Detection of serine isotopomers as a measure of mitochondrial function

Introduction: The glycine cleavage system, found loosely associated with the mitochondrial inner membrane (1), is responsible for the majority of glycine catabolism in mammals. It converts the 1-C of glycine into CO2 and transfers the 2-C to tetrahydrololate (THF), forming methylene THF (mTHF). The formation of mTHF in the mitochondria is tightly coupled to mitochondrial serine hydroxymethyltransferase (mSHMT) activity (2), a reaction that transfers the methyl group obtained from glycine onto a second glycine to form serine. A similar reaction also occurs in the cytosol, catalyzed by cytosolic SHMT (cSHMT). Pasternack et al. first exploited this compartmentation in yeast, feeding the cells labeled 2-13C glycine and observing the pattern of serine isotopomer formation (3). Since GCS is restricted to the mitochondria, serine can only be labeled in the 3 position from 2-13C glycine and if it is formed in the mitochondria (Fig 1). As a result, by monitoring formation of serine labeled at the 3 position, it is possible to assess GCS activity. Since GCS activity is stimulated by an increased NAD+/NADH ratio (4), hormonally activated by glucagon (5), and absent in diseases such as non-ketotic hyperglycemia (NKH), we hypothesize that analysis of differences in the resulting pools of 2-C serine, the cytosolic pool, and 3-C serine, the mitochondrial contribution, can be used as a non-invasive, in vivo detection system of mitochondrial function.

Methods: Rat liver cells were isolated using a standard collagenase digestion protocol as described by (6). The hepatocytes, viability > 85%, were then resuspended to a density of 2.0 million/mL in plating media (DMEM high glucose with L-glutamine and pyruvate plus 10% FBS, 100U penicillin/streptomycin, 140mM insulin, and 1mM dexamethasone) and 10mL was plated onto collagen-coated 145cm2 plates. After 1-2 h the media containing unattached/dead cells was removed and replaced with fresh plating media (37°C). After overnight incubation at 37°C, 5%CO2, hepatocyte plating media was then replaced with 10mL of appropriate test media for one hour, and then spiked with 5 mM 2-13C glycine for two hours. At this point, two plates were extracted using a 1:1:1 methanol:water:chloroform extraction method and protein was measured using the Bradford assay. Mixture was then shaken vigorously, allowed to separate overnight, lyophilized and pellet was dissolved in phosphate-buffered D2O containing 1mM TSP and 2.5mM 13C, 15N formamide, pH 8.0. The 13C spectra were obtained using an 11.7T Varian (Palo Alto, CA) INOVA equipped with a 5 mm broadband probe at 25°C. The SW = 32K Hz, AT= 2 sec, and D1 = 2 sec, and 1H decoupling was performed with a WALTZ 16 during acquisition. All spectra were normalized to formamide and peaks were fitted using ACD software.

Results: Figure 2 presents the characteristic serine isotopomers formed from 2-13C glycine treatment. The chemical shifts of the 2 and 3 positions of serine were 56.6 and 60.4 ppm, respectively. Figure 3 presents the integrated areas of the isotopomers. As proof of concept, the potent GCS inhibitor cysteamine, used at 500µM, prevents formation of mitochondrial serine isotopomers, the 2,3- and 3-13C serine isotopomers, and decreases 2-13C serine by 65%. Treatment with 5µM carbonyl cyanide m-chlorophenylhydrazone (CCCP), a mitochondrial uncoupler that increases the NAD+/NADH ratio, results in a 36% increase in 3-13C serine, a 52% increase in 2,3-13C serine, and a 23% increase in 2-13C serine compared to control. Treatment with 100mM ethanol, which causes accumulation of NADH, decreased these isotopomers by 18%, 26%, and 6%, respectively. Additionally, 100mM glucagon caused an increase in isotopomers similar to CCCP.

Discussion and conclusion: These results present new insight into the expanded use of MRS to probe mitochondrial function. This data, obtained from 2-D hepatocyte cultures, combined with previous in vivo work from our lab (Fig 4) (7), suggests that monitoring serine isotopomers after 2-13C glycine infusion is a novel research tool to probe mitochondrial function. Additionally, we also speculate that this approach may be clinically relevant in diagnosing NKH, which currently requires a liver biopsy to definitively diagnose (8).

REFERENCES:

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