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Ocena roli neprylizyny i farmakologicznego zahamowania jej aktywności w zwierzęcym modelu kardiotoksyczności antracyklin

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

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ABSTRACT

Introduction: Anthracyclines are a group of widely used anticancer drugs in clinical practice, the administration of which is associated with the risk of cardiotoxicity (AIC), leading to heart failure with reduced ejection fraction (HFrEF). To prevent AIC, new therapies to reduce the risk of this side effect are sought, as well as biomarkers allowing for assessment of the risk of AIC and its subsequent monitoring. Due to the beneficial effect of the new drug combination: angiotensin II type 1 receptor antagonist and neprilysin inhibitor (ARNI) among patients with HFrEF, the role of neprilysin (NEP) and its soluble form (sNEP) in the pathogenesis of cardiovascular diseases has recently attracted the interest of researchers again. In studies in animal models of AIC the use of ARNI in high doses has been shown to have a protective effect against the occurrence of left ventricular (LV) systolic dysfunction. However, whether this effect remains present at lower ARNI doses has not been assessed. In addition, it has not yet been investigated if there are changes in the activity, expression and level of NEP within the LV in AIC and whether sNEP may be a useful biomarker for assessing the risk of AIC.

Aims: The first aim was to assess the changes in NEP activity, expression, and protein levels within the LV, as well as sNEP activity and concentration in the serum of experimental animals in acute (Part Ia) and chronic (Part Ib) AIC models. The second aim was to assess the effectiveness of low-dose ARNI in an animal model of chronic AIC.

Material and methods: 12-week-old Sprague-Dawley rats were used in the study. Animals in Part 1a received one intraperitoneal (*i.p.*) injection of doxorubicin (DOX) and were sacrificed within 24 hours for collection of LV and blood for further analysis. Animals in Part 1b and Part II received four DOX injections *i.p*. at weekly intervals and were sacrificed after a week of the last injection. Animals in Part II additionally received ARNI at a dose of 20 mg/kg of body weight by gastric gavage (*p.o.*), daily. In Part I of the study, the control animals received *i.p.* injections of 0.9% sodium chloride (NaCl), while in Part II – DOX *i.p.* and 0,9% NaCl *p.o*. Echocardiography was performed in all animals on the first and last day of the experiment. LV fragments were subjected to histopathological evaluation. In the remaining LV fragments from animals from Part I, NEP expression, activity and protein levels were measured, while in their serum concentration of sNEP was assessed. In the serum of animals from Part II, the concentrations of cardiac troponin I (cTnI) and the N-terminal propeptide of B-type natriuretic peptide (NT-proBNP) were measured.

Results: In Part I of the study the administration of DOX resulted in a significant decrease in echocardiographic parameters of LV systolic function and the occurrence of pathomorphological changes characteristic of AIC. In the acute AIC model, significantly lower

NEP activity was observed within the LV, while in the chronic AIC model - significantly lower NEP protein levels. There were no differences in mean serum sNEP concentrations between the control and experimental groups in both models. In the acute AIC model, the serum concentration of sNEP showed a strong positive correlation with the severity of degenerative changes of cardiomyocytes in the histopathological examination. In the chronic AIC model, there was a strong negative correlation between serum sNEP concentration and stroke volume and cardiac output. These were not observed in the control groups. In Part II, on the last day of the study, animals receiving ARNI had significantly higher values of echocardiographic parameters of LV systolic function and significantly lower degree of pathomorphological changes within LV, as well as lower serum cTnI concentrations compared to the control group.

Conclusions: DOX has an inhibitory effect on NEP activity in the LV in a rat model of acute AIC and NEP protein levels in the LV in a rat model of chronic AIC. In both models, these changes do not seem to affect the severity of AIC. Due to the observed relationships between the concentration of sNEP in the serum and the severity of degenerative changes in cardiomyocytes (acute AIC model) and the degree of decrease in some echocardiographic parameters (chronic AIC model), it seems that sNEP participates in the pathophysiological processes associated with the development of AIC. However, given the lack of change in mean sNEP concentrations in the serum of DOX-treated animals, sNEP is likely not a useful biomarker in AIC. Low-dose ARNI appears to be protective against the myocardial injury associated with chronic DOX administration in SPRD rats.

Summary: In this study, it was shown that neprilysin plays a role in the development of an animal model of anthracycline cardiotoxicity. Low-dose ARNI may be justified in the primary prevention of AIC in oncological patients who are intolerant to high doses of the drug.