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Impact of cyclooxygenase inhibitors on proteolysis of fibronectin and activity of gelatinases in experimental cardiomyopathy

Wpływ inhibitorów cyklooksygenazy na proteolizę fibronektyny i aktywność żelatynaz w kardiomiopatii doświadczalnej

INTRODUCTION

Cardiomyopathy is a frequent consequence of cardiovascular diseases and one of the main causes of morbidity and mortality worldwide. The molecular mechanisms underlying the development of cardyomyopathy are still poorly understood, making optimization of medical care as well as the progress in the development of new medications difficult. Impairment of the extracellular matrix (ECM) architectonics is considered to be one of the main mechanisms leading to the development of myocardial hypertrophy. The condition of ECM is determined by balance between the synthesis and degradation of their main proteins, such as fibronectin (FN). It is likely that activation of proteolysis, e.g. by activation of metalloproteinases (MMP) in the ECM, can lower the risk of fibrogenesis in the myocard and thereby of cardiac complications [1].
The aim of this study was to investigate the possible role of various COX inhibitors in the activity of proteolysis in connective tissue in an experimental model of cardiomyopathy.

MATERIAL AND METHODS

The activity of MMP-2, MMP-9, the concentration and fragmentation of fibronectin were determined in plasma of rats. The experiment was conducted on Wistar male rats that were divided into 5 groups of 6 animals in each. The first group consisted of intact animals (control). Doxorubicin-induced model of cardiomyopathy (DRCMP) was used in the other groups [2]. The second group consisted of the animals with DRCMP, the third group was treated with ketorolac (KT, selective COX-1 inhibitor), the fourth – with lornoxicam (LC, nonselective COX inhibitor), the fifth – with celecoxib (CCX, selective COX-2 inhibitor). All experimental animals were treated according to European ethical standards. At the end of the experiment the animals were anaesthezed with sodium thiopental and then sacrificed by decapitation.

The activity of MMP-2 and MMP-9 was estimated by gelatin zymography [3], and it was calculated as a percentage relative to the activity of these enzymes in the plasma of control rats. Measurement of the concentration of fibronectin (FN) was performed by immunodot. Fragmentation of FN (fFN) was investigated by Western blotting using polyclonal rabbit antibodies which were produced in our laboratory. Commercial standard of plasma FN (Sigma, USA) was used as a positive control. Statistical data processing was performed using the software Statistics 6.0. Nonparametric test of Mann-Whitney was used to assess significance.

RESULTS

According to our data, which are listed in table 1, in DRCMP animals, the activity of pro-MMPs increased 1.4–1.5 times but the activity of their mature forms remained unaffected. The activity of MMP-9 and pro-MMP-2 was decreased (67.3±7.09% and 78.2±1.51%, respectively) after application of KT, the activity of proMMP-9 did not change significantly in respect to the untreated group of DRCMP animals. CCX led to decreased proMMP-9 and increased proMMP-2 activities. LC exhibited an intermediate effect. The concentration of FN significantly increased in all experimental groups compared to control; the main FN level was in the fifth group.

Unexpected results were revealed by analysing circulating fFN (Fig.1). We did not find significant changes in the degree of FN degradation in animals with DRCMP. Treatment with COX inhibitors leads to increasing FN degradation. The greatest number of fFN was shown in animals treated with LC and CCX.

Table 1. Activity of gelatinases and fibronectin content in plasma of experimental rats

	Activity of MMPs, %				Concentration of FN, mg/ml
	proMMP-9	MMP-9	proMMP-2	MMP-2	
Control group	100	100	100	100	0.465±0.011
DRCMP	139.4±1.16**	119.5±1.16	152.1±5.78***	105.6±0.71	0.565±0.014**
DRCMP + KT	141.7±24.1	67.3±7.09***	78.2±1.51	105.5±1.51	0.703±0.058**
DRCMP + LC	122.3±3.13	70.1±5.36***	84.5±3.57***	103.4±1.00**	0.654±0.018***
DRCMP +CCX	110.3±2.13	100.4±7.50	134.5±4.62***	130.8±0.89	0.731±0.003***

** Level of reliability during p>0.01 *** p>0.001comparatively to norm

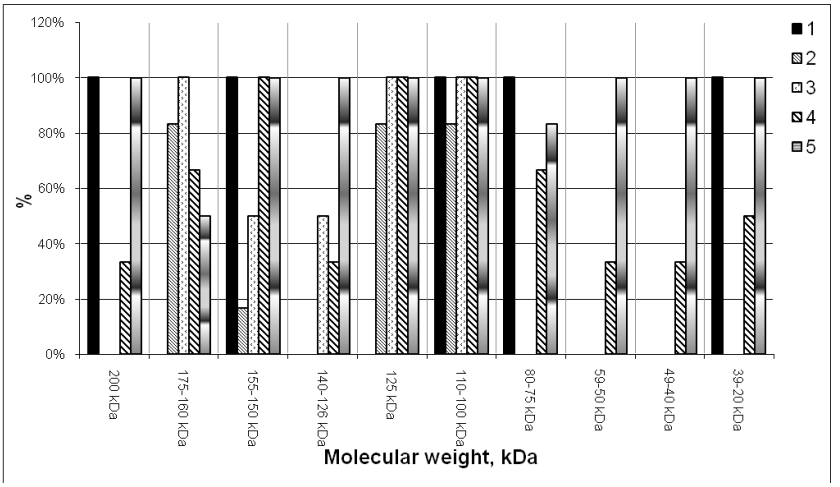

Fig. 1. Rate of fibronectin fragments detection in plasma of experimental rats: 1 – control animals, 2 – animals with DRCMP, 3 – animals treated with ketorolac, 4 – animals treated with lornoxycam, 5 – animals treated with celecoxib

DISCUSSION

The present study investigated whether selective and nonselective inhibitors of cyclooxygenase have an impact on proteolysis in the extracellular matrix, the process which plays an important role in cardiac remodelling. To our knowledge, that is the first report demonstrating the influence of the nonsteroidal anti-inflammatory drugs on the activity of MMPs and degradation of FN. The difference in effects of COX-1 and COX-2 is difficult to explain basing on the presented results. We suppose that changes in the activities of gelatinases and the degree of the FN degradation upon application of COX-inhibitors is the evidence of the diversity of mechanisms by which the investigated drugs influence fibrogenesis.

REFERENCES

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SUMMARY

The activity of gelatinases A and B, fibronectin concentration and its fragmentation in plasma of rats with doxorubicin-induced cardiomyopathy were investigated. It was shown that the treatment with COX-1 inhibitors leads to a significant decrease of MMP-9 and proMMP2 activities, and

treatment with celecoxib (COX-2 inhibitor) leads to an increase of both forms of MMP-2. Fibronectin degradation was increased in animals treated with COX inhibitors.

Key words: gelatinases A and B, fibronectin, doxorubicin, cardiomiopathy, COX-1, COX-2

STRESZCZENIE

Określono aktywności żelatynazy A i B oraz stężenie i fragmentację fibronektyny w osoczu szczurów z kardiomiopatią indukowaną doksorubicyną. Wykazano, że stosowanie inhibitorów COX1 prowadzi do istotnego spadku aktywności MMP-9 i proMMP-2, zaś zastosowanie celekoksybu (inhibitora COX2) powoduje wzrost obydwu form MMP-2. Degradacja fibronektyny była zwiększona u wszystkich zwierząt, u których stosowano inhibitory COX.

Słowa kluczowe: żelatynazy A i B, fibronektyna, doksorubicyna, kardiomiopatia, COX-1, COX-2