[U-^{13}C] Glutamine as a Tracer to Study Hepatic Metabolism

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Clayton Olinger,[1] Mukundan Ragavan,[2] Anthony Giacalone,[2] and Matthew Merritt[2]

[1]Department of Biology, [2]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

Introduction

Several liver diseases and disorders have strong metabolic components associated with them. The use of isotopic tracers is an established approach to assess metabolic alterations or to obtain basal activities. Here, we test the possibility of using [U-^{13}C] glutamine as a tracer to study hepatic metabolism. We compare the results obtained with those obtained using [U-^{13}C] propionate, an established tracer for liver metabolism. Previous work questioned propionate as a suitable tracer, due to its low inherent concentration in the blood. Glutamine should not be subject to these objections.

Methods

All procedures were approved by the IACUC at University of Florida. C57/BL6 mice were fasted overnight. Each mouse was injected with heparin 15 minutes prior to the start of surgery. Mice were anesthetized using isoflurane and an incision was made along the midline of the abdomen. Livers were perfused through the portal vein (Figure 1) with Krebs-Henseleit buffer containing 1.5% (w/v) bovine serum albumin, 10%(v/v) D_2O, [U-^{13}C] glutamine or propionate, mixed fatty acids, lactate and pyruvate (lac:pyr is 10:1). Perfusate is constantly oxygenated using 95%/5% O_2/CO_2 mixed gas. The liver was cannulated and perfusate flow was started. Cannulated livers are then removed, cleaned and attached to a glass column and perfused for 30 minutes. Efferent perfusate was collected during the perfusion.

Glucose was extracted by adding perchloric acid (concentration 6%(v/v) final) to the efferent perfusate. The solution was centrifuged, and supernatant was dried. Glucose was converted to monoacetone glucose (MAG) by the addition of sulphuric acid and acetone as described in the literature (3). MAG was purified using a C-18 column, and ^{2}H NMR spectra were measured on a 14.1T NMR spectrometer.

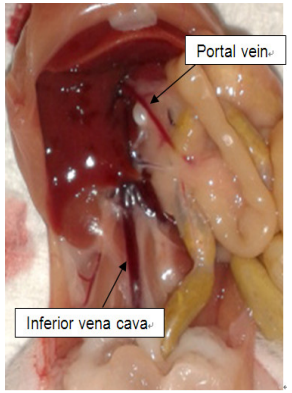

***Figure 1**: Photograph taken during the surgery showing the portal vein and inferior vena cava. Liver is perfused through the portal vein.*

Results and Discussion

Glucose present in efferent perfusate can originate from different sources including glycogen breakdown and gluconeogenesis. Since the perfusate contains 10% (v/v) D_2O, specific positions on glucose will be ^{2}H enriched. Representative ^{2}H NMR spectra of MAG are shown in Figure 1. Clearly, glucose production from glutamine perfused livers is similar to propionate perfused livers. Metabolic sources of glucose present in the efferent perfusate can be estimated using peak areas from ^{2}H spectra and the following equations (1) -

$$glycogen\ contrib. = 1 - \frac{H5}{H2}$$

$$glycerol\ contrib. = \frac{H5 - H6_S}{H2}$$

$$Krebs\ cycle\ contrib. = \frac{H6_S}{H2}$$

Based on the peak areas (Figure 3), glucose from glycogenolysis varied between 18 – 72% of total, suggesting incomplete fasting of mice. Consequently, Krebs cycle contribution to gluconeogenesis also varied from 18 – 65% of total.

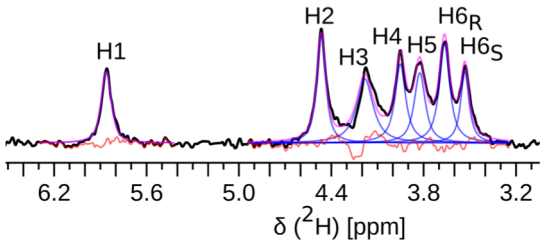

***Figure 3**: Representative ^{2}H NMR spectra showing areas obtained by peak fitting. Blue lines represent fits and red lines represent fit residuals.*

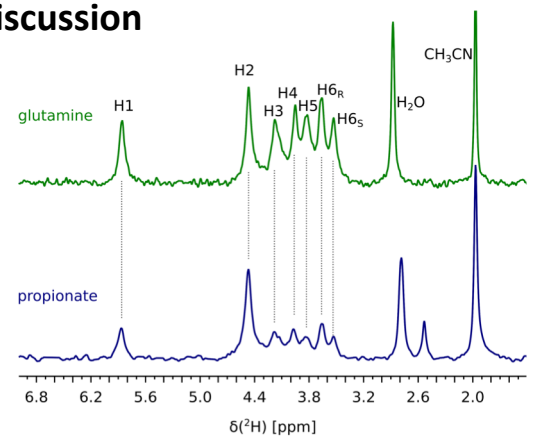

***Figure 2**: Representative ^{2}H NMR spectra of MAG synthesized from perfusions with [U-^{13}C] Glutamine (top) and [U-^{13}C] propionate (bottom). MAG resonances are indicated – H1 … $H6_S$*

Conclusions

- [U-^{13}C] Glutamine works as a tracer that is comparable to the more established [U-^{13}C] propionate
- Experiments are in progress to achieve robust fasting of mice by utilizing cage grids

References

1. Merritt, ME, et. al, MRM (2003)
2. Previs, SF and Kelly, DE. AJP-Endo (2015)
3. Burgess, SC, et. al, Anal. Biochem (2003)

Acknowledgements

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The Big Picture

- Track basal metabolic activities or metabolic alterations using isotopic tracers
- We test [U-^{13}C] glutamine's effectiveness in tracing these metabolic activities.
- Compare to [U-^{13}C] propionate, an established tracer for liver metabolism

Methods

- C57/BL6 mice were fasted overnight
- Mice were then anaesthetized, and an incision was made by the midline.
- Perfusate used has krebs buffer, [U-^{13}C] glutamine, mixed fatty acids, and lactate and pyruvate.
- Liver cannulated , attached to a column and perfusate was run through the portal vein.
- Efferent perfusate collected
- Glucose extracted from perfusate, converted to MAG and then ^{2}H NMR was collected.

Results and Discussions

- The glucose we extracted can come from different sources including glycogen breakdown and gluconeogenesis.
- Using ^{2}H NMR we can see glucose production using glutamine is similar to tracking it with propionate.
- Using three separate equations we can quantify the metabolic sources of glucose.

Conclusions

- [U-^{13}C] Glutamine works as a tracer that is comparable to the more established [U-^{13}C] propionate