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Biochemical aspects of purulent coxitis in an experiment

Biochemiczne aspekty ropnego zapalenia stawu biodrowego w badaniu eksperymentalnym

INTRODUCTION

The purpose of this work was to research dynamic changes of main biopolymers of osseous connective tissue during the purulent coxitis caused by the acute hematogenous osteomyelitis and the activity of lysosomal enzymes in blood serum that take part in this process.

MATERIAL AND METHODS

The experiments were conducted on rabbits breed "Chinchilla", weight from 1.8 to 3.2 kg, with the experimental modeling of the purulent coxitis (PC) caused by acute hematogenous osteomyelitis of the proximal part of femur and aseptic infarction of femur (AI). All manipulations were made under general thiopental anesthetic. The blood serum of animals was analyzed on 1, 3, 7, 14–15, 30–35, 60–65 days after creating the model. In the blood serum the activity of collagenase, cathepsin B, cathepsin D, proteolytic activity, elastase, antielastase, hyaluronidase, antitryptic activity, hydroxyprolin fractions, elastin and summary glycosaminoglycan content was determined. Control data was the information received for intact rabbits. The animals were taken out of the experiment by means of thiopental overdose.

RESULTS

Results of experimental research showed that within the first 24 hours in animals with PC the activity of cathepsin D rises and reaches 366%, the activity of collagenase and cathepsin B stays at the level of control values. Proteolitic activity reaches 134%. The concentration of free fraction of hydroxyprolin lowers to 65%, protein binding hydroxyprolin stayed at normal levels (Tables 1, 2). In animals with model of AI activity of collagenase, cathepsin B, cathepsin D did not change as the same with intact animals. Proteolitic activity reached 126%. The concentration of free fraction of hydroxyprolin lowers to 67%, protein binding hydroxyprolin stayed at normal levels.

Table 1

Indicators	Level in control animals	Kind of pathol-ogy	Levels of indicators at observation		
			1 day	3 day	7 day
Collagenas (mkmoll/l/gr) P	1.52±0.16	PC	1.48 ± 0.10	2.16± 0.30	5.23±0.47**
		AI	1.55 ± 0.07	1.97± 0.35*	1.95 ± 0.21
		PC-AI	> 0.05	> 0.05	< 0.01
Fraction of hydroxyprolin : Protein binding (mkmoll/l). P	10.14±0.55	PC	9.30 ± 1.15	9.92 ± 0.50	10.03 ± 0.18
		AI	11.80±0.14*	11.45 ± 0.15	11.60±0.28**
		PC-AI	> 0.05	< 0.05	< 0.05
Free (mkmoll/l). P	11.63±0.28	PC	7.55 ± 0.76	14.80 ± 2.43	16.93±0.54**
		AI	7.75±0.21*	7.43± 0.13**	8.85 ± 0.35**
		ГК-AI	> 0.05	< 0.05	< 0.001

* P < 0.05 **P < 0.01 compared with control

Table 2

Indicators	Level in control animals	Kind of pathology	Levels of indicators at observation		
			1 day	3 day	7 day
Cathepsin D (mkmoll/l/gr). P	0.50 ± 0.006	PC	1.16±0.10*	1.96±0.18**	2.38± 0.10**
		AI	0.45± 0.05	0.53± 0.015	0.66± 0.28**
		PC-AI	<0.001	<0.001	<0.001
Cathepsin B (mkmoll/l/gr). P	7.15 ± 0.49	PC	7.60± 0.22	6.32± 0.10	10.38±0.72**
		AI	7.55± 0.35	8.38± 0.08*	7.75 ±0.21
		PC-AI	>0.05	<0.001	<0.05
Proteolitic activity (mkmoll/ min/100ml) P	5.62 ± 0.13	PC	7.53±0.24**	8.44±0.77**	11.05±0.47**
		AI	7.10± 0.14	8.15±0.15**	6.40± 0.85
		PC-AI	>0.05	>0.05	<0.05

*P< 0.03 **P< 0.01compared to control

On the 3rd day (Tables 1, 2) in animals with PC the activity of cathepsin D reaches 392%, the activity of cathepsin B is a bit smaller then in intact animals and it was 88%. The research discovered increased activity of collagenase reaching 142%. Increased activity of callagenase occurs together with increased concentration of free fraction of hydroxyprolin to 127%, which appears under destruction of the main protein of connective tissue of the bone-collagen, so we found a straight correlation between the activity of collagenase and the concentration of free fraction of hydroxyprolin. In the concentration of protein binding hydroxyprolin we observed a tendency of lowering. In that time of observations (Tables 1, 2), in animals with model AI the data that were researched stayed at the normal level, except proteolitic activity which increased to 145% compared to the level in intact animals. Also, we observed not a big rise in the activity of collagenase 129% and cathepsin B 117%. In the concentration of fractions of hydroxyprolin we observed changes – lowering of free fraction to 64% and a rise of protein binding hydroxyprolin to 113%.

On 7th-day of observation (Tables 1, 2) in the blood plasma of animals with model of PC the activity of cathepsin D reaches maximum to 476%, or it is 4.7 times bigger then compared with intact animals. Analyzing results compared with animals with AI the results were 3.61 to 1. Proteolitic activity rises to 196%. The activity of collagenase and the concentration of free fraction of hydroxyprolin rises and reaches 160%, so at this time we see a straight correlation between the activity of colagenase and the level of free fraction of hydroxyprolin. First, we observe the activity of cathepsin B of 145%. The concentration of protein binding hydroxyprolin stays at normal levels with a light shift to the side of lowering.

In animals with model with AI the activity of cathepsin B stays at normal level, proteolitic activity lowers to the normal level, and on the 3rd day it goes up to 145%. The activity of cathepsin D reaches 132%. The activity of collagenase stays on the same level as on 3rd day of the experiment. The concentration of free fraction of hydroxyprolin rises to 76%, and the concentration of protein binding hydroxyprolin stays at the normal level.

On 14–15 days (3–4) in animals with PC the activity of cathepsin D stays on the level of 7th day, the activity of collagenase and cathepsin B rises and reaches maximum at 356% and 168%. Proteolitic activity lowers to 176%, the concentration of free fraction of hydroxyprolin stays on the level of 3rd day 130% and the concentration of protein binding hydroxyprolin a bit low at 89%.

In animals with AI (Tables 3, 4) the activity of cathepsin D stays at the normal level. The activity of collagenase stays at same level of day 7. Proteolitic activity is 112%. The activity of cathepsin B rose not significantly to 116%. The concentration of free fraction of hydroxyprolin lowers to 89% and the concentration of protein binding hydroxyprolin stays at the same level.

On 30–35 days of observation (Tables 3, 4) in model of PC the activity of cathepsin D stays at the level of 14th day with slight changes, compared to 14th day no changes were found in levels of collagenase, cathepsin B, proteolitic activity and concentrations of fractions of hydroxyprolin.

In animals with AI no differences were found in results compared to day 14, except that collagenase level was 125%.

On 60–65 days we observed no big changes in results compared to day 30. The levels stayed high. In animals with AI the activity of cathepsin D was little bit increased to 130% compared to intact animals (Tables 3, 4).

Table 3

Indicators	Level in control animals	Kind of pathology	Levels of indicators at observation		
			14-15 day	30-35 day	60-65 day
Collagenas (mkmoll/l/gr) P	1.52± 0.16	PC	5.41±0.13*	5.10±0.49*	5.31± 0.33
		AI	1.81± 0.12	1.90± 0.41	1.77± 0.14
		PC – AI	> 0.05	> 0.05	< 0.05
Fraction of hydroxyprolin : Protein binding (mkmoll/l). P	10.14± 0.55	PC	9.0 ±0.91*	10.0±0.39	8.73±0.59**
		AI	10.3±0.23	11.1± 0.29	10.5± 0.37
		PC – AI	< 0.001	<0.05	< 0.05
Free (mkmoll/l). P	11.63± 0.28	PC	15.1± 1.2	15.9± 0.91	14.77 ±1.20
		AI	7.97± 0.41	8.90± 0.31	11.53± 0.16
		PC - AI	> 0.05	< 0.001	< 0.05

*P< 0.05 **P< 0.01 compared to control

DISCUSSION

Thus, at the basis of changes of biochemical indicators of blood serum in experimental animals with PC and AI lie difficult tissue pathological processes. The rise in the level of cathepsin D in an early stage of the experiment was apparently connected with the liberation, after administration of bacteria, of lysosomal enzymes from hemopoietic cells that were directly damaged by toxins contained in the marrow of the pus-forming agents.

Accumulation of pyoinflammatory mainly neutrophilous exudation in the bone at the PC, break-up of leucocytes, liberation and suction to blood lysosomal enzymes lead to the subsequent increase of cathepsins activity, collagenase and proteolytic activity of blood serum. Intensive widespread destructive processes in bone – collagenolysis and bone resorption – also determine the absorption and growth of free hydroxyprolin fractions concentration in blood serum.

Table 4

Indicators	Level in control animals	Kind of pathology	Levels of indicators at observation		
			14 day	30-35 day	60-65 day
Cathepsin D (mkmoll/l/gr). P	0.50± 0.006	PC	2.14±0.35*	1.98± 0.14	2.20± 0.12
		AI	0.59± 0.02	0.61± 0.022	0.66± 0.03
		PC – AI	>0.05	<0.001	>0.05
Cathepsin B (mkmoll/l/gr). P	7.15± 0.49	PC	12.0± 0.98	11.40±0.87**	10.90±0.14
		AI	8.30± 0.29	7.90± 0.41	7.70± 0.12
		PC – AI	<0.05	< 0.001	< 0.001
Proteolitic activity (mkmoll/ min/100ml) P	5.32± 0.55	PC	10.10±0.98	9.10± 0.74*	11.97±0.93*
		AI	6.30± 1.10	7.20± 0.35	5.95± 0.28
		PC - AI	<0.05	<0.05	<0.05

*P<0.05 **P<0.01 compared with control

CONCLUSIONS

Some of these biochemical indices, taking into account their chronological movement can be recommended as diagnostic tests of PC. Thus, the earliest diagnostic characteristic was proven to be cathepsin D activity (1 day). In a later period from the onset of the disease additional diagnostic information can also be received by determination of collagenase activity (7 day), cathepsin B (14 day) and free hydroxyprolin fraction (7 day) (Table 5).

Table 5

Indicators	Time of informity
1. Cathepsin D	from 1 day
2. Collagenase	from 7 day
3. Fraction of hydroxyprolin	from 7 day
4. Cathepsin B	from 14 day

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SUMMARY

The aim of this research was to investigate the dynamics of changes of catabolism in the main biopolimers in bone tissue under purulent coxitis caused by acute hematogeneus osteomeitis and the activity of lysomal enzymes in blood serum that take part in this process. As a result, it was found that at the basis of changes of biochemical indicators in blood serum in animals with PC and AI lie difficult tissue pathological processes.

Key words: purulent coxitis, aseptic infarction, experiment

STRESZCZENIE

Celem badań było określenie zmian dynamicznych katabolizmu głównych biopolimerów tkanki kostnej w warunkach ropnego zapalenia stawu biodrowego, spowodowanego przez ostre zapalenie szpiku, i aktywności enzymów lizosomalnych w surowicy krwi, biorących udział w tym procesie. Wykazano, że u podstaw zmian w zakresie wskaźników biochemicznych surowicy krwi u zwierząt z PC i AI leży toczący się tkankowy proces patologiczny.

Słowa kluczowe: ropne zapalenie stawu biodrowego, zawał aseptyczny, eksperyment