Xenobiotic-mediated redirection of glucose flux through the D-glucuronic acid pathway in animal liver and isolated hepatocytes

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Introduction and Aim

The biosynthetic pathway for vitamin C (ascorbic acid) has been retained by most vertebrates with the exception of a few including humans [1]. With the exception of gulono-γ-lactone oxidase (GLO), all enzymes participating in ascorbate biosynthesis are active in all vertebrates. Ascorbate biosynthesis in vertebrates is carried out by the D-glucuronic acid pathway, which is an essential pathway for the detoxification of toxic compounds such as acetaminophen (APAP) by glucuronidation. Among a variety of compounds [2,3], a xenobiotic-mediated induction of the D-glucuronic acid pathway has been known for many years; however, its cellular implications have not been elucidated. We were particularly interested, how the ascorbate synthesis pathway is related to hepatocellular glucose- and glycogen metabolism.

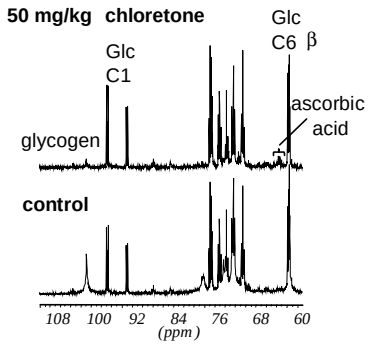
Methods

Isolation of rat hepatocytes. Rat hepatocytes were isolated using collagenase perfusion method [4]. Upon anaesthetization with pentobarbital (Somnitol), the portal vein was cannulated and perfused with Hanks buffered salt solution (HBSS) containing 0.65g/ml of bovine serum albumin (BSA). The perfusate was changed to HBSS containing collagenase (type IV). Hepatocytes were washed and resuspended into KBSS and incubated in continuously-rotating round bottom flasks at an atmosphere of 95%O_2,5% CO_2 at 37°C. ***HPLC analysis.*** Ascorbate content was measured in rat hepatocytes by HPLC with amperometric detection as described previously [4]. APAP and its metabolites were extracted with metaphosphoric acid 0.15%. The reduced (GSH) and oxidized from (GSSG) of glutathione in isolated rat heptocytes were analyzed by an HPLC method as described previously [4]. ***NMR spectroscopy.*** For NMR experiments, mice were intravenously injected with [1-^{13}C]- or [U-^{13}C]glucose (500 mg/kg) concomitant to the administration of chloretone (50 mg/kg) or vehicle (corn oil). Isolated hepatocytes were treated with 2.5 mM [U -^{13}C]glucose or [1-^{13}C]glucose concomitant to APAP and/or chloretone addition. Liver tissues and cells were freeze-clamped or snap frozen in liquid nitrogen, respectively, and metabolites were extracted in 2% perchloric acid (PCA) [5]. The lyophilized samples were dissolved in D_2O. Metabolite concentrations were calculated from ^{1}H-NMR spectra; the ^{13}C-enrichments were calculated from ^{13}C-NMR spectra [5]. 1D ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on Bruker spectrometers DRX 600 or AVANCE-NB/WB 360. Gradient selected 2D-NMR inverse homonuclear (COSY) and heteronuclear (HSQC) correlations were applied to verify chemical shift data.

Results

1) Effects of chloretone on ascorbate synthesis in isolated rat hepatocytes. ^{13}C-labelled ascorbate was detected as labeled on carbon 6 due to the inversion pathway during ascorbate biosynthesis from [1-^{13}C]glucose. Chloretone (100 – 500 µM) was found to time-dependently elevate cellular ^{13}C-labelled ascorbate up to 22-fold (after 7 h) of controls. Ascorbate synthesis occurred almost instantaneously as a significant 1.5 fold increase was observed 15 minutes following chloretone administration. The increase in ascorbate occurred linearly thereafter. The stimulating effect of chloretone on ascorbic acid synthesis was maximal at a concentration of 100 µM at which the concentration of ascorbate reached 45 nmol/10^6 cells by 3 hours. Interestingly, the chloretone-mediated dose-dependent increase in ascorbate synthesis (EC_{50} = 12 µM) was similar to the chloretone-mediated dose-dependent inhibition of acetaminophen glucuronidation (IC_{50}=20 µM). ***2) Effect of chloretone on glycolysis and gluconeogenesis.*** Chloretone treatment (500 µM) significantly decreased the synthesis of [3-^{13}C]lactate from [1-^{13}C]glucose to 67±4.3 % of controls. The formation of [6-^{13}C]glucose from [1-^{13}C]glucose (gluconeogenesis) decreased to 47±0.7% of controls within 2 h. Furthermore, chloretone treatment decreased the glycogen content in isolated hepatocytes to 68±10% of controls within 2 h, which further decreased to 41.2±8.7% of controls after 7 h. However, changes in glucose levels were not observed with chloretone. This was compared with the effect of glucagon, which do not stimulate ascorbic acid synthesis, but rapidly stimulated glycogenolysis and glucose synthesis from glycogen. **3) *Ex vivo* ^{13}C-analysis of ascorbate synthesis and glycogenolysis in mice.** Chloretone (50 mg/ml; i.p., 1 h) significantly increased the synthesis of ^{13}C-labelled ascorbate, which was hardly detectable in the liver of controls, from [U-^{13}C]glucose. A significant reduction in the concentration of ^{13}C-labelled glycogen indicated to an inhibition of glycogen synthesis from glucose (Fig. 1).

Fig. 1. Segments of ^{13}C-NMR spectra of mice liver extracts



Conclusions

The results of the present study show that the dose-dependent effect of chloretone in stimulating ascorbate synthesis in isolated rat hepatocytes was directly associated with its ability to inhibit acetaminophen glucuronidation. Furthermore, this NMR analysis showed that chloretone stimulates ascorbate synthesis through a redirection of glucose metabolism into the D-glucuronic acid pathway.

References

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