LABILE ZINC AND ITS ROLE IN REGULATION OF PRO-CASPASE-3 AND NF-κB ACTIVATION IN MAST CELLS

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TABLE OF CONTENTS

SUMMARY

The main aim of this thesis was to further investigate the relationship between Zinquin-detectable intracellular pools of labile Zn and caspase activation since caspases have now been shown to be important effector enzymes in apoptosis. This was largely studied in mast cells and some findings confirmed in neuronal cells, a cell type with quite different function and cell physiology.

Mast cells were chosen because these cells are important inflammatory cells that have been shown to contain Zn rich granules. However, there have been no previous studies of labile Zn in these cells, the mechanisms by which it is accumulated and its function in relationship to apoptosis. Labile Zn was shown to be rich in mast cells by Zinquin fluorescence with a granular like staining pattern and the fluorescence decreased during degranulation. A major issue is how mast cells regulate their uptake of Zn from their environment. The experiments reported here are the first to describe the localization of a Zn transporter, ZnT_4 in mast cells. There was high expression of both the mRNA and its protein in mast cells, suggesting that it is likely to be involved in the transportation of Zn either across the plasma membrane or into the granules. The absence of overlap between Zn and ZnT_4 may imply that only a subset of granules require ZnT_4 as their major Zn transporter. Other transporters need to be investigated.

The distribution of pro-caspases, important components in apoptosis signaling has not been a focus of mast cell biologists. It was important to determine whether these were

present in mast cells and their relationship to mast activation. In mast cells, expression of pro-caspase-3 mRNA was shown and the detection of its protein by immunocytochemistry indicated that it was translated. One unexpected finding was that pro-caspase-3 (and -4) were localized within granules of the mast cells as shown by immunoelectron microscopy. This was confirmed by their loss during degranulation.

Explanations for why pro-caspases are localized in mast cell granules include: 1) pro-caspases may be released and activated during degranulation and cleave extracellular substrates, 2) caspases maybe released and induce apoptosis in other cells, 3) caspases may have a special function in cleaving proteins within the granules and 4) in order to prolong their survival during activation, mast cells may shed their caspases during degranulation and thereby become more resistant to noxious stimuli.

Depletion of Zn by one mechanism (TPEN chelation) but not by degranulation increased the activation of pro-caspase-3, either spontaneously or in cells treated with the apoptotic inducer butyrate. The experiments described in this thesis show that at least under *in vitro* conditions, degranulated mast cells can recover their granular content of Zn and do not undergo apoptosis spontaneously. Similar results were obtained for a range of degranulators and various mast cell types. These findings suggest that there are two labile pools of Zn; one regulates caspase activation and apoptosis while the other (the granular pool) has other functions.

To further understand the mechanism by which Zn may regulate apoptosis in mast cells, the effects of Zn supplementation and depletion on activation of NF-κB were

investigated. NF-κB is thought to be an anti-apoptotic factor prolonging the survival of inflammatory cells. Zn supplementation by the ionophore pyrithione blocked nuclear translocation of NF-κB, an important step in the activation of this transcription factor. Furthermore, Zn depletion by TPEN was found to be an effective inducer of nuclear NF-κB translocation. This has not previously been demonstrated. These findings suggest that Zn is a regulator of NF-κB translocation and raise questions as to whether abnormalities in NF-κB activation occur in Zn deficiency.

The studies with neuroblastoma cells confirmed some of these findings. BE(2)-C neuroblastoma cells had cytoplasmic/cytoskeletal pools of intracellular labile Zn which were further increased by Zn ionophore or decreased by Zn chelator TPEN. As for mast cells there was synergy between TPEN and butyrate in caspase activation and this was suppressed by Zn supplementation, suggesting that the effect of Zn on caspase regulation is a general phenomenon.

As a summation of the findings reported in this thesis, a general model is proposed describing possible interactions between Zn, pro-caspases and NF-κB. Some of these findings were reported in Ho et al 2000 and form part of a submitted manuscript Ho et al 2003.