XYLITOL METABOLISM AND OXALATE SYNTHESIS IN THE RAT

A Thesis submitted by

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SUMMARY

The intravenous administration of xylitol to patients in the Royal Adelaide Hospital resulted in several adverse metabolic effects, including the increased production of lactic and uric acids, and the deposition of oxalate crystals in the kidneys and brains. The unexplained nature of this latter effect has culminated in this investigation, which has been conducted at the cellular level using the rat as an experimental model. This thesis contains three major areas of investigation:

1. The comparative metabolism of xylitol and sorbitol in isolated rat hepatocytes.

The hepatic metabolism of xylitol was compared with other carbohydrates, including sorbitol and fructose. In many respects the utilisation of xylitol was similar to that of sorbitol, with the initial dehydrogenation step being a major determinant in the degree of metabolism of both polyols. The increased NADH levels accompanying this step resulted in a shift in the cellular redox couples to a reduced state, leading to increased lactate and glycerophosphate production. This latter phenomenon was not observed in comparative studies with D-fructose and D-xylulose. The increased production of glycerophosphate during xylitol and sorbitol metabolism appeared to place greater emphasis on the glycerophosphate hydrogen shuttle, particularly with xylitol as substrate. A marked stimulation of polyol metabolism was observed in the presence of the artificial electron acceptor, phenazine methosulphate. Facilitation of hydrogen flux through the glycerophosphate shuttle was one postulated effect of this compound. Inhibition of the malate aspartate shuttle with the transaminase inhibitor, amino-oxyacetat indicated that this route of hydrogen transfer into the mitochondria is also significant in polyol metabolism, with more hydrogen from sorbitol than xylitol being transferred by this mechanism. Although the differences in the metabolism of xylitol and sorbitol were not great, it can be suggested that as xylitol causes a greater accumulation of glycerophosphate than sorbitol, then the binding of intracellular phosphate, the decrease in adenine nucleotide levels, and the associated increase in uric acid production may be more pronounced during xylitol metabolism.

2. The mechanism of oxalate synthesis from the immediate precursors glycollate and glyoxylate.

Hepatocytes were found to effectively produce oxalate from the major precursors, glycollate and glyoxylate. Inhibition studies revealed that glycollate oxidase rather than lactate dehydrogenase was the most significant enzyme in hepatic oxalate synthesis. An oxidised cellular redox state was found to promote oxalate production from both precursors. The accepted oxidative
sequence of glycollate $\rightarrow$ glyoxylate $\rightarrow$ oxalate was not supported by studies with hydroxypyruvate and the transaminase inhibitor, amino-oxyacetate, for glycollate produced more oxalate than glyoxylate. Subcellular investigations provided further evidence that the peroxisomal enzyme, glycollate oxidase, is the major enzyme of hepatic oxalate synthesis. However, due to differences between physiological systems and purified enzymes, it is not possible to exclude the participation of lactate dehydrogenase in oxalate synthesis. A compartmentalised model involving both these enzymes in hepatic oxalate synthesis is proposed with peroxisomes as the major site of hepatic oxalate synthesis. Experiments with clofibrate, a drug which causes peroxisomal proliferation, showed variable effects in vivo and in vitro, the most interesting being a reduction in oxalate synthesis from glycollate in vitro. This potentially useful experimental approach awaits further investigation.

3. Oxalate production from xylitol and other carbohydrates.
Labelled xylitol was shown to produce oxalate in vivo and in vitro, an effect which was enhanced by pyridoxine deficiency. In rat hepatocytes, a xylitol concentration of 1 mM was found to be optimal for oxalate synthesis. As seen with the major oxalate precursors, an oxidised cellular redox state facilitated oxalate synthesis from xylitol. This effect was demonstrated with the artificial electron acceptor phenazine methosulphate, which has the dual effect of enhancing xylitol metabolism, and increasing carbon flux through the oxalate biosynthetic pathway. Isotope dilution studies and in vivo labelling experiments have identified glycollate as a key intermediate in the pathway from xylitol to oxalate.

A major finding in these investigations was that at a concentration of 1 mM, in hepatocytes; xylitol produced more oxalate than any of the other carbohydrates examined. This finding has increased significance when viewed in light of the similarity in the metabolism of xylitol and sorbitol. Evidence for three mechanisms of oxalate synthesis from xylitol are discussed. The first involves the production of an oxalate precursor from a glycolaldehyde - thiamine pyrophosphate intermediate in the pentose phosphate pathway; the second postulates the action of aldolase on a fortuitously formed xylulose-1-phosphate and the third mechanism concerns reactions of the glucuronic acid cycle which are involved in oxalate synthesis from ascorbate. The first hypothesis remains the most tenable, for the second is untested and the third is not supported by data from isotope labelling experiments.