ABSTRACT

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Title of Doctoral Thesis **Influence of polyphenolic substances with iron chelating activity in model of acute myocardial infarction and oxidation stress induced by catecholamines**

Background and aim: Ischaemic heart disease (IHD) and particularly its most serious form - acute myocardial infarction (AMI) - represents significant health problem in the developed countries with relatively high mortality. Within multifactorial pathogenesis of AMI, reactive oxygen and nitrogen species (RONS) generated by free iron catalytic effect and increased activity of the sympathetic nervous system accompanied with a release of the catecholamines play a significant role. Large doses of the synthetic katecholamine isoprenaline has been used as a suitable experimental model of AMI.

AIM is considered as typical form of ischaemic-reperfusion (I-R) injury. RONS, which are excessively released during the first minutes of reperfusion, play a significant role in the pathogenesis of myocardial I-R injury. Free iron significantly increases the process of RONS generation.

Use of iron chelators could have protective influence in I-R injury and AMI, which is obvious from the mentioned pathogenesis. Iron chelators represent large group of drugs with very variable chemical structure. In clinical practice they are used in cases of acute iron overdose and in chronic iron overload accompanying mainly frequent blood transfusions in patients with thalassaemia. These substances are experimentally studied also in another pathological conditions.

Aim of this study was therefore focused on substances with iron chelation potential. By in vitro method we compared iron-chelating activity of the tested polyphenolic substances in different patho/physiological pH conditions, their relation to the structure. Their chelating activity was compared with the standard chelating agent deferoxamine. The last part of the doctoral thesis deals with in vivo analysis of the effects of iron chelating substances in catecholamine model of AMI, induced by the administration of high dose of ISO.

Methods: In in vitro studies, 26 flavonoids from different subclasses and a series of naturally and synthetic 4-methylcoumarins were analysed for their iron chelating activity and stability of the formed complexes in four patho/physiologically relevant pH conditions (4.5/5.5/6.8/7.5) and compared with clinically used iron chelator deferoxamine. Ferrous ions and total iron chelating activity was assessed by a simple spectrophotometric assay. Iron chelators were mixed with ferrous or ferric ions in various ratios. Concentration of free iron was measured by specific indicator of ferrous ions ferrozine at 562 nm. Absorbance was measured immediately after addition of ferrozine and 5 minutes later. Concentrations of ferric ions is determined indirectly by means of ferric ions reduction by hydroxylamine to ferrous ions, which are subsequently determined by ferrozine. By this indirect method, the total iron chelation has been assessed too.

In in vivo experiments we used male Han:Wistar rats weighing approximately 360 g. Rats were pretreated i.v. with flavonoid rutin (46 mg.kg-1 and 11,5 mg.kg-1) or with standard iron chelator dexrazoxane (20,4 mg.kg-1) (DEX) alone or in combination with ISO (100 mg.kg-1 s.c.). 24 hours after drug(s) administration, animals were anaesthetized with uretane (1,2 g.kg-1 i.p.). Haemodynamic parameters of cardiac function were assessed by thermodilution method using Cardiosys (Experimetria Ltd., Maďarsko). In the case of DEX, continual 2-hours measurements of haemodynamic and ECG parameters using apparatus PowerLab (AdInstruments, Austrálie) were performed. Blood sample was withdrawn for biochemical

measurement. Following euthanasia (1 mM KCl i.v.), the heart ventricles were removed for the measurement of their wet weight, concentration of the selected elements and for histopathological analysis.

Results: In vitro study demonstrated that the most effective iron binding site of flavonoids represents 6,7-dihydroxy structure, as in the case of baicalein, which had the similar activity in all tested pH as compared to deferoxamine. The 3-hydroxy-4-keto conformation together with 2,3-double bond and catecholic B ring, for example myricetin and quercetin, were also associated with a substantial iron chelation, but they have no significant role in the acidic conditions. They have similar activity like baicalein and deferoxamine only in the neutral conditions. The 5-hydroxy-4-keto site was less efficient and isolated keto, hydroxyl, methoxyl groups or an ortho-methoxy-hydroxyl groups were not associated with iron chelation.

Among the tested coumarins, ortho-dihydroxyderivatives were the most potent iron chelators. 7,8-dihydroxy-4-methylcoumarins reached the efficacy of deferoxamine in neutral pH, but they did not bind iron firmly in acidic conditions. In addition, they reduced ferric ions to ferrous ones, which could lead to intensification of the Fenton chemistry. Other tested coumarins did not substantially chelate iron with exception of ortho-diacetoxycoumarins.

In in vivo study, ISO alone caused 30% mortality, a decrease in blood presure, cardiac output, total peripheral resistance and an increase in heart rate, marked elevation of cardiac troponin T concentration, wet ventricle weight increase, myocardial calcium overload and histopathological abnormalities. Regarding ECG parameters, ISO caused rapid elevation in ST junction and a depression in T-wave and R-wave amplitudes.

Rutin decreased peripheral resistance elevation following ISO administration, but generally, rutin rather aggravated catecholamine myocardial injury. Higher dose of rutin increased mortality, intensified an increase in serum cTnT concentration and increased calcium overload in myocardium.

DEX decreased mortality likely due to an attenuation of dysrhythmias and inhibition of myocardial calcium overload. Although DEX tended to insignificantly decrease mean blood pressure, it had no significant influence on other haemodynamic and ECG parameters.

Conclusion: Ability of particular substances to chelate iron depends on their chemical structure and pH conditions. Using of some iron-chelating flavonoids or 4-methylcoumarins could be unsuitable mainly in acidic conditions (e.g., in acute myocardial infarction), as a consequence of ferric ions reduction and subsequent participation of ferrous ions in Fenton chemistry.

In the case of in vivo effects, rutin was not able to decrease catecholamine cardiotoxicity, probably due to its hydrophilic character and pro-oxidant properties. Contrarily, rutin in higher dose rather aggravated this myocardial injury. DEX demonstrated some cardioprotective effects in a model of AMI, which were probably caused, at least partly, by antidysrhytmic activity due to iron chelation and partial prevention of calcium overload of the myocardial tissue.