PD98059 PROMOTES APOPTOSIS IN PC12 CELLS EXPOSED TO PEROXYNITRITE VIA A MECHANISM INDEPENDENT ON ITS EFFECTS ON ERK 1/2 PHOSPHORYLATION.

Cerioni L.,1° Anno di Corso, Dottorato in Metodologie Biochimiche e Farmacologiche, XVII° ciclo, durata del corso anni 3. Istituto di Farmacologia e Farmacognosia, Università degli Studi di Urbino.

Nitric oxide (NO) is a free radical that is endogenously produced by the enzyme NO synthase which catalyzes the oxidation of L-arginine, yielding NO and L-citrulline. NO regulates numerous cell functions via cyclic GMP-dependent and independent mechanisms but an excessive or inappropriate formation of NO might cause deleterious effects, including neurodegeneration. Although NO can be directly detrimental to target cells, most of its toxic effects appear to be mediated by peroxynitrite, the coupling product of NO and superoxides. The cytotoxic potential of peroxynitrite has long been attributed to its ability to react with all the major classes of biomolecules. Indeed, peroxynitrite causes an array of effects, including lipid peroxidation, protein nitration and nitrosylation, DNA damage and oxidation of thiols, which most likely represent upstream events leading to inhibition of mitochondrial respiration, mitochondrial permeability transition and/or other dysfunctions promoting cell death.

It is important to note, however, that a paradigm shift has recently been occurring whereby reactive nitrogen species are appreciated as signaling molecules. In particular, peroxynitrite was shown to activate an array of kinases which may affect cellular processes regulating the balance between cell survival and death.

We recently observed that exposure of PC12 cells to concentrations of peroxynitrite as high as 500 µM does not produce obvious signs of toxicity and asked the question of whether resistance was associated with the activation of a major subfamily of the mitogen-activated protein kinases, namely the extracellular signal-regulated kinases (ERK1 and ERK2).

We found that the MEK inhibitor PD98059, that binds directly to the non-phosphorylated form of MEK after blocking its activation by Raf, promotes a rapid and extensive apoptosis in cells treated with peroxynitrite; this response was associated with and preceeded by formation of reactive oxygen species. Apoptosis was also detected in cells post-incubated in complete culture medium for up to 48 hr, so that very few cells survived treatment with PD98059/peroxynitrite. Interestingly, PD98059 did not suppress but, rather, significantly enhanced the peroxynitrite-dependent ERK phosphorylation.

In contrast with these results the MEK inhibitor U0126, which inhibits both MEK activation and activity, on the one hand abolished ERK1/2 phosphorylation and on the other hand failed to promote delayed formation of reactive oxygen species. Under these conditions, a small proportion of cells underwent early apoptosis and the surviving cells showed a reduced proliferation rate but did not undergo delayed apoptosis, or necrosis.

Thus, peroxynitrite activates ERK 1/2 via a mechanism that is abolished by U0126 but enhanced by PD98059. Since ERK 1/2 phosphorylation induced by the cocktail PD98059/peroxynitrite was sensitive to U0126, it might be inferred that ERK 1/2 phosphorylation is mediated by MEK which in turn must be phosphorylated by a Raf-independent mechanism. U0126 did not affect delayed formation of reactive oxygen species and the extent of secondary DNA fragmentation detected after exposure to the cocktail PD98059/peroxynitrite. As consequence, it appears that ERK1/2 hyperphosphorylation mediated by PD98059 in PC12 cells exposed to peroxynitrite is an epiphenomenon unrelated to the observed apoptotic response.