View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Yamagata University Academic Repository

Yamagata Med J 2004；22（1）：103

A Cellular Mechanism that Reversibly Inactivates Pancaspase Inhibitor zAsp-CH2-DCB

Kaori Sakurada

Department of Neurosurgery, Yamagata University School of Medicine, Yamagata, Japan

ABSTRACT

Apoptosis is a form of programmed cell death (PCD) characterized by cell shrinkage as well as by early and prominent nuclear chromatin condensation. Our understanding of the molecular mechanism involved in the final common pathway of apoptosis has advanced remarkably in recent years, and it is now well established that a family of cysteine proteases called caspases play a pivotal role in the process. On the other hand, there is evidence to suggest that there exist PCDs essentially distinct from apoptosis. These PCDs (described as apoptosis-like or necrosis-like depending on the extent to which they retain the morphological features of apoptosis) are regulated independently of caspases. As a natural consequence of the complexity of PCD, it is becoming increasingly important to know whether the cell death model of interest is caspase-dependent or independent. Cell-permeable pancaspase inhibitors such as zAsp-CH2-DCB and zVAD-fmk are widely used to examine the involvement of caspases in cell death models. While examining the caspase-dependence of staurosporine (STS) -induced neuroblastoma cell death, I found that zVAD- fmk but not zAsp-CH2-DCB inhibits apoptosis, and that *in vitro* and *in vivo* caspase assays may give divergent results in the presence of zAsp-CH2-DCB. Time course analysis revealed that, in contrast to zVAD-fmk which constantly inhibited the processing of endogenous caspase substrates, zAsp-CH2-DCB inhibited substrate processing only for the first few hours after its addition to the culture medium. However, when the caspase activity in lysates prepared from cells treated with STS and zAsp-CH2-DCB was measured *in vitro*, quite unexpectedly, it was found that zAsp-CH2-DCB completely inhibited the STS-mediated activation of caspases throughout the observation period even when it apparently failed to inhibit the processing of caspase substrates within intact cells. These findings together suggest that there exists a cellular mechanism that inactivates zAsp-CH2-DCB in a reversible manner. This reversible inactivation was an active, intracellular process requiring de novo protein synthesis that inhibited by protein synthesis inhibitor cycloheximide. And the inactivation was observed in another human cancer cell line HeLa and with different apoptotic stimuli such as UV irradiation. These results have important implications that require consideration when designing experiments involving the use of caspase inhibitors as well as interpreting their results.