UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE AND TECHNOLOGY OF TÂRGU MUREȘ

DOCTORAL SCHOOL IN MEDICINE AND PHARMACY

DOCTORAL THESIS

SUMMARY

The role of lymphangiogenic markers in sepsis

PhD: Almasy Emoke

PhD Coordinator: Prof. Dr. Copotoiu Sanda Maria

UNIVERSITATEA DE MEDICINĂ, FARMACIE, ȘTIINȚE ȘI TEHNOLOGIE „GEORGE EMIL PALADE" DIN TÂRGU MUREȘ

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Introduction

Lymphangiogenesis occurs at the tissue level during inflammation, tissue or tumor injury. Endothelial lymphatic cells are differentiated cells, distinct from endothelial blood cells. This attracted the discovery of specific vascular lymphatic molecules, as a result: Prox-1 gene, podoplanin, endothelial growth factor type – C (VEGF-C) with its receptor, VEGFR-3. Inflammation is a common feature of various conditions, characterized by the pathological formation of new vessels, hemangio- and lymphangiogenesis. Lymphangiogenesis within inflammation is induced by macrophages and dendritic cells. It involves the reduction of tissue edema, the initiation of the immune response and the elimination of antigen.

The production of VEGF-C is induced in response to proinflammatory cytokines such as TNFα by activating the NF-kB pathway. Lymphangiogenesis occurs at the level of tissue inflammation, for example in bacterial infection and is induced by macrophages and granulocytes, producing VEGF-C. It has been shown that PDPN stimulates lymphangiogenesis, the inflammatory process and tissue edema. It is expressed on a variety of epithelial surfaces in several tissues, including the brain, heart, lungs, kidneys, osteoblasts and lymphoid organs.

The aim of the thesis

The first study aimed to evaluate plasma levels of VEGF-C as a diagnostic and prognostic biomarker for sepsis and septic shock.

In the second experimental study we analysed the tissue expression of PDPN in combination with the serum VEGF-C level in sepsis induced by living bacteria versus lipopolysaccharide.

Study no. 1. The Diagnostic and Prognostic Role of Vascular Endothelial Growth Factor C in Sepsis and Septic Shock

This study included 58 patients with sepsis, 49 septic shock patients diagnosed according to sepsis-3 criteria, and a control group of healthy volunteers. The plasma level of VEGF-C was determined in all groups. We analysed the difference in plasma VEGF-C values between the three groups. VEGF-C was correlated with severity scores, laboratory parameters, and intensive care days.

VEGF-C levels were significantly higher in patients with septic shock than in the control group, and VEGF-C was lower in patients with sepsis than septic shock patients.

VEGF-C correlated significantly with the APACHE II and MODS severity scores, but not with the SOFA score.

The temperature and number of leukocytes showed positive correlation with VEGF-C in septic shock. We found significant negative correlation between VEGF-C and respiratory rate in sepsis and with PaO2 in septic shock. The dose of the vasoactive drug used in the septic shock group correlated significantly with VEGF-C.

The specificity of the plasma level of VEGF-C was 71.43%; respectively, the sensitivity was 61.22% for the diagnosis of septic shock.

VEGF-C can be used as a prognostic marker of sepsis and septic shock, due to its correlation with APACHE II values. It is an early biomarker for determining the likelihood of developing multiple organ dysfunction. VEGF did not prove to be an ideal early marker for diagnosing patients with sepsis or septic shock because of its relatively low specificity and sensitivity. This study draws attention to the prognostic value of the level of VEGF-C in sepsis and septic shock, not least due to its quality of expressing the severity of tissue hypoxia.

Study no. 2. VEGF-C and podoplanin, as biomarkers of sepsis.

The study comprised 22 Wistar rats, divided into three groups: two experimental groups (n=8/group) and a control group (n=6).
Sepsis was induced by intraperitoneal injection of pathogens:

For group A we administered live E. Coli bacteria, group B was injected with lipopolysaccharide (LPS) from E. Coli, and group C received 1 ml of sterile saline solution.

Blood samples for serum VEGF-C levels for all groups and for blood culture in group A were collected under deep anesthesia. Tissue samples were collected from the liver, kidneys and lungs for the histopathological evaluation of the PDPN.

We achieved significantly higher VEGF-C values in groups A and B compared to the control group. During septic shock, LPS is released from the site of infection as a result of bacterial lysis and is transported to the circulatory system in a complex with the LPS binding protein. LPS is a surface component of all gram-negative bacteria.

The expression of the PDPN was present in all the organs examined by us: the kidney, lung and liver, in all the groups. Since the highest level of VEGF-C was found in Group A with positive blood culture, but PDPN expression was lower in proximal tubules in group A compared to group B, we conclude that the increased level of VEGF-C could reflect LPS-induced damage to proximal tubules as well as podocytes. Since the cases from group A had a high expression of PDPN in alveolocytes, it can be assumed that it exerts a protective role against lung damage during inflammation in sepsis induced by living bacteria.

The serum level of VEGF-C could be used as a potential biomarker in the proinflammatory stages of sepsis and as a predictor biomarker for the development of IRA in septic shock. The increase in VEGF-C in the critical septic patient could be used in modulating treatment to the exclusion of medication with implications for the development of IRA. PDPN is a biomarker with strong renal expression in the experimental model of sepsis, induced by LPS. Due to the high expression of PDPN in alveolocytes in sepsis induced by live bacteria, we can assume that PDPN has a protective role against lung injury during inflammation in this pathology.

Originality of the thesis

The originality of the work lies in the evaluation of lymphangiogenesis in sepsis and septic shock, using two biomarkers whose value has not yet been elucidated in this pathology.

The present study was carried out on two levels, one clinical, performed on patients and one in murin model.

We established the diagnostic value of VEGF-C in sepsis and septic shock at humans. We made a correlation between VEGF-C and the severity scores used in the ICU, the literature being less extensive on this aspect. To assess the involvement of various bacterial components in sepsis, we used LPS or live bacteria to trigger the immune response in rats.

For the induction of sepsis, we used increased doses of live bacteria and LPS. Moreover, the strains of E Coli used to induce sepsis come from local intensive care. Our experimental models raise the threshold of the amount of bacteria used by two orders of magnitude, indirectly demonstrating that established doses may not actually induce sepsis in experimental rat models. This observation is an alarm sign inciting to revise the established and overused models of induction of sepsis in experimental rat models.