

[P-7]**PKC Downstream of PI3-Kinase Regulates Peroxynitrite Formation for Nrf2-Mediated GSTA2 Induction**

Won Dong Kim, Sun Ok Kim and Sang Geon Kim

College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul

The protective adaptive response to electrophiles and reactive oxygen species is mediated by the induction of phase II detoxifying genes including glutathione S-transferases (GSTs). NF-E2-related factor-2 (Nrf2) phosphorylation by protein kinase C (PKC) is a critical event for its nuclear translocation in response to oxidative stress. Previously, we have shown that peroxynitrite plays a role in activation of Nrf2 and Nrf2 binding to the antioxidant response element (ARE) via the pathway of phosphatidylinositol 3-kinase (PI3-kinase) and that nitric oxide synthase in hepatocytes is required for GSTA2 induction. In view of the importance of PKC and PI3-kinase in Nrf2-mediated GST induction, we investigated the role of these kinases in peroxynitrite formation for GSTA2 induction by oxidative stress and determined the relationship between PKC and PI3-kinase. Although PKC activation by phorbol 12-myristate-13-acetate (PMA) did not increase the extents of constitutive and inducible GSTA2 expression, either PKC depletion by PMA or PKC inhibition by staurosporine significantly inhibited GSTA2 induction by tert-butylhydroquinone (t-BHQ) a prooxidant chemical. Therefore, the basal PKC activity is requisite for GSTA2 induction. 3-Morpholinopyridone (SIN-1), which decomposes and yields peroxynitrite, induced GSTA2, which was not inhibited by PKC depletion, but slightly enhanced by PKC activation, suggesting that PKC promotes peroxynitrite formation for Nrf2-mediated GSTA2 induction. Treatment of cells with S-nitroso-N-acetyl-penicillamine (SNAP), an exogenous NO donor, in combination with t-BHQ may produce peroxynitrite. GSTA2 induction by SNAP + t-BHQ was not decreased by PKC depletion, but rather enhanced by PKC activation, showing that the activity of PKC might be required for peroxynitrite formation. LY294002 a PI3-kinase inhibitor blocked GSTA2 induction by t-BHQ, which was reversed by PMA-induced PKC activation. These results provide evidence that PKC may play a role in formation of peroxynitrite that activates Nrf2 for GSTA2 induction and

that PKC may serve an activator for GSTA2 induction downstream of PI3-kinase.

Keyword : Glutathione S-transferase, Peroxynitrite, PKC, PI3-kinase, Nrf2