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Study of the pro-oxidant and antioxidant properties of glutathione on proteins and lipids oxidative damage : the relevance to atherogenesis

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SUMMARY

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Oxidative damage of biomolecules (proteins, lipids, ADN) caused by free radicals is involved in pathogenesis of different diseases like cancer, atherosclerosis, inflammation, etc. Glutathione (GSH), the major intracellular non protein thiol, is mainly known as an important protector against free radical damage by providing reducing equivalents for several key antioxidant enzymes and also by scavenging hydroxyl radicals and singlet oxygen. However, it has been reported that the GSH metabolism by gamma-glutamyltranspeptidase (GGT) in the presence of iron leads to reactive oxygen species (ROS) generation, by the autoxidation of the metabolite of GSH, cysteinylglycine (CysGly) (Stark et al, 1993, Paolicchi et al, 1997). We proposed to study in vitro the pro-oxidant role of GGT/GSH and the oxidative modifications that it induces on proteins and lipids, known vulnerable targets of free radical attack. The measurement of ROS generation in the presence of different thiols and iron, by using dihydrorhodamine 123 (DHR 123), a leucodye nonspecifically oxidized by ROS to fluorescent rhodamine 123, showed an antioxidant role of glutathione and a pro-oxidant role of cysteine and CysGly. On the other hand, cleavage of g-glutamyl moiety of GSH by GGT initiated the ROS production in the presence of chelated iron. In analogy with GGT, we hypothesized that GGT-rel, a distinct g-glutamyl cleavage enzyme have also pro-oxidant properties. Using a HPLC method for thiols dosage we showed that 3T3/GGT-rel transfected cell line metabolize extracellular GSH to CysGly, and that induces a ROS production in the presence of chelated iron. We studied the consequences of the GGT/GSH generated oxidative stress in the model of recombinant apolipoprotein E (apo E) oxidation (Jolivalt et al, 1996). We found a diminution of immunoaffinity towards specific antibody (by Western Blotting technique) and a modification of HPLC profile of apo E samples submitted to GGT/GSH/Fe3+ system. However, these results do not allow to conclude an oxidative modification of protein structure and identification of observed modifications by analytical chemistry techniques remains quite difficult. We also studied the lipid peroxidation (LPO) of polyunsaturated fatty acids induced by GGT/GSH/Fe3+. LPO secondary aldehydic products, derived as 2,4-dinitrophenylhydrazones were separated on TLC, HLPC and analyzed in mass spectrometry. By these techniques we identified a great complexity of carbonyl products (alkanals, alkenals, alkadienals, hydroxyalkenals and dialdehydes), most of them being similar to those produced in a known model for LPO (Fe2+/ascorbate). We found a significant increase (1.3 to 5 fold) for different carbonyl compounds in the samples containing GGT in the oxidation mixture, as compared to the control, that clearly proves the role of GGT in lipid peroxidation. In addition, generation of highly reactive 4-hydroxyalkenals (such as 4-hydroxynonenal) with known toxic effects on cell membranes and functions (Comporti, 1998) suggests that the GSH metabolism by GGT in the presence of iron might represent a biological way for a LPO toxic process. The present data justify a more detailed study of GGT/GSH oxidant action on serum lipoproteins and membrane phospholipids in order to correlate with mechanisms of atherogenesis and carcinogenesis. In conclusion, our in vitro studies demonstrate that the metabolism of GSH by GGT (or GGT-rel) leads to ROS generation and oxidation of lipids and must be taken into account as one of the physiopathological oxidation system. Further studies will focus on the importance of GGT/GSH in the pro-oxidant/antioxidant balance with particu-

larly attention on its involvement in the pathologies of cardiovascular system.

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