Changes in specific metabolic pathways are essential steps in the early apoptotic process in the liver

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Introduction and Aim

Apoptosis is the main mode of cell death in most liver injuries.¹,² It is believed that mitochondrial dysfunction is a late event during apoptosis.³ It is noteworthy that mitochondrial involvement in the apoptotic process occurs in viable cells with adequate ATP stores. For example, ATP depletion can prevent activation of executioner caspases upstream of cytochrome c release.⁴ Another characteristic of the apoptotic response is its dependence on the cellular redox status.⁵ An intriguing observation was made between apoptosis and glucose metabolism: Danial et al.⁶ demonstrated that BAD, a pro-apoptotic member of the Bcl-2 family, resided in a functional complex with glutathione. To clarify whether changes in glucose metabolism occur prior to the already known events in the apoptotic process, we used NMR spectroscopy to characterize metabolic pathways in mice liver during Fas-induced apoptosis.

Methods

Animal model. 1) To induce hepatocyte apoptosis, BALB/C mice were injected with anti-Fas antibody (10 µg; i.p.). The investigations were done at 7 time-points (15 min - 7.5 h). 2) Protection against apoptosis was afforded by treatment of Fas-injected mice with EGF (7.5 µg; i.p.) (45 min prior to Fas, concomitantly with Fas (t = 0) or at 45 min - 4.5 h postinjection). All mice were injected with [U-¹³C]glucose or [1,2,6-¹³C]succinate (2.2 mmol/kg, i.v.). 45 min after administration of the ¹³C-labelled substrates, the mice were killed by cervical dislocation. The livers were freeze-clamped using liquid nitrogen. Extraction. Tissue samples were powdered over liquid nitrogen and homogenized in 5% perchloric acid (PCA) at 0°C. Blood (taken from the carotid artery) was immediately mixed with PCA, dual-extracted, and used for the analysis of serum alanine aminotransferase (ALT) levels and ¹³C-NMR analysis of the blood. NMR analysis. After lypohilization, the samples were redissolved in 0.5 ml and centrifuged. ¹H-, ¹³C- and ³¹P-NMR spectra were recorded on Bruker spectrometers DRX 600 or AVANCE-NB/WB 360. Metabolite concentrations were calculated from ¹H-NMR spectra; the flux of ¹³C through metabolic pathways was followed up by ¹³C-isotopomer analysis.⁷

Results

1a) Serum ALT and metabolic parameters in the liver of FAS-(+ EGF)-treated mice (7.5 h). ATP administration (7.5 µg) prior or simultaneously to Fas treatment prevented massive apoptosis (as indicated by histological investigations), and delayed the demise of the animals from 7-9 h to > 2 days. After concomitant treatment with EGF and Fas, ATP depletion and impaired flux of [U-¹³C]glucose through PDH and PC, and glutathione depletion at late stages (7.5 h after Fas) were completely prevented (Fig. 1a). 4) EGF reverses the Fas-induced upregulation of specific pathways of glucose metabolism at early stages (t = 1.5 h). 1.5 h after concomitant treatment with Fas and EGF, flux through PDH and PC decreased considerably, in complete opposition to the upregulation of the same pathways observed with Fas alone (Fig. 1b). In particular, the reversal of Fas-induced metabolic upregulation was associated with abolition of the apoptotic process, which preceded its protective effect on apoptosis. 5) Fas causes metabolic changes in the intermediary stage (t = 5 h) of the apoptotic process, which are different from very early and very late changes. When entry of [U-¹³C]glucose into the TCA cycle via PDH and PC was impaired by 50% (p<0.001), the de novo synthesis of glucose from [2,3-¹³C]succinate (gluconeogenesis) rose to 203±38% (p<0.01). Furthermore, increased de novo synthesis of lactate (to 262±42%) suggested stimulation of pyruvate recycling involving phosphoenolpyruvate carboxykinase (PEPCK) and/or malic enzyme (ME) during the later, but still progressing apoptotic process.

Conclusions

Increased mitochondrial glucose oxidation and de novo glutathione synthesis occur very early and specifically following the initiation of apoptosis. We suggest that these metabolic events are responsible for the maintenance of adequate energy stores and a reduced cell environment necessary for apoptosis to proceed. The Fas-induced upregulation of specific pathways also precede the protective effect of EGF on the apoptotic process. Furthermore, these early mechanisms are different from those occurring at later stages, when the apoptotic process is still progressing, as well as from those occurring at very late stages, e.g. when the cells lose their capacity to maintain adequate energy stores. These phenomena provide useful hints for the understanding of the early mechanisms controlling apoptotic cell death.

References
