THE CITRUS FLAVONOID NARINGENIN SLOWS DOWN MYOCARDIAL AGEING BY INCREASING SIRTUIN 1ENZYME EXPRESSION IN MICE

Eugenia Piragine[1], Lara Testai[1], Alma Martelli[1], Valentina Citi[1], Lorenzo Flori[1], Ilaria Piano[1], Maria Claudia Gargini[1], Andrea Pirone[2], Vincenzo Miragliotta[2], Lorenzo Di Cesare Mannelli[3], Carla Ghelardini[3], Maria Cristina Breschi[1], Vincenzo Calderone[3]

[1]Università degli Studi di Pisa - Dipartimento di Farmacia, Pisa - Italy, [2]Università degli Studi di Pisa - Dipartimento di Scienze Veterinarie, Pisa - Italy, [3]Università degli Studi di Firenze - Dipartimento di Neuroscienze, Area del Farmaco e Salute del Bambino (NEUROFARBA), Firenze - Italy

Introduction: Ageing represents one of the main risk factors for the development of several cardiovascular diseases, such as hypertension, myocardial infarction and heart failure. Indeed, the average lifespan is increasing worldwide and the incidence of cardiovascular diseases drammatically increases with age. Emerging evidence suggests that sirtuine 1enzyme (SIRT1) plays a crucial role in heart ageing. Phisiologically, SIRT1is involved in the regulation of important biological processess, including inflammation, oxidative stress, cardiac remodeling, mitochondrial biogenesis as well as cell senescence and consequent ageing. Unfortunately, both SIRT1expression and activity gradually decrease with age. In previous works, the Citrus flavonoid naringenin showed interesting SIRT1-stimulating activity and promising cardioprotective properties. The aim of this study was the evaluation of the potential cardiac anti-ageing effects of naringenin chronic treatment in mice.

Materials and methods: Six months old C57bl/6male mice were daily treated for six months with naringenin 100 mg/Kg or vehicle (DMSO 1%) diluted in drinking water. Three months old mice were used as young control. Mice were weekly weighed and water intake was daily monitored during the experimental period. At the end of the treatment, mice were anestethized and sacrificed. Blood samples were collected and hearts were isolated for further investigations. Firstly, the effects of naringenin chronic treatment on cardiac SIRT1expression were determined by densitometric analysis of Western Blot assay. Then, reactive oxygen species (ROS) production in the hearts was evaluated by fluorescence microscopy with the fluorescent probe dihydroethidium. Cardiac levels of two different biomarkers of inflammation (interleukin 6and tumor necrosis factor alpha) were determined in all experimental groups using the enzyme-linked immunosorbent (ELISA) assay. Moreover, mitochondrial functionality was evaluated in the myocardium by measuring both mithocondrial membrane potential ($\Delta\Psi$) with a TPP+-sensitive minielectrode and citrate synthase activity in the myocardium. Furthermore, cardiac fibrosis was histologically assessed with the Mallory's trichrome stain. Finally, blood samples were used for the measurement of glycaemia, glycated haemoglobin (HbA1c) and serum lipid levels (total cholesterol, HDL, LDL and tryglicerides). Cardiovascular risk was calculated as the ratio between total cholesterol and HDL.

Results: Data showed that naringenin chronic treatment significantly increased the expression of SIRT1in the aged myocardium, protecting the hearts from oxidative stress and inflammation. Moreover, naringenin increased citrate synthase activity and preserved mitochondrial membrane potential ($\Delta\Psi$), thus mantaining cardiac mitochondrial function in old mice. Finally, naringenin prevented age-related myocardial fibrosis and significantly reduced cardiovascular risk in old animals.

Discussion and conclusions: These results suggest that naringenin is a Citrus flavonoid of pharmacological, nutraceutical and phytoterapic interest; indeed, it represents a suitable anti-ageing candidate useful in slowing down cardiac cell senescence. Accordingly, naringenin may have a translational potential in the prevention of several age-related cardiovascular diseases.

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