I. SUMMARY

Background: Iron is an essential element necessary for many physiological processes involving oxygen transport, DNA-synthesis and ATP-formation. The fate of iron in the organism is tightly regulated especially at the absorption and distribution level probably mainly due to lack of specific active iron excretion mechanism. Any derangement of iron homeostatis may lead to appearance of free (unbound or loosely bound) iron, which can catalyse reactive oxygen species (ROS) production by Haber-Weiss chemistry.

Cardiovascular diseases, particularly coronary heart disease (CHD), remain notwithstanding recent scientific advances important therapeutic problem. The most serious form of CHD represents acute myocardial infarction (AMI). The pathophysiology of AMI involves in most cases initial ischaemic period caused by coronary blood flow derangement due to a thrombus formation. Ischaemia alters substantially tissue homeostasis with subsequent cytosolic free iron appearance. Reconstitution of coronary blood flow (reperfusion) represents the only way for myocardial tissue recovery although on the other hand, it is linked with a release of free redox-active iron in the circulation and formation of ROS both intracellularly as well extracellularly.

Iron chelators are a large group of drugs with very diverse structure. They are traditionally used as protective medication in conditions with suggested involvement of iron in ROS generation (e.g., thalassemic patients treated with blood transfusions and antracycline cardiotoxicity). Due to mentioned involvement of iron in the pathogenesis of AMI, drugs chelating iron may be useful in prevention of AMI-associated tissue impairment. The only drug

which has been tested extensively in occlusional models of AMI was deferoxamine. Regrettably, the published results have been contradictory.

Aim and methodology: This study was aimed to investigate the effects of drugs with iron chelating properties on catecholamine model of AMI. For this purpose, a synthetic catecholamine isoprenaline (ISO) with non-selective β-agonist activity was used in a dose of 100 $mg.kg^{-1}$ to evoke a pathological state in many aspects similar to AMI. Male Han:Wistar rats were pretreated i.v. with agents as follows: deferoxamine mesylate (50 $mg.kg^{-1}$), equivalent doses of 2-pyridylcarboxaldehyde-2-thiophenecarboxyl hydrazone (PCTH, 20.4 $mg.kg^{-1}$), rutin (46 $mg.kg^{-1}$) and lactoferrin in a dose of 50 $mg.kg^{-1}$ five minutes before application of ISO. Additional smaller doses of PCTH (10.2 $mg.kg^{-1}$) and rutin (11.5 $mg.kg^{-1}$) were administered to other rats for obtaining dose-reponse effects. 24 hours after drug application, animals were anaesthetized with urethane (1.2 $g.kg^{-1}$ i.p.). Cardiac function was assessed in terms of a thermodilution method using Cardiosys® apparatus (Experimetria Ltd.®, Hungary), blood was withdrawn for biochemical measurement and heart ventricles removed for weight, elements and histological analysis.

Results: Isoprenaline alone caused 30% mortality, a decrease in cardiac output associated with an increase in heart rate and peripheral resistance, marked elevation of serum cardiac troponin T (cTnT) concentration, an increase in wet ventricle weight, myocardial calcium overload and substantial impairment of cardiac tissue within 24 hours in comparison to the controls. There were no significant changes in plasma TBARS levels, serum vitamin C, total glutathione, erythrocyte antioxidant enzymes (SOD, GPx) and myocardial levels of zinc, selenium and iron.

SUMMARY

Deferoxamine did not demonstrate any positive effect on ISO-impairment. PCTH in equimolar dose to that of deferoxamine hindered mortality, markedly reduced release of cTnT and partly but significantly reduced an increase in wet ventricles weight. Others parameters seemed not to be positively influenced by PCTH although some of its effect may be obscured due to used solvent – 20% water solution of propylene glycol, which alone demonstrated some myocardial derangement (e.g. a drop in cardiac index).

Both lactoferrin and rutin inhibited an increase in peripheral resistance caused by ISO. Notwithstanding these positive effects, rutin rather negatively influenced ISO-impairment. Namely, higher dose of rutin increased mortality to 53% and tended to intensify cTnT release, as well as calcium overload. Additionally, only higher dose of rutin elevated myocardial calcium levels in control animals too, and caused dose-dependent myocardial zinc homeostasis derangement. Except for the mentioned positive effect on peripheral resistence, lactoferrin restored cardiac index dropped by ISO and partly decreased calcium overload.

Discussion and conclusion: This study demonstrated for the first time that iron chelators can at least partly prevent myocardial injury caused by catecholamines and may probably protect myocardium from AMI consequences. The failure of complete inhibition of catecholamine myocardial injury seems to depend on the pathogenesis of ISO-cardiotoxicity. This is not fully understood but involves at least two main features – cardiac overstimulation by activation of ß-adrenoreceptors and ROS-generation due to released iron, as well due to catecholamines themselves. Lipophilic iron chelator PCTH may revert, at least partly, this injury probably because the fact that it chelates both intravascullar as well as intracellular free iron. Contrarily, hydrophilic, therapeutically standardly used chelator deferoxamine had no possitive effect on this injury. Endogenous hydrophilic ferric chelator lactoferrin had modest influence on this injury,

which were probably mediated by extracellular iron chelation (peripheral resistence, calcium overload) and/or by another unknown mechanism. By contrast, rutin appeared to aggravate this injury in the larger dose, which can be ascribed: i) to its proposed possible pro-oxidant properties; ii) to an increase in myocardial calcium level which may increase physiologically contractile force but under pathological condition may lead to acute heart failure or arrhythmias.

SUMMARY