ABSTRACT

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Title of doctoral thesis:	**Screening of iron-chelating substances and their potential use in the therapy of acute myocardial infarction**

Iron is an essential element virtually for all living organisms. The concentration of free/unbound iron is very low at physiological conditions. However, in several pathological states, its homeostasis is impaired which may lead to an organ damage due to the increased production of reactive oxygen species.

Coronary heart disease is the main cause of morbidity and mortality worldwide. Its most serious form is acute myocardial infarction (AMI). During the early ischaemia, catalytically active elements, particularly iron and copper, are released into the blood circulation. After restoration of blood flow (reperfusion), these elements may participate in the production of biologically the most potent oxidant – hydroxyl radical via iron/copper catalysed Fenton reaction. Therefore, a therapy based on administration of iron/copper-chelating agents could be a potential pharmacotherapeutic approach in the treatment of this disease.

The main aim of this doctoral thesis was a screening of iron-chelating substances and their influence on isoprenaline model of AMI including characterisation of several previously unknown aspects of this model.

In vitro study analysed effects of the tested substances at (patho)physiologically relevant pH conditions by: 1) the spectrophotometric ferrozine methodology detecting iron-chelating properties, and 2) the HPLC analysis determining anti/pro-oxidative activities by the use of salicylic acid as the indicator of the formation of hydroxyl radical. Moreover, a new inexpensive but precise analytical approach for a determination of a stoichiometry of the complex chelator:iron using UV–Vis spectrophotometry was evolved. In vivo part of the study was aimed at an evaluation of effects of dexrazoxane (20.4 mg/kg, i.v.) on isoprenaline model of AMI (100 mg/kg, s.c.) in Wistar:Han rats. Furthermore, early pathological changes and relationships among various biochemical and functional parameters of cardiac dys/function were described after the administration of isoprenaline. In these in vivo studies, the thermodilution method or the invasive measurement of pressure and volume in the left heart ventricle were used.

In flavonoids, the 6,7-dihydroxy structure was the most effective substitution for iron-chelation. Baicalein, in which this group is incorporated, possessed a similar ability to chelate iron as a reference iron chelator deferoxamine. However, its influence on the inhibition of Fenton reaction was lower. The 3-hydroxy-4-keto conformation together with 2,3-double bond and the catecholic B ring (e.g. quercetin) were associated with a substantial iron-chelating properties as well. On the other hand, the influence of these structures on Fenton reaction was rather minimal, and in several cases, even undesirable pro-oxidative effect was observed.

Although synthetic iron chelators from the group of 1-phenyl-3-methyl-4-acyl-pyrazol-5-ones have been known for many years, data on their biological activity are rather limited. Some of the tested substances were even more potent iron chelators at pH 4.5 than the clinically used standard – deferoxamine. Of particular interest is a prototype compound H_2QpyQ, i.e. 2,6-bis[4(1-phenyl-3-methylpyrazol-5-one)carbonyl]pyridine, which iron-chelating affinity increased when pH was decreasing. To our knowledge, it is the first compound having such properties. Moreover, most of the tested acylpyrazolones were powerful inhibitors of Fenton chemistry as deferoxamine.

One of the most important features of iron chelators is the stoichiometry of a formed complex in relation to pH. Moreover, in certain substances, e.g. flavonoids, the stoichiometry of the complex has been still unknown, therefore, a new analytical approach using UV–Vis spectrophotometry was evolved. The major benefit of this approach, compared to the standard Job's method, seems to be its capability to reveal the stoichiometry and the kinetic aspects of formation of the complex in chelators with moderate affinity to iron, as well.

The study focused on the analysis of early pathological changes after administration of the cardiotoxic dose of isoprenaline showed that diastolic dysfunction preceded systolic dysfunction and β2-adrenoreceptor stimulation alone was not sufficient for its induction. Moreover, serum concentration of cardiac troponin T (cTnT) correlated strongly with the degree of myocardial injury in rats (e.g. calcium overload – positive correlation, stroke volume – negative correlation). On the other hand, correlations between cTnT and oxidative stress parameters were weak (for glutathione and vitamin C) or were not found (for serum vitamin E and TBARS – thiobarbituric acid reactive substances levels). Relationships between cTnT and other parameters were exponential with the exception of myocardial calcium, where a power function was found.

In a 24-hour experiment, the administration of dexrazoxane resulted in the partial decrease in mortality, reduction of myocardial calcium overload and improvement in histological impairment and peripheral haemodynamic disturbances. Continuous 2-hour experiments showed that dexrazoxane did not influence isoprenaline-induced atrioventricular blocks and had little effect on the measured haemodynamic parameters. Complementary in vitro experiments suggested that iron-chelating properties of dexrazoxane apparently did not play the major role in the cardioprotective mechanism.

It can be concluded that in vitro analysis of iron chelators may at least partially predict their positive or even negative influence on isoprenaline model of AMI. On the other hand, some aspects should be confirmed by additional in vivo experiments.