Nitric oxide biosynthesis in red blood cells: impairment in coronary artery disease

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Background. Recently it has been shown that erythrocytes are not only scavenger and transporter of bioactive forms of nitric oxide (NO) in the bloodstream, but they also synthesize and release NO. All the enzymatic factors/cofactors involved in NO metabolism, as well as a functional NO synthase, are present in red blood cells, to indicate that these cells may contribute to the regulation of vascular homeostasis in physiological and in pathological conditions. Increased oxidative stress can impair the NO synthesis and inactivate the produced NO, by transforming it into peroxynitrate, resulting in decreased NO bioavailability. Oxidative stress and reduced bioavailability of endothelial NO have been described in coronary artery disease (CAD) patients. Thus the aim of the study was to highlight a potential dysfunction of the metabolic profile of NO in red blood cells and in plasma from CAD patients compared with healthy subjects.

Methods. The measurement of the analytes involved in L-arginine/NO pathway was performed using the liquid-chromatography tandem mass spectrometry and high performance liquid chromatography methods. The ratio of oxidized and reduced forms of glutathione, as index of oxidative stress, was measured by liquid-chromatography tandem mass spectrometry method. NO synthase expression and activity were evaluated by immunofluorescence staining and *ex-vivo* experiments of [$^{15}N_2$] L-arginine conversion to [^{15}N] L-citrulline respectively.

Results. Higher amounts of asymmetric and symmetric dimethylarginines, the endogenous inhibitors of NO synthase, were found both in red blood cells and in plasma of CAD patients with respect to controls. Interestingly, NO synthase expression and activity were reduced in CAD red blood cells. In contrast, oxidized/reduced glutathione ratio was increased in CAD and this increment was associated to arginase activity.

Conclusion. In the study we have analyzed for the first time the whole metabolic pathway of L-arginine/NO in red blood cells and in plasma, highlighting an impairment of NO pathway in erythrocytes from CAD patients, associated with a decrease of NO synthase expression/activity and an increase of oxidative stress. Thus, therapeutic interventions aimed at reducing intracellular oxidative stress, might be effective to improve the balance between NO synthase and arginase activities, resulting in enhanced NO bioavailability.