Impaired angiogenesis in Duchenne muscular dystrophy - is there a role for heme oxygenase-1 and statins?

Duchenne muscular dystrophy (DMD), an X-linked recessive disease, affecting around 1 in 3,500 boys, is the most common and one of the most severe forms of muscular dystrophy. It was first described by the French neurologist Guillaume Benjamin Amand Duchenne in the 1860s, and up to date, it is an incurable disease. Early signs of motor impairment manifest between the ages of 2 years, but the disease progresses rapidly and between 10 and 14 years of age, patients have serious problems with walking and are usually wheelchair-dependent by adolescence. Finally, they begin to suffer from respiratory and cardiac failure what leads to the patient's death around the 2nd or 3rd decades of life.

DMD is caused by mutations in the gene coding for the protein dystrophin, large actin-binding cytoskeletal protein that is essential for the proper connection between the actin cytoskeleton filaments and extracellular matrix proteins. In the absence of dystrophin, the continuous activation of skeletal muscle satellite cells (i.e. stem cells, which normally are activated, for example in the injured muscles or after training), induction of inflammation, fibrosis or oxidative stress occur. All these processes lead to a progressive loss of muscle mass and muscle dysfunction. Some studies suggest that the abnormal process of blood vessel formation (angiogenesis), can also affect the development of DMD. In patients with DMD, dystrophin is absent also in endothelial cells, and this affects their proper angiogenic functions and cause dysfunction of endothelium.

In the current project we will test the hypothesis that modulation of angiogenesis by the cytoprotective enzyme - heme oxygenase 1 (HO-1) and statins could have therapeutic effect in the treatment of DMD. HO-1 possess well-documented anti-apoptotic, anti-inflammatory and antioxidant effects, and on the other hand, it positively regulates the production of angiogenic factors and controls angiogenesis. Our results indicate also that this protein is important in the biology of muscle cells. Interestingly, statins, drugs commonly used to lower cholesterol level, exert pleiotropic action and as demonstrated by our research they can stimulate angiogenesis and regulate the expression of HO-1.

To examine our hypotheses, we will conduct experiments in a mouse model of DMD disease – *mdx* mice, lacking expression of dystrophin. We will also use the unique model of double knockouts - mice lacking both HO-1 gene and the dystrophin. Part of the experiments will be performed on genetically modified myoblasts, with HO-1 overexpression or silencing. In order to verify whether the mechanisms, presumed to exist in the mouse model of DMD are recapitulated in human model, we propose innovative strategy of generation of induced pluripotent stem cells (iPSC), from fibroblasts of healthy subjects and fibroblasts obtained from DMD patients (such fibroblasts are commercially available), and their differentiation to endothelial cells, pericytes and skeletal muscle cells. In the project we apply multiple angiogenic models *in vitro*, including tube formation assay on special matrix called Matrigel or spheroid test as well as *in vivo*, using hind limb ischemia model and implantation of iPSC-derived endothelial cells into mouse cells in so called *in vivo* plug angiogenic test.

The result of the project will broaden our knowledge about the role of HO-1 and statins in the modulation of angiogenesis in DMD disease. We believe that planned analysis will broaden our knowledge about the pathology of DMD. This will be possible thanks to versatile, innovative and not yet used on such a large scale research strategy using animals lacking specific genes, gene therapy and induced pluripotent stem cells.