 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. 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 suture line. Blood supply, bacterial contamination of suture line and many other factors influence on regeneration of sutured tissues of intestine [7, 9]. Speed of regeneration depends on the processes of formation and destruction of the connective tissue controlled by the activity of the proteolytic and fibrinolytic systems. Individual papers are partially devoted to a study of the some biochemical processes in the tissue of the hollow organs of digestion [4]. The state of the proteolytic and fibrinolytic activity of tissues of the intestine in the region of sutures in case of their incompetence remains obscure.

The object of the research

To study changes of proteolysis, fibrinolysis and lipid peroxidation of the tissues of intestine in the region of sutures under conditions of the development of their insufficiency.

Material and methods

The experiments have been carried out on 56 albino nonlinear male rats, weighting 180±20g. 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 proteolytic activity by the lysis of: azoalbumin (AA), azocollagen (ACg), azocasein (ACs), and the indices of fibrinolitic activity: total (TFA), nonenzymatic (NFA), enzymatic (EFA) have been researched according to Kukharchuk's procedure (1996). The indices of the 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). Statistical processing of the results of the investigation was performed on PC by means of the application "Primer of Biostatistics". 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).

Discussion of results of the investigation

Results of the investigation are presented on the table. According to the obtained data the parameters of the proteolytic and fibrinolytic activity under study were reliably higher in the animals of the experimental group as compared with the control

Table

Indices of proteolysis, fibrinolysis and lipid peroxidation in the tissues of the rat cecum in the region of the suture line (x±Sx)

Indices	Intact	12 hours		24 hours		48 hours		72 hours	
		C	E	C	E	C	E	C	E
Azoalbumin lysis (E440/h × g)	43,80±1,27	56,80±1,19	77,76±1,33 P<0,001	74,40± 1,73	101,80± 1,24 p<0,001	83,52±0,86	114,04± 1,47 p<0,001	80,08±0,98	124,96± 1,84 p<0,001
Azocollagen lysis (E440/h × g)	14,68±0,92	17,40± 1,296	31,52± 1,602 p<0,001	18,04±1,62	55,92± 1,602 p<0,001	32,84±1,48	48,24± 1,68 p<0,01	23,36±1,36	46,88±0,91 p<0,001
Azocasein lysis (E440/h × g)	56,78±1,45	81,84±1,54	106,64± 1,401 p<0,001	67,00±1,84	120,00± 1,77 p<0,001	103,56±1,39	116,64± 1,97 p<0,01	90,20±1,45	111,84± 1,19 p<0,001
Total fibrinolytic activity (E440/h × g)	40,48±1,56	55,80±1,48	82,60±1,024 p<0,001	43,04±1,99	86,64±1,12 p<0,001	48,76±1,97	80,32±1,12 p<0,001	45,52±2,19	83,44±1,34 p<0,001
Nonenzymatic fibrinolytic activity (E440/h × g)	21,20± 1,079	28,80±1,29	44,36± 0,995 p<0,001	22,32±1,64	45,04± 1,072 p<0,001	24,40± 1,035	40,16±0,54 p<0,001	21,96±1,19	40,40±0,95 p<0,001
Enzymatic fibrinolytic activity (E440/h × g)	19,28±0,64	27,00±0,43	38,24± 0,508 p<0,001	20,72±0,49	41,60±0,32 p<0,001	24,36±0,94	40,16±0,58 p<0,001	23,56± 1,007	43,04±0,57 p<0,001
Diene conjugates (nmole/mg of protein)	-	0,333±0,017	0,470± 0,021	0,385± 0,037	0,675± 0,018 p<0,001	0,131± 0,015	0,793± 0,012 p<0,001	0,223± 0,023	0,589± 0,007 p<0,001
Malonic aldehyde (nmole/mg of protein)	-	0,154±0,024	0,594± 0,057 p<0,001	0,331± 0,046	0,461± 0,021	0,286± 0,006	1,211± 0,089 p<0,001	0,545± 0,074	1,578± 0,110 p<0,001
Superoxide scavenger (Units/mg of protein/min)	-	0,627±0,041	0,237± 0,018 p<0,001	0,962± 0,089	0,469± 0,025 P<0,01	0,572± 0,070	0,476±0,024	1,130±0,118	0,270±0,031 p<0,001
Catalase (mmole H_2O_2/min/mg of protein)	-	20,734±1,31	0,309±0,041 p<0,001	29,82±2,091	0,316± 0,057 p<0,001	23,941±1,16	0,554±0,034 p<0,001	29,966±2,030	0,429±0,045 p<0,001
Glutathione peroxidase (Gsh/mg of protein/min)	-	0,076±0,018	0,013±0,003 p<0,05	0,186±0,030	0,009± 0,0003 p<0,001	0,230±0,037	0,010± 0,0003 p<0,001	0,207±0,035	0,010± 0,0007 p<0,001

Notes. C – control; E – experiment

one. The 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 in the ISI group in comparison with control one 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 with the animals without this one throughout the entire period of observation.

When analysing the obtained findings it has been established that the steady activation of tissues proteolysis take place in the animals with ISI. 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 also contributes the intensified lysis of fibrin in the junction area at the expense of a direct enzymatic action [1]. 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 non-enzymatic fibrinolysis is a counterbalance of a stress reaction [2]. 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 [6]. 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 firinolysis 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 [5]. 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. 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

1. On the model of intestinal sutures insufficiency an increase of proteolytic and fibrinolytic activity with accumulation of products of lipid peroxidation are observed in the tissues of the junction area. 2. In the early terms (12-24 h.) increased proteolytic and fibrinolytic activity may be one of the mechanisms of disturbance of the primary (biological) leak-resistance of the suture line. 3. 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.

Prospects of further research

We consider it expedient to study correlations between the fibrinolytic and proteolytic activity and degree of microbial contamination of the region of the interintestinal connection in case of sutures insufficiency.

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ДЕЯКІ АСПЕКТИ НЕСПРОМОЖНОСТІ КИШКОВИХ ШВІВ

С. І. Рябий

Резюме. Досліджені зміни показників протеолізу, фібринолізу та перекисного окиснення ліпідів у зшитих тканинах кишечника на експериментальній моделі неспроможності кишкових швів. Встановлено, що порушення первинної біологічної герметичності швів у ранні терміни (12-24 год.), спричинені надмірною активацією тканинного фібринолізу, а також порушення регенерації ділянки з'єднання (24-72 год.), зумовлені різкою та тривалою активацією тканинного протеолізу і перекисного окиснення ліпідів у поєднанні з виснаженням антиоксидантного захисту, можуть бути основою для розвитку неспроможності кишкових швів.

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

НЕКОТОРЫЕ АСПЕКТЫ НЕСОСТОЯТЕЛЬНОСТИ КИШЕЧНЫХ ШВОВ

С. И. Рябой

Резюме. Исследованы изменения показателей тканевого протеолиза, фибринолиза и перекисного окисления липидов в сшитых тканях кишечника на экспериментальной модели несостоятельности кишечных швов. Установлено, что нарушения первичной биологической герметичности швов в ранние сроки (12 час.), вызванные чрезмерной активацией тканевого фибринолиза, а также нарушения регенерации зоны соединения (24-72 час.), обусловленные резкой и продолжительной активацией тканевого протеолиза и перекисного окисления липидов в сочетании с истощением антиоксидантной защиты, могут быть основой для развития несостоятельности кишечных швов.

Ключевые слова: несостоятельность кишечных швов, протеолиз, фибринолиз, перекисное окисление липидов.

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