CELLULAR METABOLISM AND APOPTOSIS – DEXAMETHASONE, A PROMISING NEW CANDIDATE TO INTERVENE ON THE METABOLIC LEVEL

S. Gottschalk[1], M. Hohnholt[2], D. Leibfritz[2], C. Zwingmann[1], and M. Bilodeau[1]

[1]Department of Medicine, University of Montréal, Montréal, Québec, Canada, [2]Department of Organic Chemistry, University of Bremen, Bremen, Germany

Introduction :

Apoptosis – a regulated form of cell death – is the main mode of cell death in most liver injuries. Mitochondria, besides their recognized role as energy-providing metabolic centers, are also key regulatory centers during the genesis of apoptosis. Consequently, increasing interest has developed around the relations between cell metabolism and cell death/survival. We have recently shown that the initial phase of the apoptotic process is associated with alterations in specific glucose metabolic pathways.

The synthetic glucocorticoid Dexamethasone (DEX) is a commonly used therapeutic agent in several inflammatory disorders. Besides its clinical use, DEX is known to inhibit apoptosis *in vitro*. So far, the exact underlying mechanisms of the anti-apoptotic effect of DEX are poorly understood and are currently being investigated.[1,2] Interestingly, with regards to our earlier observations DEX has been shown to modify metabolic pathways (in particular, anaplerosis and gluconeogenesis) both *in vivo* and *in vitro*.[3,4]

Aims :

DEX was used to further study the links between glucose metabolism and apoptosis in murine hepatocytes. **A)** We characterized DEX-induced changes in metabolites and metabolic pathways in primary mouse hepatocytes by multinuclear NMR-spectroscopy and **B)** we measured the anti-apoptotic effect of DEX on Fas-induced apoptosis in primary mouse hepatocytes.

Methods :

Cell culture: Hepatocytes were isolated from the livers of Balb/c mice and put in primary cultures. After a period of attachment to the Petri dish overnight, cells were exposed to increasing concentration of DEX (0.01; 0.1; 1; 10; 100 µM) in a glucose-free culture medium (DMEM) containing [5mM] of [3-^{13}C]pyruvate. Cells were extracted after 6 h of incubation with this medium.

Extraction: Cells were immediately frozen in liquid nitrogen and extracted with 5% perchloric acid. After centrifugation the supernatant was neutralized and lyophilized.

NMR analysis: Lyophilized samples were redissolved in 0.5 ml D_2O. 1D ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on a Bruker DRX 600 MHz spectrometer. Metabolite concentrations were calculated from ^{1}H-NMR spectra. The flux of ^{13}C through metabolic pathways was followed up by ^{13}C-isotopomer analysis.

Biochemical assays: Apoptosis was induced by exposing cells to anti-Fas antibody (0.25µg/ml) for 6 hrs. Cell viability was quantified by MTT-assay. Apoptosis was assessed by quantification of nuclei showing chromatin condensation/fragmentation after staining of formaldehyde-fixed cells with the DNA-binding dye Hoechst 33258. Standard enzymatic assays were used for caspase-3 determination.

Results :

A) Analysis of ^{1}H-NMR spectra showed decreased concentrations of lactate after 6 hours of incubation with 1, 10 and 100µM DEX (to 49±25; 57±15 and 51±10 % of controls, respectively, $P<0.01$). On the other hand, the enrichment in [3-^{13}C]lactate was unchanged by DEX. Branched chain amino acids (valine, isoleucine, leucine) were also significantly decreased upon DEX-exposure (to 57±22 and 61±13 % of controls, respectively with DEX 1 and 10 µM, $P<0.05$). Another amino acid, glutamine was also decreased with 1 and 10 µM DEX (to 62±19 and 66±6 % of controls, respectively, $P<0.05$). Flux analysis from ^{13}C-NMR spectra revealed diminished [3-^{13}C]pyruvate-metabolism trough pyruvate dehydrogenase (PDH) after incubation for 6 hours with 1, 10 and 100µM DEX (to 53±24; 75±23 and 35±17 % of controls, respectively, $P<0.05$). This reduced flux trough PDH is also substantiated by a lesser *de novo* synthesis of glutathione (indicated by a lower incorporation of [4-^{13}C]glutamate into glutathione).

Further analysis of ^{13}C-NMR spectra suggests higher flux trough pyruvate carboxylase (PC) (to 151±47 and 149±44 % of controls, respectively with DEX 1 and 10 µM). Analysis of ^{31}P-NMR spectra revealed no changes in the cellular energy status (as assessed by the concentrations of high energy phosphates, e.g. ATP) with any concentration of DEX,

B) DEX exerted no toxicity up to 6 hours of exposure at concentrations up to 100µM. Cell viability was slightly decreased with 500µM DEX (to 82.6±2.2% of control, $P<0.05$). DEX significantly diminished anti-Fas-induced increase in Caspase-3 activity (anti-Fas-only: 33.9±2.2 U/L; anti-Fas + 5µM DEX: 21.2±0.4 U/L; anti-Fas + 10µM DEX: 23±2.3 U/L; both $P<0.001$ vs. anti-Fas-only). The amount of apoptotic nuclei was significantly reduced with DEX (anti-Fas-only: 8.9±1.1% apoptotic cells; anti-Fas + 1µM DEX: 3.6±1.0%; anti-Fas + 5µM DEX: 4.3±2.4%; both $P<0.05$ vs. anti-Fas-only).

Conclusions :

The observed metabolic changes indicate that DEX induces gluconeogenesis in primary mouse hepatocytes after 6 hours of treatment. In particular, glucogenic amino acids were reduced and flux through PC was enhanced, while at the same time flux through PDH was diminished. The protective effect of DEX against Fas-mediated apoptosis was also demonstrated. These results further support the strong relationship noted between changes in cellular metabolism and apoptosis. This paves the way toward the therapeutic modulation of cell metabolism in order to impact upon cell survival/death particularly in the context of liver injury.

References :

1) Oh et al. Cell Death and Differentiation 13(2006)512–523; 2) Bailly-Maitre et al. Cell Death and Differentiation 9(2002)945-955; 3) Jones et al. Biochem J 289(1993)821-828 4) Martin et al. Biochem J 219(1984)107-115