6. SUMMARY

The role of iron chelation in the mechanism of protection offered by flavonoids to cells exposed to oxidative stress

The role of Mediterranean diet in human health has been the subject of a large number of studies. One of the most underestimated constituents of this diet is honey. In the present study, the ability of Greek honey extracts to protect cellular DNA against H_2O_2-induced damage was evaluated. The observation that some butanolic honey extracts, which were rich in phenolic substances, were able to offer protection, led to isolation and evaluation of some of their constituents. Among the bioactive constituents of honey, flavonoids were selected for further investigation.

Flavonoids, with minimal structural differences, were selected and evaluated for their abilities: a) to protect cellular DNA against H_2O_2-induced damage, b) to scavenge free radicals, and c) to chelate intracellular iron, in order to elucidate the molecular mechanism of the offered protection. It was revealed that despite the prevailing idea that flavonoids exert their beneficial effects through their ability to scavenge free radicals; this ability did not correlate with their protective capacity against H_2O_2-induced DNA damage. Instead, it was shown an excellent correlation between the protection offered by flavonoids and their capacity to chelate intracellular iron existed. It was concluded, that chelation of intracellular redox iron by flavonoids plays a crucial role in the molecular mechanism of the protection against H_2O_2-induced DNA damage.

In addition the effects of two selected flavonoids, namely luteolin and apigenin, against H_2O_2-induced apoptosis were evaluated. It was revealed that luteolin, which is able to chelate intracellular iron, but not apigenin, that does not chelate iron, is able to protect against H_2O_2-induced apoptosis. The pathway of the apoptotic procedure was examined step by step, in an effort to indicate the exact point of interaction. It was revealed that a crucial step in the apoptotic pathway is the translocation of Bax from cytosol to mitochondria, which is suppressed by luteolin, as well as desferioxamine, an iron-chelating agent. The earliest step in H_2O_2-induced apoptotic pathway seems to be the destabilization of the lysosomal membrane, which

is blocked when cells are preincubated with luteolin. Lysosomes contain the high concetrations of redox-active iron, and it is plausible to speculate that luteolin exerts its protective action through iron-chelation in lysosomes. Further investigation is necessary in order to elucidate the exact mechanistic details that connect lysosomal destabilization with Bax translocation to mitochondria, and the following release of cytochrome c to the cytosol.

In conclusion it is revealed that chelation of intracellular redox-active iron is crucial for the protective action offered by flavonoids against H_2O_2 induced DNA damage and apoptosis.However, further investigations are nessescary for the elucidation of the exact molecular mechanisms underlying these effects.

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Protection against nuclear DNA damage offered by flavonoids in cells exposed to hydrogen peroxide: the role of iron chelation.

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The ability of a number of flavonoids belonging to the flavone, flavonol, flavanone, and flavan-3-ol subclasses to protect cellular DNA from H2O2-induced single-strand breaks and the underlying molecular mechanisms were investigated in this work. Formation of single-strand breaks on nuclear DNA, after exposure of Jurkat cells to continuously generated H2O2 in the presence or absence of the flavonoid compounds, was evaluated by the comet assay (single-cell gel electrophoresis). The results indicate the following structural requirements of flavonoids for effective DNA protection: (a) the ortho-dihydroxy structure in either ring A or ring B, (b) the hydroxyl moiety on position 3 in combination with the oxo group at position 4, and (c) the presence of a C2, C3 double bond in ring C. In contrast to free flavonoids, the ability of complexes of [Fe2+]/[flavonoid] to protect nuclear DNA was decreased as the ratio increased, and the complex was completely inactive when the ratio reached a certain value. Moreover, it was observed that several of the flavonoids tested were able to remove iron from calcein loaded into cells and that this property was in excellent correlation with their ability to protect DNA (Spearman's correlation coefficient, rho = 0.9, p = 0.005). The antioxidant (electron donating) capacities of the same flavonoids were also evaluated by a conventional method, but no relation with their DNA-protective ability could be established even when their membrane-penetrating abilities were taken into account (p = 0.64). In conclusion, the results presented in this work strongly support the notion that intracellular binding of iron is responsible for the protection offered by flavonoids against H2O2-induced DNA damage.

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