mgr Ewa Waszkiewicz

temat pracy: *„Wpływ wieku i interleukiny 6 na proces uszkodzenia mięśnia sercowego indukowany lipopolisacharydem bakteryjnym”*

SUMMARY

Circulatory failure caused by septic shock is one of the most important causes of death in intensive care units. Patients in the intensive care units are particularly susceptible to infection because of the often older age, presence of concomitant diseases and severe general condition that all impair immune responses. Septic shock developing in the course of infection is characterized by a lower vascular resistance and myocardial contractility. Injured tissues and immune cells are the source of large amounts of cytokines and inflammatory mediators, which are responsible for the occurrence of septic shock.

Bacterial lipopolysaccharide (LPS), which is a component of the outer membrane of Gram-negative bacteria is a very potent inducer of acute inflammatory response. Interleukin-6 (IL-6) is among cytokines released during septic shock. It functions as both proinflammatory and immunomodulatory mediator. The precise mechanisms responsible for the observed pleiotropic effects of IL-6, is not fully elucidated. It is not established what is the efficacy of the protective mechanisms of Il-6 in older age.

The aim of the study was to evaluate the effect of bacterial lipopolysaccharide (LPS) on activation of intracellular signaling pathways in myocardial cells in relation to age and to the presence interleukin-6 in laboratory mice.

The study was conducted on 43 male mice of strain lacking a functional IL-6 gene (IL6KO - C57BL6 / $J^{IL6\ -\ /\ -\ TMKopf}$) and 44 animals control mice (C57BL6 / J – WT). In order to compare the effect of age on the studied phenomena experiments were performed on animals aged 3 months (49 mice) and 24 months (38 mice). Control groups of each genotype constituted animals treated with intraperitoneal with 0.9% sodium chloride solution at the dose 10 ml/kg, whereas study groups received LPS from E. coli according to the following regimens: group "0.1 LPS" received low-dose LPS in 0.1 mg / kg; group "LPS 10" received a high dose of LPS (10 mg / kg m. c.) in order to induce septic shock; the last group (“0.1 LPS + 10”) received two doses of LPS - first low dose (0.1 mg / kg b.w.), and after 24 hours the high dose (10 mg / kg

b.w.). The volume of the substance administered was the same in all cases. 24 hours after the last dose of LPS administration mice were sacrificed by cervical dislocation and the blood and the heart for further study. The base of the hearts fixed in 10% buffered formalin, and the remaining portions of the left ventricle were placed in liquid nitrogen for later analysis by molecular biology methods. Assessment of the severity of the inflammatory infiltration of the myocardium was performed on histological sections stained with hematoxylin and eosin and immunohistochemical staining for the common leukocyte antigen CD45. Degree of apoptosis was assessed based on the number of nuclei stained by the TUNEL method. The expression and phosphorylation of the proteins in the left ventricular myocardium were evaluated using Western Blotting. Following intracellular pathways' caomponents were evaluated: STAT3, ERK1/2, Akt1/2/3, and proteins affecting apoptosis: antiapoptotic Bcl-2 and pro-apoptotic p53. Moreover, expression of regulators of the JAK / STAT pathway: SOCS1/ 3 and PIAS proteins was evaluated.

Results: In young animals administration of high dose of LPS leads to activation of JAK/STAT and MEK/ERK pathways, with concomitant decreased expression of inhibitory proteins (PIAS and SOCS3), and without significant change of the IP3K/Akt cascade, which is activated by a low dose of LPS. Lack of IL-6 does not substantially change the response of JAK/STAT and MEK/ERK pathways to administration of LPS, but leads to reduction of PIAS expression and cancellation of inhibiting effect on SOCS3.

Administration of high dose of LPS to wild-type animals leads to downregulation of apoptosis-regulating proteins Bcl-2 and p53 and augmentation of apoptosis in the myocardium. Initial administration of the preconditioning dose of LPS is able to partially prevent septic shock-induced apoptosis. Absence of IL-6 downregulates p53 expression in the myocardium, and increases the severity of Bcl-2 downregulation after LPS administration. In senescent mice the dynamics of the activation of the JAK/STAT and MEK/ERK in response to LPS is blunted and high dose of LPS stimulates the IP3K/Akt cascade.

Lack of IL-6 does not change the response to intracellular signaling pathways after LPS administration. In senescent wild-type mice, LPS decreases PIAS expression, and absence of IL-6 makes this effect more pronounced. In contrast to the young animals, LPS administration did not significantly affect expression of SOCS3 protein, regardless of presence of IL-6.

Conclusions: Intraperitoneal injection of LPS causes changes in the activity of intracellular signaling pathways JAK/STAT and MEK/ERK related to cytoprotection, cell injury and apoptosis in the myocardium. In old age dynamics of the activity of later pathways is

significantly diminished and the IP3K/Akt responds to LPS with its upregulation. The impact of the absence of IL-6 activity changes of signaling pathways in response to LPS is limited and is characterized by lowering the expression of antiapoptotic proteins.