Proposed Project is the result of previous cooperation of few teams of scientists within the consortium "Application of polyisoprenoid derivatives as a drug carriers and metabolism modulators". Polyisoprenoid alcohols (polyprenols and dolichols) are long-chain, linear polymers composed of isoprene units (from several up to more than 100 units) found in almost all living organisms. They are involved in cell response to environmental stress, and intensify the fusion and increase the permeability of model membranes. The subject of abovementioned consortium were cationic, semi-synthetic derivatives of polyisoprenoid alcohols, called amino-prenols. Obtained results confirmed that the tested compounds possess lipofecting properties (*in vitro* and *in vivo* studies), which means that they facilitate the transfer of genetic material into the cells, hence the idea to use amino-prenols as components of transfection mixtures. It was also confirmed that tested derivatives are not toxic (*in vivo* studies on rats and *in vitro* in various cell cultures).

In this project we would like to address the following issues and accomplish the following tasks:

1. *To compare lipofection efficacy of new lipofecting mixtures containing derivatives of polyisoprenoid alcohols (amino-prenols) with commercially available reagents for transfection in targeted therapy*

Gene therapy, a therapeutic approach consisting of delivering nucleic acids into patient's cells in order to supplement or correct impaired genes responsible for disease development, holds a great promise for treatment of many diseases. According to Gene Therapy Clinical Trials Worldwide Database more than 2 thousands clinical trials had been conducted or approved by January 2014 to treat many different health problems, such as cancer, AIDS, cardiovascular diseases or cystic fibrosis. **Development of efficient and, most of all, safe vectors for transfection is one of the major challenges in modern genetic engineering**. Viral vectors are considered more effective, but plagued by safety concerns as opposed to non-viral vectors which are non-pathogenic and non-immunogenic for human cells.

Obtaining effective carriers of genetic material seems to be extremely important for the development of many fields, such as medicine (developing new models of diseases, vectors in gene therapy) or molecular biology (gene function and protein expression study).

2. *To examine if local enhancement of expression of genes responsible for VEGF-A synthesis in renal medulla (area predisposing to hypertension) will lower the blood pressure of spontaneously hypertensive rats (SHR)*

In the present project we would like to use amino-prenol-based lipofecting mixtures, to deliver plasmid DNA encoding VEGF-A protein and reporter protein Green Fluorescence Protein (GFP), needed for the visualization of transfection effectiveness, to spontaneously hypertensive rats (SHR) in order to increase the level of VEGF-A and investigate its impact on blood pressure and renal haemodynamic and function.

Hypertension is major worldwide health problem because of its high prevalence and associated risks of chronic kidney disease, heart failure, ischaemic heart disease, but also stroke and other cerebrovascular or retinal diseases. D**espite decades of research, hypertension is still inadequately diagnosed, largely because of incomplete understanding of the underlying pathogenic mechanisms.**

Recent studies provide an ample evidence indicating important role of vascular endothelial growth factor (VEGF) signalling pathway in the prevention of the development of hypertension. VEGF is a principal regulator of various physiological processes including angiogenesis, mediating increased vascular permeability, proliferation and lymphangiogenesis. The first indication of an association of VEGF-A and hypertension was when VEGF inhibitors were applied for anticancer therapy. VEGF has been shown as a mediator of neoangiogenesis, which is a crucial phenomenon enabling the growth and metastasis of tumours. The most common adverse effect, occurring after treatment with VEGF inhibitors, is hypertension. The mechanism of hypertension associated with VEGF inhibition is still not clear and it is uncertain what is crucial for the development of suitable therapeutic strategies for patients. It is proposed that hypertension which develops after VEGF inhibition is caused by multiple factors. First to mention is deficient production of nitric oxide (NO), which leads to systemic vasoconstriction and a rise in blood pressure. Several studies indicate that VEGF induces NO-dependent relaxation in coronary arteries by up-regulation of the endothelial nitric oxide synthase (eNOS). A decrease in the number of small arteries and arterioles (microvascular rarefaction) observed after treatment with VEGF inhibitors may be another mechanism responsible for the rise in blood pressure. Microvascular rarefaction occurred consistently as a result of defective angiogenesis in adults with high blood pressure (especially those with genetic predisposition to hypertension).

Recent studies performed in our Department of Renal and Body Fluid Physiology also indicate the very important role of VEGF-A in hypertension. Our studies demonstrated two phenomena: lowered perfusion of the renal medulla and relatively low immunoreactivity of VEGF-A in this region observed in spontaneously hypertensive rats (SHR) in comparison to normotensive animals. Numerous mechanisms were described to protect renal circulation, especially in the inner medulla (IM) of the kidneys, which suggest the medulla's important role in hypertension. It seems that the reduced VEGF-A immunoreactivity in IM could be a predisposing factor for the development of hypertension.

Answering the question, through basic research, if enhanced expression of VEGF-A in the inner medulla of the kidneys will increase its circulation and reduce blood pressure seems to be very beneficial for fulfilling the gaps in the knowledge of pathogenesis of hypertension and mechanisms underlying blood pressure regulation.

Work plan

First, we would like to investigate the effectiveness of newly designed amino-prenol-based mixtures per se in introducing plasmid DNA encoding GFP reporter gene *in vivo*. Selected mixtures will be administered into the renal artery of SHR under short-term anaesthesia. After 48 h rat will be anaesthetised again and respective organs (kidneys, heart, liver) will be harvested. Efficacy of transfection will be analysed on the basis of fluorescence intensity of GFP and compared to effectiveness of commercially

available reagents.
In the second phase of the study we plan to administer lipofection-mixtures (selected in the first step) directly into the renal medulla of SHR. Rats will be anaesthetized (short-term anaesthesia) and both kidneys will be exposed. Micro-cannulas will be placed into the renal medulla, allowing the infusion of respective solutions. Such targeted therapy appears to be very beneficial since we want to enhance expression of VEGF-A exclusively in this specific renal region, which seems to be crucial for blood pressure regulation. After careful administration, rats will be awake from anaesthesia and monitored for 7, 14 and in the following group for 21 days.
In all groups we intend to measure blood pressure by telemetry method, which is considered as "golden standard" in laboratory animals. Blood sampling and metabolic studies (24-h observation in metabolic cages) combined with urine collection will be also performed in all groups. In the end of each chronic experiment, the rats will be divided into two groups. One group will be anaesthetized and short observation of renal haemodynamics and functions will be performed, since alterations of medullary blood flow may be expected to be associated with hypertension. The second group will be also anaesthetized and tissues for morphologic, morphometric, biochemical and molecular analysis will be harvested.
Collected biological material (tissues, urine, plasma) will be used for measuring various substances involved in blood pressure regulation (concentration of ion's, nitric oxide), markers of kidney damage (microalbuminuria, creatinine), as well as concentration and localization of VEGF-A (qualitative and quantitative VEGF-A levels). Due to angiogenic activity of VEGF-A, we also plan to evaluate changes in vascular network (using different microscopic technics and various stainings and labelings with factors specific for angiogenesis).
In the third phase we also would like to make an attempt to enhance expression of VEGF-A in long-term manner by transfection mixtures, administered in osmotic mini-pumps directly into renal medulla of SHR. Cannulas will be inserted directly into both kidneys. Such approach is possible with ALZET® Brain Infusion Kits. Such original and unconventional approach seems to be very beneficial for long-term up-regulation of expression of VEGF-A.

Interdisciplinary research proposed in this Project on novel carriers of genetic material used to locally increase expression of VEGF-A in the renal medulla, if successful, not only can help to clarify the basic mechanisms and to understand diverse pathogenesis of hypertension, but also provide a powerful tool in the form of effective mixtures for transfection, which will allow the rapid development of many fields, such as molecular biology or medicine.