Mice liver metabolism and defence mechanisms under oxidative stress-related conditions: Hypotaurine as selective hepatic antioxidant?

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Introduction
Mitochondrial damage is being recognized as a key step in liver injury associated with oxidative stress. Mitochondria also have necessary roles in the genesis of apoptosis in certain cell types [1]. As mitochondrial function is tightly coupled to both production of free radicals and cellular energy production, changes in mitochondrial oxidative metabolism may be a central feature in liver injuries. Interestingly, a link between glucose metabolism and apoptosis has been reported recently [2]. The most widely used antidote to prevent oxidative damage to the liver is N-acetyl-cysteine (NAC), which is believed to exert its beneficial effect by the replenishment of the antioxidant glutathione (GSH). To clarify the involvement of hepatic intermediary metabolism in liver injury, we used ex vivo multinuclear NMR spectroscopy combined with in vivo injection of different 13C-labelled substrates to characterize key metabolic pathways in mice liver at rest, 2) to investigate whether metabolic changes are associated with a) peroxide-mediated oxidative stress, b) oxidative stress due to mitochondrial impairment or c) anti-FAS mediated apoptosis, and 3) to address whether NAC is involved in yet undescribed metabolic pathways of the liver under normal and oxidative-stress related conditions.

Methods

Animal model. 1) BALB/c mice were injected with different 13C-labelled substrates ([U-13C6]glucose, [U-13C3]propionate, [1,2,3-13C]acetate, [3,4,5-13C3]pyruvate, [3-13C]alanine, [U-13C]taurine, 22 mmol/kg, i.p.). 2) Mice were treated with a) tert-butylhydroperoxide (t-BHP; 50 mg/kg, i.p., 5 h), b) 3-nitropipionic acid (3-NPA; 50 mg/kg, i.p., 5 h), an inhibitor of succinic dehydrogenase, or c) anti-FAS antibody (0.5 µg/g; 1.5 - 7.5 h). 3) Mice were injected with NAC (100-600 mg/kg or 3 x 100 mg/kg; i.p.) alone or after treatment with t-BHP, 3-NPA or anti-FAS, 30 min after administration of the 13C-labelled substrates, the mice were killed by decapitation. The liver (and other organs for comparison) were removed and immediately snap-frozen in liquid nitrogen. Extraction. Tissue samples were powdered over liquid nitrogen and homogenized in perchloric acid (PCA) at 0°C [3]. To obtain lipid extracts from the same tissues, the pellets were extracted with CHCl3/CH3OH. Blood (taken from the neck) was immediately mixed with PCA and dual-extracted as well. NMR analysis. After lyophilization, the samples were redissolved in 0.5 mL D2O (water-soluble metabolites) or in 0.8 mL CDCl3/CD3OD (2:1) (lipid components) and centrifuged. 1D 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 percentage 13C-enrichments were calculated from 13C-NMR spectra as described previously [3]; the flux of 13C through metabolic pathways was followed up by 13C-isotopomer analysis of the 13C-13C coupling pattern in amino acids. Gradient selected 2D-NMR inverse homonuclear (COSY) and heteronuclear HSQC correlations were applied to verify chemical shift data.

Results

1) Under physiological conditions, mitochondrial metabolism was most active using glucose, followed by propionate > acetate > alanine > pyruvate as 13C-labelled substrate. The major fraction of pyruvate from [U-13C6]glucose was metabolized anaplerotically via pyruvate carboxylase (PC; > 70%). While flux of carbon through glycolysis was low compared to the TCA cycle flux, the gluconeogenetic pathway led to considerable de novo synthesis of glucose and glycogen from propionate, followed by pyruvate > alanine > acetate. With the exception of glutamine, synthesized in the muscle from [1,2-13C]acetate, only minor contributions of other organs to the labelling pattern of liver metabolites were detected. A significant de novo synthesis of glutathione (GSH) (the 13C-glutathione residue of GSH was quantified from 13C-NMR spectra) was observed selectively in liver tissue, consistent with much higher basal concentrations of GSH (8.72 ± 0.93 µmol/g ww) compared to any other organ (p<0.001). 2) After treatment of mice with either t-BHP or 3-NPA, oxidative stress and mitochondrial injury in the livers were observed as indicated by increased blood GSH levels concomitant to >60% decreased GSH tissue concentrations (p<0.05). 3) Arsenic was defined as a control condition and after treatment with t-BHP and/or NAC after injection of different 13C-labelled substrates 1) to characterize key metabolic pathways in mice liver at rest, 2) to investigate whether metabolic changes are associated with a) peroxide-mediated oxidative stress, b) oxidative stress due to mitochondrial impairment or c) anti-FAS mediated apoptosis, and 3) to address whether NAC is involved in yet undescribed metabolic pathways of the liver under normal and oxidative-stress related conditions.

Conclusions

The present data demonstrate the usefulness of ex vivo multinuclear NMR spectroscopy to study various aspects of liver intermediary metabolism associated with oxidative stress-associated events. Furthermore, changes in energy metabolism may explain some mechanisms involved in apoptosis. In particular, initially activated glucose metabolism and subsequently sustained energy depletion might trigger death receptor engagement by the provision of the needed energy and render the liver cells more susceptible to oxidative stress at later stages, respectively. Furthermore, the observed NAC-induced alterations in hepatic metabolism improve the understanding of its beneficial effect in liver injuries. We suggest that, apart from GSH replenishment, several other metabolic actions of NAC contribute to the maintenance of the liver’s normal redox state. In particular, formation of HTau, which can be oxidized to taurine, may represent a further antioxidant defence mechanism in the liver.

References