ABSTRACT

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Title of Doctoral Thesis: Screening of novel chelators of microbiogenic metals

Iron, copper and zinc are microbiogenic elements which play crucial roles in a series of physiological processes in human organism. Homeostasis of these transition metals is strictly regulated since, among others:

a) free or loosely bound iron or copper can catalyse the production of hydroxyl radical;
b) lack of zinc but also of the previously mentioned metals is associated with significant impairments.

Hereditary hemochromatosis, transfusion-induced secondary iron overload and Wilson´s disease are known as pathological conditions associated with metal overload in the organism. Chelator agents have vital relevance for the treatment of these impairments. There are also numerous diseases with homeostatic imbalances in iron, copper and or zinc: neurodegenerative diseases, cardiovascular diseases, cancer and diabetes mellitus. Different chelating compounds have been examined for the treatment of these impairments.

The aim of this doctoral thesis was to perform a screening of metal chelating properties of different compounds, at (patho)physiologically relevant pH, with the objective of their potential therapeutic use or detection of toxicity toward removal of non-targeted essential metals (e.g. mentioned zinc), and characterization of the formed complexes. In order to fulfil these aims, two major approaches: the competitive (the hematoxylin and bathocuproine methods) and non-competitive approach (the Job and our complementary methods) were applied, and also novel methodologies were developed (zinc chelation and very sensitive method for hydroxyl radical detection). Different compounds were tested: flavonol isoquercitrin, flavonoid derivatives flavonolignans from silymarin, metabolites of isoflavonoids formed by human microflora and coumarins.

Within the screening, following novel finding were published:

1. Detailed analysis of isoquercitrin complex with iron and copper at 4 (patho)physiological pH conditions (7.5, 6.8, 5.5 and 4.5);
2. Screening of copper chelation and reduction properties of 4-methylcoumarins;
3. Detection of interactions of microbial metabolites of isoflavonoids with copper and zinc.

Concerning methodologies, the following outcomes were reached:

1. Antioxidants can profoundly interfere with the hematoxylin assay aimed at initial cupric chelation screening. This was documented by use of a set of different antioxidants with no chelating sites.
2. The research of metal chelators would not be sufficient without testing their effect on the Fenton reaction since even the potent chelator can behave as pro-oxidants (e.g. EDTA). For this purpose a HPLC method coupled with coulometric detection has been developed.
3. Another important objective of this work was to develop a method for zinc chelation. This was accomplished using zinc detection by a spectrophotometric indicator dithizone.

It can be concluded that this research opened novel possibilities not only for our research group but possibly also for the other investigators interested in pharmacology of trace metals.