Description for the general public

The central problem of this project is the urgent need to develop an innovative method of the therapy of inflammatory bowel diseases (IBDs), which pathological cause remains unclear. IBD is a complex condition arising as a result of cross-talk between environmental and genetic factors leading to altered immunological responses and inflammation in the intestine. Inflammatory bowel diseases are most commonly diagnosed in young patients aged between 15 and 30 years old. These disorders typically follow a relapsing-remitting course, with symptoms such as diarrhea and abdominal pain. IBDs may lead to extraintestinal complications, such as peripheral arthropathies of small and large joints, primary sclerosing cholangitis or deep vein thrombosis. In extreme cases an increased risk of colorectal cancer may occur. Until now, no fully effective therapy of IBDs has been developed.

Mesalazine (5-aminosalicylic acid, 5-ASA) is one of the basis of modern treatment methods of IBDs. Its main anti-inflammatory activity involves inhibition of signaling through nuclear factor-κB (NF-κB), a transcription-regulating protein complex playing a key role in control of immunological processes such as cytokine and chemokine production. The drug also proved to be a potent free radical scavenger, as well as inhibitor of activity of cyclooxygenases and prostaglandin synthesis. However, applicability of 5-ASA is notably limited due to its rapid inactivation and removal from the organism, as well as the need for efficient, membrane transporter-dependent cellular uptake of the drug to reach its molecular targets and exert therapeutic effect. Mesalazine treatment is also responsible for numerous side effects, involving myelosuppression and dysregulation of nervous, cardiovascular, digestive and excretory systems, further limiting the efficacy of therapy.

In order to increase its intestinal concentrations and enhance clinical effectiveness, several formulations and delivery vehicles for mesalazine have been developed. Current research is aimed at application of innovative micro- and nanocarrier systems, of which dendrimers attract particular attention. Due to their sphere-shaped, symmetrical architecture with external branches terminated with chemical moieties, these macromolecules are featured with high solubility and biopermeability (ability to cross biobarriers), enabling efficient cellular uptake. Terminal groups are usually highly reactive and may be further modified to change the physicochemical features of a dendrimer, or to generate desirable activity, e.g. therapeutic or catalytic. Drug-dendrimer conjugates have been shown to improve the solubility of therapeutics, extend their systemic circulation time and protect against degradation. Such constructs proved to be capable of efficient transport of drugs directly to its destination, thus contributing to elimination of adverse side effects. Covalent bonding may provide higher stability in comparison to non-covalent complexes, as well as controlled, enzymatic or pH-triggered drug release at the site of action, giving a chance to reduce the dose while maintaining the same therapeutic effect. All these features make dendrimers promising candidates for nanocarriers of mesalazine.

The present project aims to develop an innovative nanocarrier of 5-ASA, based on poly(amidoamine) (PAMAM) dendrimer of the 4th generation (G4), in order to obtain higher concentrations of the drug in the intestinal epithelial cells, thus increasing its anti-inflammatory potential. The project involves synthesis and *in vitro* characterization of covalent conjugate of PAMAM G4 macromolecule and multiple particles of mesalazine with the use of succinic linker. The experiments will be carried out in two *in vitro* models of intestinal epithelium, CaCo-2 and HT-29 cell lines. The models of inflammation will be obtained by incubating cells with well-known pro-inflammatory activators. Cellular parameters related to anti-inflammatory activity of 5-ASA will be evaluated: inhibition of NF-κB signaling pathway, down-regulation of synthesis of cytokines and prostaglandins, and free radical scavenging. Selected experiments will be repeated in the presence of inhibitors of mesalazine-specific cellular uptake in order to fully evaluate the therapeutic potential of PAMAM-5-ASA. In addition, drug release from conjugate and cellular uptake of mesalazine and PAMAM-5-ASA in the presence or absence of inhibitors of 5-ASA-specific membrane transporters will be evaluated as well.

The use of PAMAM macromolecule as a carrier for mesalazine may increase the solubility and thus the bioavailability of the drug. The covalent bonding is meant to protect 5-ASA against inactivation and premature release. The dendrimer-based carrier may ensure enhanced cellular uptake, bypassing the need to utilize mesalazine-specific membrane transporters. All these characteristic will potentially translate into an improved efficacy of mesalazine. The use of modern methods of molecular biology will allow to fully characterize the mechanism of action of both the drug itself and drug-dendrimer conjugate, thus helping to fill gaps in knowledge concerning central aspects of this project.