THE ROLE OF CYTOSOLIC PHOSPHOLIPASE A_2 IN PEROXYNITRITE-INDUCED U937 CELL DEATH

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Exposure to authentic peroxynitrite promotes rapid U937 cell death mediated by mitochondrial permeability transition (1). Inhibition of complex III, leading to formation of H_2O_2, was identified as a critical upstream event playing a pivotalrole in this toxicity paradigm (2). Since under the same conditions a release of arachidonic acid (AA) due to stimulation of phospholipase A_2 (PLA_2) was also observed, we investigated the role of this process in the peroxynitrite-dependent lethal response.
Peroxynitrite stimulates release of AA sensitive to various PLA_2 inhibitors, including arachidonyl trifluoromethyl ketone ($AACOCF_3$), which specifically inhibits cytosolic PLA_2 ($cPLA_2$). This response linearly increases using non toxic concentrations of the oxidant, and reaches a plateau at levels at which toxicity becomes apparent. Three separate lines of evidence are consistent with the notion that AA generated by $cPLA_2$ promotes survival in cells exposed to peroxynitrite. Firstly, toxicity was suppressed by nanomolar levels of exogenous AA, or by AA generated by the direct PLA_2 activator melittin. Secondly $AACOCF_3$, or other PLA_2 inhibitors, promoted cell death after exposure to otherwise non toxic concentrations of peroxynitrite; exogenous AA abolished the enhancing effects mediated by the PLA_2 inhibitors. Finally, U937 cells transfected with $cPLA_2$ antisense oligonucleotides were killed by concentrations of peroxynitrite that were non-toxic for cells transfected with nonsense oligonucleotides. This lethal response was insensitive to $AACOCF_3$ and prevented by exogenous AA.
As mentioned above, delayed formation of H_2O_2 plays a pivotal role in the peroxynitrite-dependent lethal response (2). Using an array of approaches involving respiratory chain inhibitors, as well as respiration-deficient cells, we were able to demonstrate that H_2O_2 is not directly toxic for the cells but, rather, reduces the extent of $cPLA_2$ activation mediated by peroxyntrite. Thus, it appears that peroxynitrite stimulates $cPLA_2$ activity and that the released AA, or its metabolites generated by the cycloxygenase or lipoxygenase pathways, stimulates a signaling pathway leading to cell survival. Cell death occurs under conditions of limited AA formation due to inhibition of $cPLA_2$ caused by H_2O_2 generated at the mitochondrial level via peroxynitrite-dependent inhibition of complex III. Since AA and peroxynitrite are concomitantly produced at the inflammation sites, we speculate that autocrine or paracrine generation of AA may allow survival of peroxynitrite producing cells.
The observation that AA generated by a different PLA_2 isoform mediates peroxynitrite-dependent toxicity in PC12 cells (Palomba and Cantoni, poster presented at this Conference) further supports the role of cell signaling in toxicity induced by peroxynitrite and demonstrates that AA plays a central role in different cell types but with even opposite consequences. The identification of the signaling cascades triggered by AA in different cell types may provide the means for manipulating the toxicity elicited by peroxynitrite in a cell/tissue-specific manner.

References

1. Sestili P., Tommasini I. and Cantoni O. *Free Rad. Res.*, 34, 513, 2001.
2. Tommasini I., Sestili P. and Cantoni O. *Mol Pharmacol.* 61, 870, 2002.