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The effect of rosemary and lemon balm extracts and rosmarinic acid on collagen type I metabolism in fibroblasts from Osteogenesis Imperfecta patients

Doctoral dissertation based on a series of scientific publications

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Promoter

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SUMMARY

Osteogenesis imperfecta (OI) is an inherited disease of the connective tissue manifested mainly by defects in the skeletal system (bone fragility, skeletal deformities, reduced bone mineral density, short stature), but also a number of extra-skeletal symptoms such as blue sclera, hearing impairment, skin fragility, joint laxity, muscle weakness, tooth abnormalities (dentinogenesis imperfecta), cardio-respiratory defects and impaired pulmonary function. The prevalence of OI is estimated at 1 in 15 000 to 20 000 live births. The molecular mechanisms underlying the disease are also complex. The vast majority of cases (85-90%) are caused by dominant mutations in the *COL1A1* and *COL1A2* genes encoding type I collagen. The classification of OI due to the collagen type I mutations includes four clinically defined types, the phenotype of which ranges from mild non-deforming type I, moderate type IV, severe deforming type III to the lethal type II. Many other recessive mutations in non-collagenous genes code for proteins involved in type I collagen biosynthesis, modifications, quality control, secretion as well as osteoblast differentiation and bone mineralization.

Mutations of collagen type I genes, depending on the type, can result in quantitative or structural defect in the collagen type I. The decrease in biosynthesis of structurally normal type I collagen in OI type I is caused by a null *COL1A1* allele due to premature stop codons. The causative mutations of severe and lethal OI include mainly substitutions of glycine residues with another amino acid (80%) and are defined as the dominant-negative. The resulting mutant collagen is secreted into the ECM but also accumulates inside the cell, which can cause phenotype-related ER stress. The cell can activate the UPR and improve the conformation of mutant collagen or destined for degradation most commonly via autophagy or ERAD pathway. During chronic stress, ER promotes cell apoptosis.

The currently used anti-resorptive treatment mainly uses the administration of bisphosphonates. Many various approaches remain experimental. According to the latest reports, 4-PBA, the molecular target of which is ER stress caused by intracellular accumulation of mutant collagen, is of great interest.

In this study it was hypothesized that rosemary (*Rosmarinus officinalis* L.) and lemon balm (*Melissa officinalis* L.) extracts, and rosmarinic acid, as one of their main ingredients, which attract particular attention of pharmacists due to their high therapeutic potential, can improve the quantitative defect of normal collagen type I in OI type I and/or minimize the accumulation of mutant collagen in OI type II and III fibroblasts with substitutions of glycine in α1(I) chain.

RE and LBE at concentrations of 0.1, 1 and 10 μg/mL and RA at concentrations of 0.1, 1 and 10 μM significantly reduced or completely eliminated the quantitative defect of collagen type I in OI type I fibroblasts by their stimulating effect on collagen biosynthesis and inhibiting effect on MMP (MMP-1, MMP-2 and MMP-9) activity.

RE at concentrations of 50 and 100 μg/mL significantly reduced the level of accumulated mutant collagen type I in fibroblasts of patients with severe OI type III and lethal OI type II by inducing autophagy. Activation of this process was evidenced by the increase in LC3-II/LC3-I ratio and degradation of p62 as well as localization of collagen type I in lysosomal fraction and its co-localization with autophagy (LC3-II) and lysosome (LAMP2A) markers by confocal fluorescence microscopy. The RE-induced decrease in intracellular accumulation of mutant type I collagen was associated with the decrease in expression of UPR proteins suggesting alleviation of cell stress. This was confirmed by the decrease in the pro-apoptotic markers (Bax, CHOP and cleaved caspase 3) levels under influence of RE.

RE, despite partial inhibition of proteasome activity, also increased the degradation of unfolded procollagen chains in OI cells with a mutation in C-propeptide, but did not affect the level of total protein in lysates. The obtained results reveal new clinically important properties of RA and extracts (RE and LBE) that may have some implications in OI therapy, but need to be confirmed in future *in vivo* experiments.