Induction of Heme oxygenase-1 and Overproduction of Carbon Monoxide Protect Cells from Apoptosis Caused by Peroxynitrite

Young-Nam Cha

Department of Pharmacology, Inha University College of Medicine

Stimulated macrophages undergo oxidative burst and oxidative stress by overproducing O_2^- through activation of NADPH-oxidase, a heme-containing membrane enzyme. Cells with minimal oxidative stress can survive from the toxicity of O_2^- both by utilizing the SOD and GSH-peroxidase pathway. While severe oxidative stress can kill the cell, moderately stressed cells can survive by eliminating the O_2^- via induction of iNOS and overproduction of NO to convert the overporoduced O_2^- into peroxynitrite ($ONOO^-$), a strong oxidizing product that is detoxified by intracellular GSH. However, when the production of $ONOO^-$ is excessive, cellular GSH is depleted and cells undergo apoptotic cell death. In response to the GSH depletion and to survive from the $ONOO^-$-induced cell death, cells upregulate the expression of heme oxygenase-1 (HO-1). HO is the rate-limiting enzyme degrading heme into bilivedin (bilirubin) and carbon monoxide (CO). Induction of HO-1 and elevation of HO activity will accelerate the heme degradation and prevent Fenton reaction, limiting the generation of hydroxyl radical (HO*) and additional expression of heme-requiring enzymes like the NADPH-oxidase and iNOS that produce O_2^- and NO, respectively. Additionally, bile pigment antioxidants (biliverdin and bilirubin) produced by the enhanced HO activity will detoxify $ONOO^-$ and the CO also produced by the HO activity will inhibit additional productions of O_2^- and NO by binding to the heme contained in NADPH-oxidase and iNOS, respectively. Such multi-level cross talks between the enzymes that produce O_2^-, NO and CO will protect the oxidatively stressed aerobic cells from the $ONOO^-$-derived cell death. Thus, the cells with upregulated HO-1 overproducing CO were insensitive to the stimulation by LPS or peroxynitrite. Alternatively, when the cells were pre-exposed to increasing doses of CO generated from CO-releasing molecule, the LPS-derived overproductions of O_2^- (oxidative burst) and NO (iNOS induction) were inhibited in a dose-dependent manner. This caused cells to undergo a smaller degree of oxidative stress, allowing better survival from a given dose of LPS with smaller inductions of iNOS and HO-1. Furthermore, the cells overexpressing HO-1 or exposed to exogenous CO-donor were resistant to the $ONOO^-$-inducible apoptotic cell death. This CO-dependent inhibiton of $ONOO^-$-derived apoptotic cell death was causatively associated with prevention or reversal of $ONOO^-$-dependent depolarization of mitochondrial transmembrane potential ($\Delta\psi_m$).