The hepatoprotective effect of N-acetylcysteine in acetaminophen intoxication is due to early glutathione replenishment via stimulation of anaplerotic mechanisms.

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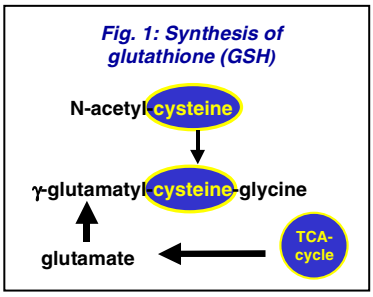
Introduction.

Acetaminophen (AAP) is converted by the cytochrome P450 pathway to a toxic intermediate, N-acetyl-p-benzoquinone-imine (NAPQI), which is usually completely detoxified through combination with glutathione (GSH) [1]. In the event of an overdose, the P450 pathway is accelerated, and glutathione levels begin to deplete [2-4]. Once the pathway consumes approximately 70% of the available glutathione, NAPQI is no longer detoxified and can bind to slufhydryl groups, leading eventually lead to cell necrosis [5]. N-acetylcysteine (NAC), the most widely used antidote in acetaminophen intoxication, is believed to act by regenera-ting glutathione (GSH) stores [3]. NAC is a delivery form of L-cysteine, which serves as a major precursor and rate-limiting amino acid to the antioxidant GSH. BUT: GSH synthesis depends not only on the availability of cysteine, but also on the provision of glutamate, which is regulated by Krebs cycle associated metabolic pathways (Fig. 1).



Aim.

Our aim was to identify the effect of NAC on the ^{13}C-carbon turnover through different metabolic pathways associated with the Krebs cycle in mouse liver in relation to a) liver injury, b) liver GSH stores and c) GSH *de novo* synthesis via different associated metabolic pathways after AAP intoxication.

Methods.

Animal model. Fasted BALB/C mice were injected with 300 mg/kg AAP (i.p.), which causes massive non-lethal liver injury within 6 h. Mice were sacrificed between 0.5 and 12 h. Other groups were treated with NAC (300 mg/kg) 0 - 9 h after AAP injection. [U-^{13}C]glucose, [2,3-^{13}C]acetate, [U-^{13}C]acetate and [U-^{13}C]glutamine (each 2.2 mMol/kg) were injected 30 min before sacrifice to investigate metabolic pathways associated with the Krebs cycle and anaplerosis simultaneously. The mice were killed by cervical dislocation. The livers were freeze-clamped. ***Extraction.*** Tissue samples were powdered over liquid nitrogen and homogenized in 5% perchloric acid (PCA) at 0°C [3]. To obtain lipid extracts from the same tissues, the pellets were extracted with $CHCl_3$/CH_3OH. 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 ^{1}H-NMR analysis of the blood. ***NMR analysis***. After lyophilization, the samples were redissolved in 0.5 ml and centrifuged. 1D ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on Bruker spectrometers DRX 600 or AVANCE-NB/WB 360. 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. Standard enzymatic tests and Western Blot analysis were performed for the analysis of serum ALT, caspase-8 and 3 activities and Bid (tBid) protein expression.

Results.

AAP caused maximal increases in serum ALT values at 3 h to 2681 U/L. NAC decreased ALT values following AAP injection by 58 ± 7.0%, when given at t = 0, but by only 17 ± 1.8% when given 3 h after AAP treatment. AAP caused decreased GSH levels to 12 ± 0.9% of controls after 1.5 h, which partly recovered to 32 ± 6.3% after 3 h. NAC completely prevented GSH depletion at 3 h, whin given at t = 0, but only by 54 ± 10.2% when given 1.5 h after AAP injection. Using [U-^{13}C]glucose, the pathways through PC (pyruvate carboxylase) and PDH (pyruvate dehydrogenase), contributing to the *de novo* synthesis of GSH, were investigated. [^{13}C]GSH synthesis from [U-^{13}C]glucose decreased early after AAP treatment, whereby this was due to decreased PC flux rather than due to changes in PDH ($p<0.001$). NAC only partially prevented the decreased PDH-mediated GSH synthesis at t = 3 h. However, NAC treatment completely prevented the decrease in PC-mediated GSH synthesis at 3.0 h, when given at t = 0, but not when given at t = 1.5 hours. Thus, anaplerosis was activated by NAC, but the final activation of whole Krebs cycle metabolism via anaplerosis was dependend on PDH. Succinate is an anaplerotic substrate. After entry into the Krebs cycle, [2,3-^{13}C]succinate is metabolized via Krebs cycle metabolism to oxaloacetate and subsequently to glutamate and GSH. Early after AAP treatment, the anaplerotic supply of succinate was considerably stimulated, which contributes to [2,3-^{13}C]GSH *de novo* synthesis. NAC did not further change [2,3-^{13}C]succinate metabolism. [1,2-^{13}C]acetate is not an anaplerotic substrate and has to condense with oxaloacetate to replenish Krebs cycle intermediates. After [1,2-^{13}C]acetate metabolism through the Krebs cycle, [4,5-^{13}C]GSH is formed. Different to the metabolism of succinate, [1,2-^{13}C]acetate metabolism did not change after AAP treatment. However, while NAC did not further change [2,3-^{13}C]succinate metabolism, it significantly stimulated [1,2-^{13}C]acetate metabolism to GSH, possibly due to stimulation of PC and therefore supply of oxaloacetate. Detailed ^{13}C isotopomer analysis of GSH and Glu showed specific early changes in Krebs cycle activity that occurred before the increase in GSH synthesis. In particular, stimulation of Krebs cycle flux arised through an enhanced provision of acetate/acetyl-CoA which was observed 0.5 h following concomitant NAC and AAT injections. This was then followed by condensation of acetyl-CoA with oxaloacetate enhabling replenishment of Krebs cycle intermediates (anaplerosis) and subsequent catabolism of succinate. This was later followed and linked to the increase in the *de novo* synthesis of GSH.

Summary/Conclusion.

After AAP treatment, a rapid depletion of GSH stores parallels a considerable inhibition of flux through PC. Furthermore, Krebs cycle metabolism after anaplerotic entry of succinate is stimulated early after AAP treatment. On the other hand, Krebs cycle metabolism of acetate, which can not serve as an anaplerotic substrate, is unchanged after AAP treatment, but stimulated by NAC, possibly due to stimulation of PC and provision of oxaloacetate. In conclusion, these data suggest that anaplerotic fluxes play a key role in the degradation and replenishment of GSH in mouse liver. We suggest that concomitant administration of anaplerotic substrates entering the Krebs cycle could promote the action of NAC on GSH synthesis and might counteract against feedback inhibition early after AAP intoxication.

References.

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