Rutin as an effective protector of fibroblasts against UVA irradiation

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Background. UVA irradiation is one of the most ubiquitous and penetrating environmental stress factor to human skin and it is crucially involved in various forms of skin damages including the photoaging and development of cancer. Mentioned skin damages are connected mainly with imbalance in redox status and consequently with cellular metabolic disturbances including changes in phospholipid mediators, including lipid peroxidation products and endocannabinoids that effect different fibroblasts functions. To prevent the changes in redox status and its consequences in skin cells many natural antioxidants which reduce the amount of ROS, resulted from UV radiation in particular, in the cells thereby protecting them from their toxic effects are used. One of them is well known plant-derived polyphenol – rutin (quercetin-3-rutinoside). Therefore, the aim of the study was to exam the effect of rutin on redox balance and lipid mediators after UVA irradiation of skin fibroblasts

Methods. Fibroblasts were subjected to UVA [30 J/cm^2] and were treated and pretreated with – rutin [25μM]. The UVA exposure dose was chosen corresponding to 70% cell viability. The redox status was estimated by xanthine and NADPH oxidases activity and ROS generation (ESR/spectrophotometry), enzymatic and non-enzymatic antioxidants activity/level (HPLC, spectrophotometry), and lipid mediators: lipid peroxidation products (LCMS; GC/MS) and endocannabinoids (LC/MS) and their receptors were examined. The cannabinoids receptors, transcriptional factor Nrf2 and its activators/inhibitors, as well as pro-inflammatory, pro- and anti-apoptotic protein levels were also measured (Western blot).

Results. Obtained results demonstrate that rutin significantly reduced UVA-induced xanthine and NADPH oxidase activity what was accompanied by changes in ROS generation. Rutin also enhanced antioxidants activity [SOD] and non-enzymatic antioxidant level [GSH, vitamin E and C], which decreased due to UVA exposure. It was shown that UVA-induced Nrf2 activity has been reduced by rutin. Simultaneously, rutin led to increase in Nrf2 inhibitor Bach2 in UVA irradiated cells. It was shown that UVA caused significant decrease in linoleic and arachidonic acids levels and in consequence a strong increase in lipid peroxidation products - MDA and 4-HHE level were observed. Also another lipid mediators – endocannabinoids level was changed during UV irradiation. The significantly reduced level of cytosolic AEA and 2-AG and accompanied higher expression in the level of their receptors CB1, CB2, VR1 and GPR55 were demonstrated. Rutin given after irradiation prevented the most changes caused by UV irradiation. Rutin also protect cells against inflammatory

effect after UV irradiation, and decrease apoptotic fibroblasts activity, confirmed by increase in Bcl-2, decrease in caspases levels, and reduced cytochrome c release from mitochondria.

Discussion. Obtained results show that rutin efficiently prevents oxidative stress and inflammatory response formation as well as changes in phospholipid metabolism including endocannabinoids and lipid peroxidation products level, what may be important point to search the skin protecting method against harmful ultraviolet radiation.