SCIENTIFIC REPORT

Project title: Impact of high glucose in valvular endothelial cells-monocyte crosstalk: molecular signatures and role in early valvular dysfunction
Acronym: VALDYSIGN
Phase 2: Characterization of the molecular profile induced by short-term exposure to diabetic conditions in the VEC-monocyte interaction (part II); Evaluation of the function of relevant molecules found to be modified in endothelial permeability and monocyte adhesion and transmigration (part I).
Implementation period: 01.01.2023-31.12.2023
Activity 2.1: Investigation of protein expression of relevant molecules induced by high glucose in VEC-monocyte co-culture;
Activity 2.2: Investigation of endothelial permeability under high glucose conditions: the role of modified expression of junctional and cytoskeletal proteins;
Activity 2.3: Establishing the role of diabetes in monocyte adhesion and transmigration under dynamic conditions.

SUMMARY OF PHASE 2

Valvular endothelium represents an important protective barrier against metabolic, mechanical, and inflammatory aggression and is believed to play a role in valvular homeostasis and interactions with circulating blood cells and molecules. Regulation of endothelial barrier function is orchestrated by the quantity and arrangement of intercellular junctions, particularly adherens and tight junctions, of actin cytoskeleton organization and focal adhesions. Together, these elements govern tissue permeability. There is evidence that the phenotype of valvular endothelial cells (VEC) is progressively modified in diabetes, but the specific mechanisms by which diabetes contributes to early endothelial dysfunction remain incompletely elucidated. However, it is certain that both VEC and monocytes (Mo) play an important role and the diabetic environment perturbs cellular homeostasis. Therefore, the hypothesis of our study is that the interaction between monocytes and VEC under diabetic conditions triggers molecular modifications that lead to impairment of endothelial integrity and induction of monocyte adhesion and transmigration. The objective of this study is to establish the molecular signatures of VEC after their brief interaction with monocytes under high glucose conditions and to propose new mechanisms underlying early valvular dysfunction in diabetes.
In the second phase of the VALDYSIGN project, the gene and protein expression of modified molecules in VEC by interaction with monocytes under high glucose conditions were investigated, targeting the molecules involved in cell-ECM and cell-cell interactions, cell adhesion, and cytoskeleton regulation (pathways identified by VEC transcriptome sequencing analysis). The results showed that the interaction between VEC and monocytes under high glucose conditions modifies focal adhesions by decreasing focal adhesion kinase phosphorylation, reducing paxillin expression, vinculin internalization, and decreasing the expression of integrins that function as ECM receptors. In addition, VEC-monocyte interaction under high glucose conditions activates PI3K and RhoA-ROCK1 signaling pathways, reduces intercellular junctions by decreasing cadherin-2, increases E-selectin expression, and α4 integrin expression on the cell surface.
Since our study identified modifications of molecules involved in cell-cell and cell-ECM interactions, we investigated the functional role of these modifications in cellular permeability.

Our data showed that valvular endothelium permeability increases in interaction with monocytes under high glucose, compared to VEC maintained in culture medium with normal glucose concentration. Moreover, VEC interacting with monocytes under high glucose conditions are less adhesive to collagen I, fibronectin, and vitronectin, corresponding to observations on integrin expression. Also, our data suggest that increased glucose levels lead to increased monocyte adhesion and transmigration, which may contribute to the progression of aortic valve lesions.

DISSEMINATION

The results obtained in Phase 2 of the VALDYSIGN project were disseminated at two international conferences:

- Oral presentation: Bioinformatic analysis of molecular mechanisms underlying the aortic valvular dysfunction in diabetes. Monica Tucureanu. Participation in the "Crossing bridges between bioinformatics and clinical research - Genetoberfest GO2023" conference, from 16-19 October 2023, Munich, Germany;
- Poster: Molecular mechanisms underlying the aortic valvular dysfunction in diabetes. Monica Tucureanu, Letitia Ciortan, Razvan Macarie, Andreea Mihaila, Elena Butoi, Ileana Manduteanu. Participation in the 44th ANNUAL SCIENTIFIC SYMPOSIUM OF THE INSTITUTE OF CELLULAR BIOLOGY AND PATHOLOGY "NICOLAE SIMIONESCU" held jointly with the 40th ANNUAL SCIENTIFIC SESSION OF THE ROMANIAN SOCIETY FOR CELL BIOLOGY, from 16-17 November 2023, Bucharest, Romania.