

Liver tissue repair in a mouse model of toxicant-induced liver injury is associated with increased hepatic energy metabolism: A multinuclear magnetic resonance study

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Introduction :

Administration of acutely toxic doses of thioacetamide (TAA) is a model widely used in experimental animal settings to investigate the mechanisms of toxin-induced liver injury, regeneration and the development of fibrosis. TAA is oxidized in the liver by microsomal CYP2E1 in two consecutive steps to TAA-S,S-dioxide. Covalent binding of this highly reactive compound to macromolecules is thought to cause hepatocyte death and consequently liver injury. Mangipudy et al.[1] have reported onset of liver tissue-repair mechanisms 24-48hrs after sub-lethal doses of TAA. However, only little is known about the associated changes in cellular metabolic pathways. We hypothesize that energy-intensive tissue repair processes and cell proliferation should be reflected in alterations of energy metabolism.

Aims :

We applied *ex vivo* multi-nuclear magnetic resonance (NMR) spectroscopy to follow up changes in cellular metabolites and energy metabolism that are associated with the onset of tissue repair mechanisms.

Methods :

Animal model: BALB/c-mice were injected intraperitoneally with one non-lethal and two lethal doses of TAA (200, 600, 1000 mg/kg, ip). Mice were sacrificed by cervical dislocation 24hrs after TAA-injection and the livers were snap-frozen in liquid nitrogen. [U-¹³C]glucose (500mg/kg, ip) was injected 45min prior to sacrifice. **Extraction:** Frozen tissue was extracted with 5% perchloric acid on liquid nitrogen. After centrifugation, the supernatant was neutralized and lyophilized. **NMR analysis:** Lyophilized samples were redissolved in 0.5 ml D₂O. 1D ¹H-, ¹³C- and ³¹P-NMR spectra were recorded on a Bruker DRX600 MHz spectrometer. Metabolite concentrations were calculated from ¹H-NMR spectra. The flux of ¹³C through pyruvate carboxylase (PC) and pyruvate dehydrogenase (PDH) was followed up by ¹³C-isotopomer-analysis. **Biochemical assays:** Standard enzymatic assays were used for serum alanine (ALT)/aspartate (AST)-aminotransferase. Reduced (GSH) and oxidized (GSSG) glutathione were analyzed by HPLC. Liver sections were derived by a standard fixing and staining technique (HPS (hematoxylin phloxine-saffron)-staining).

Results :

All TAA-treated mice survived 24hrs (the 600 and 1000mg/kg doses would be lethal past 48hrs). Analysis of ¹H-NMR spectra showed significant decreases of energy-related metabolites under all three treatment conditions (Tab. 1). The treated livers were also completely depleted of glycogen. The cytoprotective (e.g. against oxidative stress) metabolites taurine and glycine were decreased as well (Tab. 1). Glutamine was reduced to ~20% of control, in accordance with a predominant loss of perivenous hepatocytes, which are the main site of TAA-metabolism and glutamine-synthesis.

TAA[mg/kg]:	Control	200	600	1000
Glucose	3.11±0.41	0.72±0.27***	0.76±0.48***	0.73±0.08***
Succinate	0.63±0.13	0.16±0.08**	0.24±0.10**	0.25±0.02**
Acetate	0.55±0.02	0.15±0.05***	0.15±0.4***	0.13±0.02***
Taurine	13.19±0.59	2.57±1.23***	1.72±1.36***	2.42±0.15***
Glycine	4.18±0.08	1.27±0.12***	0.67±0.21***	1.53±0.08***

Analysis of ¹³C-NMR spectra revealed greatly enhanced glycolytic activity 24hrs after administration of 200mg/kg TAA (fractional enrichments in [3-¹³C]lactate: 415±59 and [3-¹³C]alanine: 301±105; both %control and P<0.001), while the concentrations of lactate and alanine were unchanged in comparison to the controls. Increased glycolytic activity was accompanied by an increase in anaplerotic activity (via PC-flux) after 200mg/kg TAA (372±62%control; P<0.001). This increase in mitochondrial metabolism was less pronounced using higher doses (600mg/kg: 296±113; 1000mg/kg: 246±52; both %control and P<0.001). PDH-flux, however, was only significantly increased with the two higher doses.

Serum-ALT/AST activities and histological evidence showed that TAA-induced liver injury was higher using 200mg/kg TAA (ALT/AST: 12969±1093/13204±1253; P<0.001 vs. control) in comparison to 600mg/kg (7467±1068/8527±1499; P<0.001 vs. control) and 1000mg/kg (1910±634/3385±1253; ns vs. control). The lower extent of tissue damage at the highest dose suggested that the time of onset of TAA-toxicity was inversely correlated to the dose of TAA. Levels of GSH and GSSG were considerably decreased after all three doses. Higher concentrations of both GSH and GSSG 24hrs after the non-lethal dose (200mg/kg) suggested that tissue reparation was occurring.

Conclusions :

TAA hepatotoxicity depletes the liver of vital energy stores and protective metabolites. Furthermore, the induction of tissue repair processes after toxicant-induced liver injury is associated with an increase in liver energy metabolism (increased PC- and PDH-flux and elevated lactate-enrichment). Since PC is known to be an important pathway for the anaplerotic supplementation of the TCA-cycle, these results strongly suggest that liver metabolism is directed towards the synthesis of amino and nucleic acids (derived from the TCA cycle) during tissue recovery. This is consistent with the expected requirements for rapid cell proliferation known to occur at this timepoint.

[1]Mangipudy *Environ.Health.Perspect.* 103(1995)260-267