THE ENHANCING EFFECTS OF INTRACELLULAR ASCORBIC ACID ON PEROXYNITRITE-INDUCED U937 CELL DEATH ARE MEDIATED BY MITOCHONDRIAL EVENTS RESULTING IN ENHANCED SENSITIVITY TO PEROXYNITRITE-DEPENDENT INHIBITION OF COMPLEX III AND FORMATION OF HYDROGEN PEROXIDE

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A short-term pre-exposure to dehydroascorbic acid (DHA) promotes toxicity in U937 cells challenged with otherwise non toxic levels of peroxynitrite (ONOO'). This effect is mediated by a saturable mechanism since concentration-dependence studies revealed that levels of DHA higher than 100 µM, while progressively increasing the intracellular accumulation of the vitamin, failed to enhance further the lethal response evoked by ONOO'. Toxicity was mediated by the same mechanism observed in cells challenged with intrinsically toxic concentrations of ONOO' and involved delayed formation of H₂O₂ and mitochondrial permeability transition. The following lines of evidence are consistent with the notion that the enhancing effects of DHA were related to mitochondrial events resulting in inhibition of complex III upon exposure to otherwise inactive concentrations of ONOO'. Firstly, DHA, as well as bona fide complex III inhibitors, similarly enhanced toxicity and delayed formation of H₂O₂ induced by ONOO' via a rotenone-sensitive mechanism. Secondly, bona fide complex III inhibitors were ineffective in DHA-preloaded cells. In addition, respiration-deficient cells were resistant to toxicity elicited by ONOO' and their supplementation with increasing concentrations of DHA, while resulting in the accumulation of vitamin C levels identical to those observed in respiration-proficient cells, failed to affect ONOO' toxicity. Finally, oxygen consumption experiments demonstrated that pre-exposure to DHA increases the ONOO'-dependent inhibition of complex III. In conclusion, the above results collectively demonstrate that increasing the intracellular accumulation of vitamin C promotes mitochondrial events leading to ONOO'-dependent formation of H₂O₂ and resulting in a rapid necrotic response.