

THE RELATIONSHIP OF NADH FLUORESCENCE LIFETIME AND MITOCHONDRIAL FUNCTION IN THE EARLY STAGE OF STAUROSPORINE-INDUCED APOPTOSIS

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We have previously reported that cellular NADH fluorescence lifetime significantly increased immediately after 1 mM Staurosporine (STS) induced-apoptosis. The main contribution of this phenomenon is due to the increased amount and lifetime of the bound NADH component indicating increased interactions of NADH and various proteins during STS-induced apoptosis. This NADH lifetime increase took place before caspase 3 activation suggesting that it may be a potential biological marker of noninvasive early detection of apoptosis. Because the main NADH fluorescence is from mitochondria and in order to understand the origins of this NADH fluorescence lifetime increase, we studied its correlation with mitochondria functions including the change of mitochondria membrane potential (MMP) and ATP that are known "early" signatures of mitochondria mediated apoptosis before various caspase activation. The relationship between these phenomena and the change of NADH fluorescence lifetime has not been fully understood. In this study, we have performed time-course measurements of NADH fluorescence lifetime, MMP, ATP levels, as well as mitochondria coupling effects at high (1 mM) and low (50 nM) doses of STS induced apoptosis. Apoptosis was confirmed by both cell morphology change and caspase 3 activation. The speed and the amount of the NADH fluorescence lifetime change is directly related to the concentration of STS that higher STS dose caused higher NADH lifetime change after treatment and within a fixed treatment time. In an additional study using various levels of mitochondrial dysfunction cells, higher NADH lifetime change with a fixed dose of STS treatment (100 nM) is correlated with severer mitochondrial dysfunction and earlier apoptosis. Compared to MMP and ATP changes after low and high STS treatment of HeLa cells, NADH fluorescence lifetime did not seem to correlate with these mitochondrial-induced apoptosis signatures in time. However, several trends have been observed: 1) the rise and drop of the NADH fluorescence lifetime after treatment was similar to the MMP change; 2) this increase then decrease of the NADH fluorescence lifetime took place before the drop of MMP and ATP. Lastly, we have measured the respiratory function of mitochondria because the increase of MMP indicated the possibility of the respiratory function failure. However, we found that the mitochondrial respiratory function remains the same whereas its coupling was decreased after treatment of cells with 50 nM STS for 90 minutes and longer. Therefore, the increase then decrease of NADH fluorescence lifetime might reflect the beginning of mitochondrial dysfunction of HeLa cells and starting apoptotic pathway.