Oxidative stress conditions induce differential S-nitrosation of smooth muscle cell proteins by S-nitrosothioglutathione (GSNO)

Eugenia Belcastro,*, Wu W,*, Perrin-Sarrado C,*, Fries I,*, Corti A,*, Pompella A,*, Leroy P,*, Lartaud I and * Gaucher C,*
*Université de Lorraine, France
&University of Pisa Medical School, Italy

Background & Aims: Reduced availability or depletion of nitric oxide (NO) is often involved in pathogenesis and/or progression of cardiovascular diseases, such as atherosclerosis, thrombosis, pulmonary hypertension, ischemia and arrhythmia. Several NO-related therapeutics have been developed to overcome the problem, such as e.g. organic nitrates, metal—NO complexes, nitrosamines etc., but all have relatively short half-lives and produce tolerance phenomena and oxidant stress. These drawbacks are absent in S-nitrosothiols (RSNOs), i.e. molecules in which NO is reversibly bound to SH groups, and in particular, S-nitrosoglutathione (GSNO) – the endogenous/physiological storage and transport form of NO – is under active investigation. However, the ability of GSNO to regulate NO bioavailability under oxidative stress is poorly studied. Using protein S-nitrosation (Pr-SNO), a post-translational protein modification, as a biomarker of the NO pool (1), the study aims to evaluated the capacity of GSNO to deliver NO to smooth muscle cells (SMCs) under oxidative stress. Furthermore, the implication of redox enzymes implicated in GSNO metabolism, such as gamma-glutamyl transferase (GGT) and protein disulfide isomerase (PDI), in Pr-SNO formation under oxidative stress will be assessed.

Experimental: Rat aorta embryonic SMCs (A-10 cell line) were exposed in vitro to a free radical generator, AAPH, as an oxidative stress model. Oxidative stress impact on the expression/activity of redox enzymes implicated in GSNO metabolism, as well as NO release were evaluated. The intracellular thiol redox status was also monitored in relation with the extent and distribution of GSNO-induced intracellular proteins S-nitrosation (mass spectrometry (LC-MALDI) analysis).

Results: Under oxidative stress, GSNO-metabolizing enzymes were differentially modulated: GGT (activity) was in fact decreased by 3.5-fold, while PDI (expression) was increased by 17-fold. Oxidative stress produced the increase of extracellular GSNO-catabolism into nitrite ions as well as an increase in seric proteins S-nitrosation. Moreover, oxidative stress caused both a decrease of SH groups in cellular proteins and an efflux of intracellular GSH to the extracellular space. However, only the first phenomenon was prevented by concomitant administration of GSNO. In agreement with the increased NO release, GSNO-dependent protein S-nitrosation was approx. 2-fold increased under oxidative stress. Experiments with GGT inhibitor serine-borate complex (SBC) and PDI inhibitor bacitracin confirmed that even oxidative stress modified their activity/localization, both enzymes participated in GSNO catabolism and subsequent Pr-SNO. LC-MALDI analysis revealed that the number of proteins S-nitrosated by GSNO was increased under oxidative stress (51 proteins, vs. 32 in basal condition). Compared to basal condition, oxidative stress promoted the S-nitrosation of three additional classes of proteins, implicated in cell adhesion, transfer/carryer functions and cellular transport, and in particular favored the S-nitrosation of a higher number of cytoskeletal proteins (17 vs. 8 in basal condition).

Conclusions: Data obtained so far confirmed that oxidative stress such as those occurring in inflammation can modify the activity and/or expression of two critical enzymes in GSNO metabolism, GGT and PDI. Overall, oxidative stress induced higher levels of GSNO-dependent NO release and RSNOs formation. Protein S-nitrosation effected by GSNO under oxidative stress was more extensive, due to the involvement of additional proteins of SMCs cytoskeleton and contractile machinery. Further studies will likely elucidate the pathophysiological significance of these observations.

Biography
Eugenia Belcastro is a PhD student under joint supervision between University of Lorraine (Faculty of Pharmacy, Ecole Doctoral BioSe, Nancy) and University of Siena (PhD in Genetics, Oncology and Clinical Medicine, GenOMeC) and with work station at the University of Pisa (Department of Translational Research and New Technologies in faculty of Medicine). She will complete and get the PhD in November 2016. She has already participated in several international conferences and she has published several articles in reputed journals.

eugenia.belcastro@univ-lorraine.fr