S-nitrosation Regulates Metalloproteinase-9 Activity

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Metalloproteinases (MMPs) are crucial mediators of cardiovascular diseases, controlling the vascular remodeling and participating in the process of atherosclerotic plaque rupture; nevertheless, the precise mechanisms regulating secreted MMPs' activity are still incompletely understood. Nitric oxide (NO) may modulate MMPs activity through S-nitrosation in tissue specific fashion, activating (neurons) or blocking (lungs) it. Due to the importance of MMPs to cardiovascular diseases and to the presence of high amounts of NO in the vessel wall, our objective was to determine the effect of S-nitrosation on the activity of MMPs produced by cells involved in atherosclerotic plaque rupture. Human umbilical vein endothelial cells (HUVECs) and macrophages (derived from monocitoid cell line THP-1) were co-cultured in a transwell system. MMP-9 expression and activity were determined by immunoblot and gelatinase activity was measured using a fluorescent substrate. S-nitrosation of MMP was determined by biotin switch. Under basal conditions, MMP-9 was recovered from the culture medium in an inactive form; further experiments showed that the enzyme was in an S-nitrosated state. When both cell types were activated, by exposure to interferon-γ and lipolysaccharide, increased NO production and MMP-9 concentration in the medium were measured. Under these experimental conditions, S-nitrosation of MMP-9 in the culture medium could not be detected anymore. Previous treatment of both cell types with L-NAME blocked S-nitrosation and increased MMP-9 activity either under basal conditions or after exposure to the inflamatory sitmulus. Altogether, these data suggest that, in vascular cells, MMP-9 is produced and secreted in the S-nitrosated, inactive form and it is activated both by proteolytic cleavage and denitrosation. These findings may represent an additional mechanism controlling MMP activity. Financial Support: CNPq, FAPESP, Direx-LIM