Novel insights into hydrogen sulfide-mediated cytoprotection – can H_2S prevent the progression of Duchenne muscular dystrophy?

Duchenne Muscular Dystrophy (DMD), a recessive X-linked disease affecting 1 in 5,000 - 6,000 boys, is the most common and one of the most severe forms of muscular dystrophy. The first symptoms of DMD, noticeable in young boys, are walking difficulties that progress with age. Death usually occurs around the 2nd or 3rd decades of life due to respiratory insufficiency and cardiac failure.

DMD, still an incurable disorder, is caused by mutations in the gene encoding dystrophin, a large actin-binding cytoskeletal protein that is essential for the proper connection between the actin cytoskeleton filaments and extracellular matrix proteins in muscle cells. In the absence of dystrophin, the continuous activation of skeletal muscle satellite cells, increased fibrosis and oxidative stress, and the induction of inflammatory processes, lead to progressive loss of muscle mass and impaired muscle function. It was shown recently, that dystrophin is absent not only in muscle cells but also in endothelial cells that build blood vessels. Our results suggest that the development of DMD may be affected by the impairment of angiogenesis, the process of blood vessel formation.

Glucocorticoids, which act mainly as an anti-inflammatory agent, are the main available treatment for patients with DMD currently, however they exert many side effects. As DMD still remains an incurable disease, the identification of new factors modulating its progression is needed. Our preliminary studies, including RNA-seq and proteomic analysis, showed decreased expression of H_2S-generating enzymes in dystrophic animals and human samples. **Therefore, in the current project, we will test the hypothesis that hydrogen sulfide (H_2S), a gas with anti-inflammatory, anti-oxidative, anti-fibrotic, pro-angiogenic and cardioprotective effect, can have a therapeutic potential in DMD.**

To examine our hypothesis, we will conduct experiments in a mouse model of DMD – *mdx* mice, lacking expression of dystrophin. In addition, we will also use *mdx*/utr$^{-/-}$ mice, deficient in both dystrophin and utrophin, representing a model of the more severe disease phenotype than *mdx* mice and used mainly to study cardiac complications. In order to verify whether the mechanisms, presumed to exist in the mouse models of DMD are recapitulated in humans, we propose state-of-the-art strategy of using induced pluripotent stem cells (iPSC), obtained from blood cells of healthy subjects and DMD patients as well as the generation of the isogenic cell lines using CRISPR/Cas9 technology and their differentiation to endothelial cells and cardiomyocytes. In addition to analyzing the effect of H_2S-releasing compounds, we suggest gene therapy approach by the overexpression of H_2S-generating enzymes both *in vitro* and *in vivo*. Using RNA-seq technology, functional tests assessing muscle contraction, exercise capacity, cardiac functionality, biochemical methods and assays to evaluate the properties of cardiomyocytes (e.g. electrophysiological characteristic, calcium dynamics, mitochondrial activity) and endothelial cells (e.g. angiogenic potential using Matrigel assay and spheroidal cultures in collagen gel) we will thoroughly analyze the role of H_2S in DMD progression.

The result of the project will be the verification of the hypothesis about the positive role of H_2S in the modulation of processes relevant to DMD development, including inflammation, fibrosis, autophagy, angiogenesis, and cardiomyopathy. We believe that planned research using mouse disease models, gene therapy and induced pluripotent stem cells will allow for a better understanding of disease mechanisms.