The establishment of a model system in which variants of the medium-chain ligase (ACSM) and glycine N-acyltransferase (GLYAT) can be co-expressed and analysed

Pieter Lourens

North West University, Potchefstroom Campus

The glycine conjugation pathway detoxifies xenobiotics and utilizes medium-chain fatty acidsin mitochondrial β-oxidation as an additional energy-producing pathway. Considering the increasing modern-day usage and consumption of xenobiotics, it is not clear why this pathway has not received much attention for accurate characterization of the respective enzymes (ACSM2B and GLYAT).

This study combines molecular and metabolomic analyses to characterize the effectivity of GLYAT *in situ* by expressing GLYAT in a bacterial system and measuring the amount of substrate converted to the respective product after a specific time using GC-MS. GLYAT was expressed in the C41(DE3)pLysS cell line using IPTG induction. The induced cells were split into two aliquots. The first aliquot was used for SDS-PAGE and western blot confirmation of the expressed enzymes, and the second aliquot for enzyme activity analysis using the GC-MS.

The Coomassie brilliant blue stained gel and the western blot both indicated that GLYAT was successfully expressed in the C41(DE3)pLysS cells in soluble form. Reactions were set up with Tris-HCl (25 mM, pH 8.0), glycine (10 mM), Benzoyl-CoA (80 µM) and soluble cell lysate to a 200 µL total reaction volume and were left at room temperature for 20 minutes and stopped by adding ice-cold methanol. A negative control contained water instead of soluble cell lysate with GLYAT.

The samples were prepared for GC-MS analysis by drying and derivatization. GC-MS was performed to detect the decreasingsubstrate (Benzoyl-CoA) and increasing product (Hippuric acid). The GC-MS analysisindicated a clear increase in hippurate concentration than the negative control that did not contain GLYAT. Interestingly, this technique may exclude the purification steps in future qualitative studies to determine the conversion reaction of a focused metabolic pathway and accelerate result output.

Keywords: Xenobiotics, ACSM2B, GLYAT, GLYATL1, GC-MS, glycine conjugation, benzoate,hippuric acid.

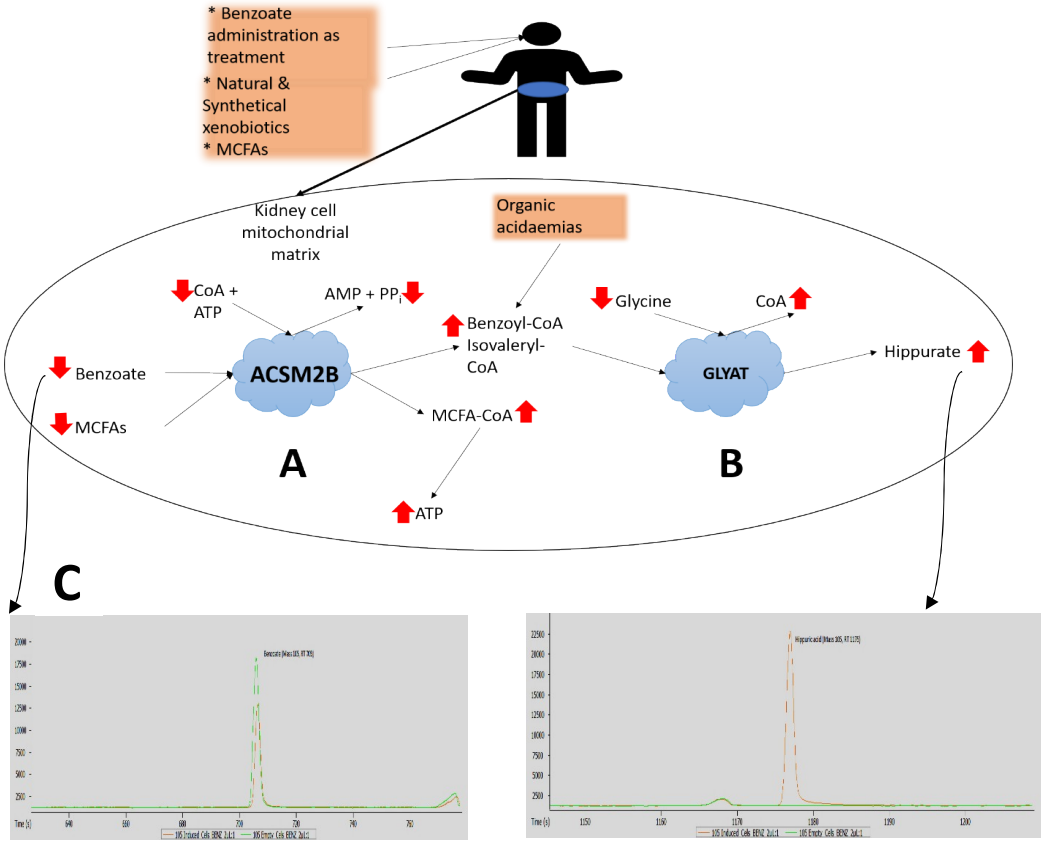

Figure 1: A and B represents a normal functioning glycine conjugation pathway indicating the changes in substrate and product levels that occur, whereas, C represents the changes measured between the reactions containing GLYAT (Orange line) and those not containing GLYAT (Green line). The GC-MS images show a clear decrease in benzoate and increase in hippurate when compared to the negative control.