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Summary of doctoral thesis „The effects of bisphosphonates used in osteoporosis therapy on aerobic metabolism of endothelial cells"

Osteoporosis is the most common disease of bone metabolism, mainly among postmenopausal women. The most commonly used drugs to reduce bone resorption are nitrogen-containing bisphosphonates (N-BPs). The mechanism of action of N-BPs is based on blocking the enzyme farnesylpyrophosphate synthase (FPPS) in the mevalonate pathway. The mevalonate pathway plays an important role in many cellular processes such as protein prenylation and post-translational modifications as well as the synthesis of molecules containing isoprenoid units, including *a*-hemes (prosthetic groups of complex IV) or coenzyme Q (CoQ). CoQ is a key electron carrier in the mitochondrial respiratory chain and an important antioxidant present in all cell membranes, mainly mitochondrial, that protects against peroxidative damage. Endothelial cells line blood vessels, which means they are in constant contact with substances transported by the blood, including drugs.

The doctoral thesis focuses on the multifaceted influence of N-BPs on the oxidative metabolism of vascular endothelial cells. The dissertation consists of two experimental papers and one review paper, which present an analysis of the mechanisms by which N-BPs, such as alendronate and zoledronate, affect energy metabolism, oxidative stress and mitochondrial function in cells.

The research material used in the experiments were human endothelial cells EA.hy926, treated for six days with 5 μM alendronate or 1 μM zoledronate, and isolated mitochondria from these cells. The analysis of changes in energy metabolism induced by N-BPs was carried out in two stages, i.e., at the cellular level (Publication 1) and the mitochondrial level (Publication 2).

Experiments conducted on whole endothelial cells (Publication 1) showed that N-BPs cause a decrease in cell viability, increased oxidative stress and inflammation, and a decrease in the activity of the prenylation-dependent ERK1/2 signaling pathway. Significant changes in cell energy metabolism were also observed, consisting in increased anaerobic respiration, decreased mitochondrial oxidation of the main respiratory substrates (except fatty acids) and a decrease in ATP level. For the first time, it has been shown that inhibition of the mevalonate pathway by N-BPs induces a significant decrease in the level of total CoQ and a loss of its reduced pool in endothelial cells. These changes are accompanied by an increase in the production of reactive oxygen species (ROS). Studies indicate that N-BPs also change the dynamics of mitochondria by reducing their fission. Thus, N-BPs modulate the energy metabolism of endothelial cells leading to changes in the energy status of cells, CoQ redox homeostasis, the activity of oxygen metabolism and mitochondrial quality control.

Further studies conducted at the level of mitochondria isolated from endothelial cells cultured for six days with N-BP (Publication 2) focused on the adaptation of mitochondrial bioenergetics to the large decrease of more than 40% in mitochondrial CoQ (mtCoQ) levels caused by the inhibition of the mevalonate pathway. In mitochondria of cells treated with N-BPs, compared with control mitochondria, the decrease in the total pool and the loss of the reduced pool of mtCoQ are accompanied by greater production of ROS and activation of antioxidant systems. mtCoQ deficiency leads to decreased oxidation of respiratory substrates (except fatty acids), decreased activity of complexes II and III and ATP synthase, decreased mitochondrial membrane potential, efficiency of ATP synthesis and reorganization of respiratory chain supercomplexes. Increased ROS production is associated with higher reduction level of mtCoQ.

It was observed for the first time that N-BPs can lead to decreased level of heme a in mitochondria. Therefore, these results indicate that N-BP-induced disruption of mtCoQ redox homeostasis can significantly affect bioenergetic activity of endothelial mitochondria.

The review presented in the dissertation (Publication 3) summarizes the results of previous studies indicating a wide spectrum of N-BP action outside bone tissue – including their effects on cytoskeletal organization, mitochondrial-dependent apoptosis, calcium metabolism and processes related to mitochondrial quality control. N-BPs, as inhibitors of the mevalonate pathway, cause a number of side effects both at the cellular level and mitochondrial function. Further studies on the complex relationship between the mevalonate pathway, mitochondrial function and overall cellular homeostasis may be important to optimize the clinical use of N-BPs, possibly including CoQ10 supplementation, and to limit side effects in off-target tissues.

The conclusions of the doctoral thesis highlight the significant, potentially adverse effects of N-BP on non-skeletal cells, exemplified by endothelial cells. The studies presented suggest that these effects may contribute to cardiovascular complications in patients treated with N-BP, and mitochondrial mechanisms may be an important target for further research and potential protective strategies.