Liver Flux Profiling (LFP) by NMR Analysis of Glucuronide from Urine

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ABSTRACT Administration of the stable isotope tracers [U-^{13}C]propionate and deuterated water followed by the analysis of plasma by NMR yields important information about liver metabolism. This approach is not satisfactory in patients for whom blood sampling is difficult or unacceptable. We demonstrate here the isolation of acetaminophen-glucuronide from the urine and conversion to the monoacetone derivative. Analysis by ^{2}H and ^{13}C NMR gives a unique liver flux profile (LFP). This procedure is completely noninvasive (other than oral tracer) and yields more material than a typical blood draw.

INTRODUCTION Recently, NMR and Mass spectroscopic (MS) studies of human plasma glucose following administration of deuterium oxide (D_2O) and ^{13}C tracers have shown that relative deuterium enrichment of the glucose H2, H5 and H6 can be used to measure relative contributions to gluconeogenic flux (1-3). This assay is an extraordinary tool for studying metabolically active diseases such as hepatitis, diabetes, and other inborn errors of metabolism. Typically, NMR studies of this type involve extracting glucose from blood draws of 30 mL or more, which is a major disadvantage when studying infants, the elderly, unstable or severely anemic patients. An alternative method for sampling the glucose pool is through the use of a chemical biopsy agent like acetaminophen, which is excreted in the urine as the acetaminophen-glucoronide. MS has been used to obtain metabolic information from urinary glucuronides with much success (4) but NMR isotopomer analysis of these compounds has been limited to ^{13}C applications.

The reason for the limited analysis of acetaminophen-glucoronide by NMR is its poor chemical shift dispersion in ^{1}H, ^{2}H and ^{13}C dimensions. We have previously shown that ^{13}C isotopomer data can be obtained after hydrolysis of the glucoronide to glucuronic acid (2). However, ^{1}H and ^{2}H dispersion still remain a difficulty. The 1,2-isopropylidene glucofuranose (MAG) derivative on the other hand has excellent dispersion in all dimensions. We report here an efficient chemical method to convert urinary acetaminophen-glucoronide to MAG. This method not only provides the needed chemical shift dispersion for the glucose resonances, but also yields a sample that is spectroscopically clean.

METHODS *Materials.* [U-$^{13}C_3$] propionate (99%) and D_2O (70%) were purchased from Cambridge Isotopes (Andover, MA). Acetaminophen (500 mg tablets) was purchased over the counter. DSC-18 Solid phase extraction (SPE) gel was obtained from Supelco (St. Louis, MO). Other common materials were obtained from Sigma (St. Louis, MO).

Experimental protocol. Volunteers were studied under a protocol approved by the institutional human studies committee. Volunteers fasted from 8 pm to 8 am overnight before coming to the clinic. After arriving, they were given a oral bolus of 50% D_2O to enrich body water to 0.5%, 10 mg/kg [U-$^{13}C_3$]propionate, and 1 g of acetaminophen. Plasma and urine samples were collected three hours after initial ingestion of isotopes.

Purification and chemical conversion. Urine was immediately treated with acetone (to 20%) and allowed to stand for 1 hour to disinfect the sample. Acetone was removed by evaporation; the sample was reconstituted in water and treated with urease. The pH of this solution was ultimately adjusted to 4.5 and the sample was lyophilized to a powder. This material was dissolved in 10 ml of water and applied to a 30 g column of C-18 Solid Phase Extraction (SPE) resin. Pure acetaminophen-glucuronide was eluted from the column with a 10% methanol/water solution and lyophilized to dryness.

The purified glucuronide was dissolved in 50 mL of dry methanol and 300 µl of methanol-trifluroborane was added drop wise over several minutes and stirred overnight. To this solution, 1.2 g of sodium borohydride was added in increments over 3 hours. The reaction mixture was evaporated, reconstituted in water and stirred with 10,000 U of β-glucosidase. The resulting glucose was converted to MAG for NMR analysis as previously reported (1).

RESULTS AND DISCUSSION The purification of acetaminophen-glucuronide and its conversion to glucose gave approximately a 60% yield. This corresponds to ~50 mg of pure glucose, a significant improvement over the ~10-15 mg obtained from a single blood draw, which translates into a significant savings in NMR scan time.

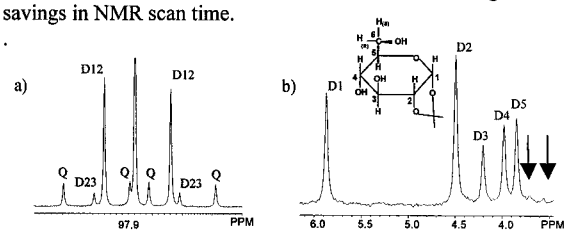

Figure 1. a) ^{13}C NMR spectrum of MAG C2. Multiplet ratios define metabolic parameters (3). b) ^{2}H spectrum of MAG. The arrows point to the position where $D6_{(R)}$ and $D6_{(S)}$ would be.

The data from ^{2}H spectra (Figure, b) provided the contribution of glycogenolysis to gluconeogenesis. In this group, 55 ± 9 % of glucose was derived from glycogen and the remainder was generated through gluconeogenic pathways. A potential disadvantage of glucose derived from glucuronides is that ^{2}H data from position 6 (which yields information about citric acid cycle contribution to gluconeogenisis) is lost when that carbon is oxidized *in vivo*.

The ^{13}C spectra (Figure, a) provided information about pyruvate recycling (2, 3). In this group of volunteers, anaplerosis was 5.3 ± 2.1 times citric acid cycle flux, and reentry of carbon skeletons into the citric acid cycle (oxaloacetate to pyruvate to oxaloacetate) was 3.7± 1.3 times citric acid cycle flux. Gluconeogenisis relative to citric acid cycle flux was 2.0 ± 1.0. These values agree with earlier (2-3) analysis blood glucose.

Urine collected after administration of acetaminophen and stable isotope tracers yielded ample glucose for LFP analysis. NMR-LFP of urine may, in some instances, be a suitable alternative to plasma glucose, which greatly increases the potential population on which this important test can be performed.

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