Metabolic and Bioenergetic Changes in the Livers of Living Mice during Acute Anti-Fas Induced Apoptosis

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Introduction:

Apoptosis is a highly regulated, ATP-requiring form of cell death involving DNA fragmentation, cellular shrinkage and condensation. Ultimately, the apoptotic cell is dismantled and phagocytized. Unlike necrosis, apoptosis does not involve disruption of the cell membrane, cell swelling or an inflammatory response. An important regulatory process in apoptosis in many cell culture systems is the mitochondrial permeability transition (MPT), in which the mitochondria depolarize and release cytochrome C to activate downstream caspases. The MPT should dramatically affect tissue bioenergetic status, as the mitochondria cannot make ATP when depolarized. Cyclosporin A (CSA) is a specific MPT inhibitor both *in vivo* and *in vitro* and can be used to delineate the role of the MPT. An *in vivo* model for hepatic apoptosis is Fas receptor stimulation by the anti-Fas monoclonal antibody (anti-Fas). Anti-Fas causes massive, selective apoptosis by activation of caspase-8, which can trigger a caspase cascade leading to MPT and apoptotic cell death. We induced rapid, massive apoptosis within the livers of Balb/c mice with anti-Fas, and followed morphological and metabolic changes by histology, electron microscopy and ^{1}H and ^{31}P NMR spectroscopy.

Methods and Materials:

Female Balb/c (17-21 g) were injected with anti-Fas (10 μg/mouse, i.v.) and sacrificed 2, 3, 4 and 5 h post-injection. Livers were rapidly excised and freeze-clamped followed by perchloric acid (PCA) extraction for NMR analysis. The PCA extracts were analysed on a Bruker Avance 400 MHz NMR spectrometer (45 degree flip angle, 2 sec repetition time). Histological analysis was by standard H&E stain. Transmission electron microscopic (TEM) samples were prepared and viewed with a JEM-100S TEM. The effects of MPT were assessed by pretreatment with CSA (100 mg/kg, i.p.) 1 hr before the anti-Fas injection.

Results and Discussion:

We obtained >70% apoptotic cells and <10% necrotic cells in livers of mice 5 hr after treatment, as judged by DNA fragmentation, TUNEL and propidium iodide labelling and H & E staining. The apoptotic index increased dramatically between 3 and 4 hr (22% to 67% apoptotic cells). Transmission electron microscopy at 5 hr post-anti-Fas showed swollen mitochondria and ruptured outer membranes. CSA pre-treatment dramatically decreased the lethality and morbidity of anti-Fas treatment. Analysis of perchloric acid extracts of freeze-clamped livers by ^{31}P and ^{1}H NMR indicated an abrupt 3-5 fold increase in phosphocholine, the glycolytic phosphomonoesters and lactate between 3 and 4 hr after anti-Fas. Lactate was 2.8 fold higher at 5 hr compared to controls. CSA prevented most of this rise in lactate and phosphocholine. However, ATP levels were stable at 3 and 4 hr, and only dropped by 35% between 4 and 5 hr. All the mice were dead by 6 hr after anti-Fas.

The results indicate the presence of the MPT *in vivo* in mouse liver during apoptosis. ATP levels are preserved until late in apoptosis in spite of the MPT, while anerobic glycolysis increases abruptly mid-way through the process. Human leukemia cells have also shown this “compensatory glycolysis” during apoptosis, with lactate buildup and acidosis following the MPT. The role of the MPT is also shown in the TEM analysis, which showed multiple mitochondria with ruptured outer mitochondrial membranes, likely caused by matrix swelling during the MPT. The ruptured outer membranes would allow release of cytochrome C from the intermembrane space of the mitochondria to the cytoplasm leading to activation of caspase-9 and downstream caspase cascade. (Financial support generously provided by the NSERC).