Evaluation of the therapeutic potential of flavonoids and their combined use with a recombinant form of alpha-L-iduronidase in murine model of mucopolysaccharidosis type I

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Mucopolysaccharidosis type I (MPS I) is a metabolic disease that belongs to the group of lysosomal storage diseases (LSD). It is caused by the mutation in the IDUA gene, which results in a complete lack or significant enzyme deficiency - α-L-iduronidase, involved in the breakdown of glycosaminoglycans (GAG) - heparan (HS) and dermatan sulfate (DS). Disruption of catabolism of these compounds results in their successive accumulation leading to dysfunction of the whole organism. The course of the disease is severe and progressive with both somatic and neurological symptoms, and the treatment options are very limited. The therapeutic strategies mostly are based on the phenomenon of cross-correction, based on external uptake of an active enzyme by deficient cell. Two therapeutic strategies have been developed to provide the deficient α-L-iduronidase to cells in the form of enzyme replacement therapy (ERT), used to treat non-neurological form of MPS I, or hematopoietic stem cell transplantation (HSCT) in severe types with central nervous system (CNS) involvement. However, none of these therapeutic strategies are able to effectively correct all functional deficits associated with the progression of the disease. In addition, both types of therapies carry a significant risk of severe complication and side effect. Delivery of a foreign protein into the body of a patient receiving ERT activates the immune response, which result in the reduction of -α-L-iduronidase activity by the produced antibodies. On the other hand the effectiveness of HSCT is related to the patient's age, and positive outcomes can only be expected if the procedure was performed in children under 2.5 years of age. Therefore, it seems reasonable to look for new therapeutic strategies that will include the delivery of the therapeutic compound to the CNS also in cases of late diagnosis.

One of alternative therapies is substrate reduction therapy (SRT), which is based on lowering the efficiency of the GAG biosynthesis process by substances of low molecular weight. Genistein a natural soybean isoflavone has the ability to modulate and reduced GAG synthesis in the cell. As part of the research described in this dissertation, using the murine MPS I model, the effectiveness of long-term administration of genistein at dose of 160 mg/kg/day had been evaluated. In addition, the efficacy of combined use of SRT with genistein and ERT with recombinant α-L-iduronidase in compare to monotherapies had been evaluated. In principle, providing two compounds with different

mechanisms of action may contribute to a better therapeutic effect. Moreover, the therapeutic potential of kaempferol, another compound from the flavonoid group, had been determined. It can be used as an active agent modulating the efficiency of GAG synthesis process, as a possibly treatment for MPS I.

The results obtained have shown that long-term oral administration of genistein in a high dose causes a significant reduction in the storage of the material in the liver and spleen of MPS I mice in compare to the animals in the control group. What's more, a statistically significant reduction of GAG accumulation in brain tissue had been noted. The tested therapy had significant outcome on the improvement of neurocognitive behavior of female MPS I mice. The combined use of genistein and recombinant α-L-iduronidase, led to improvement in all measured signs of progressive disease process: both reducing GAG storage in internal organs (liver, spleen) as well as in heart and its valves, which are resistant to ERT. The reduction in the level of GAGs excreted in the urine of animals that received combination therapy had been observed. Genistein used with ERT has resulted in a significant improvement in the behavior of MPS I females, which is not observed when only recombinant α-L-iduronidase had been delivered.

Orally administered kaempferol, effectively reduces the level of glycosaminoglycans in the liver and in the myocardium of MPS I mice in comparison to non-treated animals. In addition, it has influence on the behavior of female MPS I mice, which correspond to the behavior of wild type animals. Nevertheless, the use of kaempferol cause adverse effects and observed increase in GAG storage in spleen and kidney, which is directly proportional to the applied dose.

Due to the fact that the liver is an extremely metabolically active, during the progressive disease process of MPS I, the significant accumulation of glycosaminoglycans occurs in this organ. Therefore, the possibility of using primary mice hapatocytes as an *in vitro* model for research on new therapeutic strategies. Optimized protocol for the isolation of a large population of live mouse hapatocytes, as well as their culturing conditions has been developed. Those cells showed a significantly higher level of GAG accumulation in murine MPS I hepatocytes in compare to cells obtained from wild type animals.

The results presented in the paper are original reports on the long-term use of flavonoids, genistein and kaempferol, and their impact on the level of GAG storage in the murine MPS I model. Flavonoids can be successfully used as active agents for SRT in MPS I in both monotherapy and in combination with other types of therapy. All of the established

results are the strong basis for extending the research on the potential of using various flavonoid to treat MPS I.