Recently, diabetes has become a major health problem of people around the world. According to the IDF data (International Diabetes Federation) in 2015, the number of diabetic patients aged 20-79 years amounted to approx. 450 million, of which 193 million are undiagnosed. In the past year approximately 5 million people died because of diabetes. IDF estimates that if this growth is not being stopped, then by 2040 the number of patients suffering from diabetes will increase to 642 million.

The first-line drug for the treatment of type 2 diabetes is metformin, which in addition to its hypoglycaemic effect, possesses also beneficial effects on plasma lipid profile, exerts pro-fibrinolytic activity and reduces the risk of myocardial infarction. However, according to the scientific literature, the exact mechanism of metformin's effects on coagulation and fibrinolysis is not fully understood. Despite a number of positive pharmacological properties metformin is characterized by unfavorable pharmacokinetic properties, and high response variability associated with genetic polymorphism of transporters responsible for the processes of absorption, distribution and elimination of the drug. Therefore, to improve the physicochemical and pharmacokinetic properties of metformin, K. Huttunen et co-workers (University of Eastern Finland) synthesized a series of metformin's prodrugs.

The aim of the proposed project is to create a multidirectional model to evaluate biocompatibility of novel metformin's prodrugs with particular emphasis on plasma, platelet and vascular haemostasis. **Plasma haemostasis** studies will be based on determination of the effect of 9 metformin's prodrugs on the overall haemostatic potential (OHP), kinetic parameters of the process of clot formation and fibrinolysis (CL-test), extrinsic and intrinsic coagulation pathway, fibrinogen concentration, the enzymatic activity of thrombin, plasmin, and other coagulation factors as well as the expression of tissue factor (TF). These studies will be conducted on human plasma by means of spectrophotometric, optical and immunohistochemichal techniques. To determine the effect of metformin's prodrugs on **blood platelets** (biological material - platelet rich plasma) the following studies will be performed: activation, aggregation and adhesion of platelets using flow cytometry, and spectrophotometric methods. Studies on **vascular haemostasis** will be conducted on the cell model, and will include the following elements: the integrity of endothelial cells, apoptosis, the release of t-PA and von Willebrand factor from vascular endothelial cells, the expression of ICAM-1 (intercellular adhesion molecule-1) on endothelial cells. These experiments will be performed by means of flow cytometry and immunochemical methods.

Within this project it is also planned to verify the research hypothesis whether uptake of metformin's prodrugs can be mediated by OCT (Organic Cation Transporters) or MATE (Multidrug And Toxin Extrusion proteins) transporters. These experiments will enable to define the role of OCTs in cellular transport of prodrugs. Determination the transporters role in cellular uptake of prodrugs will be carried out on the cell model by the method of quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR). Within this group of experiments we also plan to evaluate the affinity of prodrugs for subtypes of transporters and to determine the kinetics of cellular uptake of these prodrugs.

In addition, based on the results of *in vitro* studies and determining the relationship between the structure and the prodrugs' activity it is planned to design and synthesize other metformin's prodrugs with favourable pharmacokinetic and biological properties. Structure of the new prodrugs obtained in the **synthesis process** (conventional synthesis techniques), purified and analyzed by high performance liquid chromatography (HPLC) will be confirmed by spectroscopic methods and elemental analysis. **Physicochemical and pharmaceutical properties**, such as pK_a-value, aqueous solubility, lipophilicity (log P/D), plasma protein binding, and chemical stability in various pH values and temperatures, of the novel prodrugs will be evaluated by conventional pharmaceutical techniques. The analyses will be carried out by UV, MS and HPLC methods. **Enzymatic bioconversion** of the novel prodrugs to their active drug molecules (metformin) and possible other metabolites will be evaluated in various human or mice subcellular fractions.

Accomplishment of the proposed project will enable to identify the molecular targets of potential pharmacotherapy and to determine the biocompatibility of the prodrugs in the context of their potential use as therapeutics. Furthermore, the project may also play a role in raising public awareness on the occurrence, prevention and treatment of metabolic diseases, including diabetes.