

Abstract

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Title of diploma thesis: ***In vitro* effects of 3-hydroxytyrosol on renal hypoxia and inflammation**

Chronic kidney disease (CKD) results from a group of heterogeneous disorders affecting the kidneys. The renal hypoxia and hypoxia-derived oxidative stress, renal fibrosis, and inflammation are highly prevailing conditions appearing in the diseased kidney, contributing to the progression of CKD. Phytochemicals are an essential part of contemporary therapeutic strategies for the treatment of various diseases. 3-Hydroxytyrosol (HT), a phenolic compound extracted from olives and olive-derived products (e.g. olive oil), is believed to carry a potent antioxidant, anti-inflammatory, antithrombotic, bactericidal and bacteriostatic activity. The aim of this work was to determine the preventive effect of HT in hypoxic renal cells and evaluate the effect of HT on hypoxia-related inflammation, fibrosis, and oxidative stress, in order to summarize the value of this phenolic compound as a promising novel remedy in the treatment of CKD. A cell line of human renal proximal tubular cells (HK-2) was selected to examine the effects of chemically induced hypoxia, hypoxia-derived inflammation, oxidative stress, renal fibrosis, and cell death, and the *in vitro* effect of HT under these conditions. Cobalt chloride was used to induce hypoxic conditions, and the adequate concentration to use for this purpose was determined through the MTT reduction assay (cell viability), flow cytometric analysis of intracellular oxygen levels, and expression analysis of hypoxia-related genes by qPCR. To evaluate the effect of HT on hypoxic HK-2 cells, reactive oxygen and nitrogen species production and glutathione levels were assessed as markers of oxidative stress. The effect of HT on the expression of hypoxia and related mediators was also determined by qPCR. The activation of autophagy was confirmed by the formation of acidic vesicular organelles observed under a fluorescence microscope and quantified by flow cytometry using the acridine orange dye. Though not significant, HT showed a slight preventive effect on hypoxia-induced cell death. Importantly, it showed a notable beneficial effect on oxidative stress, as well as on the expression of key genes involved in hypoxia (*GAPDH*), inflammation (*IL6*), and renal fibrosis (*TGFBI*). Together, the obtained results suggest that HT can act as a relevant therapeutic approach in the treatment of CKD in the future, though further studies on this matter are still required.