Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 571–573.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1281

JLCR

Short Research Article

Phenotype breath tests for personalized medicine†

ANIL S. MODAK*
Cambridge Isotope Laboratories Inc., Andover, MA 01810, USA

Received 21 June 2006; Revised 26 December 2006; Accepted 20 January 2007

Keywords: ^{13}C-labeled compounds; personalized medicine; breath tests

Introduction

Stable isotope ^{13}C-labeled compounds have been widely used as diagnostic probes in research laboratories for over 30 years. With the wide availability of low-cost ^{13}C-substrates and the development of bench top nondispersive isotope-selective IR spectrometers, scientists from around the world have increasingly used these tracers in nutritional, medicinal, and veterinary research.

The feasibility of administering an oral dose of a ^{13}C-substrate and procuring metabolic or diagnostic information from its metabolism and conversion to $^{13}CO_2$ is attractive due to its noninvasive nature. The salient features of the ^{13}C-breath test are that they are noninvasive, nonradioactive, safe, simple, and effective. Breath tests have the potential to be useful screening methods that can be applied at the point of care and do not require patients to wait for hours or days for results. The noninvasive method is especially attractive for infants, children, pregnant and lactating women, seniors in poor health, and subjects averse to the use of needles. The simplicity of the ^{13}C-breath test makes it very applicable in a clinical setting: the physician can obtain valuable diagnostic information by distinguishing between two groups or populations based on the recovery of $^{13}CO_2$ from the ingested ^{13}C-substrate.[1]

Several reviews on the clinical applications and visions on the future for breath tests have appeared in the scientific literature over the last 5 years. Despite over 50 diverse, accurate diagnostic ^{13}C-breath tests over a wide range of clinical applications, physicians have not been routinely utilizing them in clinical practice since only the urea-^{13}C breath test for detection of *H. pylori* has been approved by the FDA.

Phenotype breath tests using ^{13}C-substrates will enable physicians to monitor disease states or detect enzyme deficiencies. As a consequence these tests will afford physicians a tool for individualized therapy – selection of the right drug and the right dose for every individual.

A number of clinically significant *in vivo* phenotype ^{13}C-breath tests can easily make the transition from research to clinical use to personalize medication or monitor disease states or monitor recovery following therapy or surgery.

Results and discussion

2-^{13}C-uracil breath test

The dihydropyrimidine dehydrogenase (DPD)-deficient cancer patients have been shown to develop severe toxicity after administration of 5-fluorouracil. Routine determination of DPD activity is limited by time-consuming and labor-intensive methods. The purpose of this study was to develop a simple and rapid 2-^{13}C-uracil breath test, which could be applied in most clinical settings to detect DPD-deficient cancer patients.

Fifty-eight individuals (50 'normal', 7 partially, and 1 profoundly DPD-deficient) ingested an aqueous solution of 2-^{13}C-uracil (6 mg/kg). $^{13}CO_2$ levels were determined in exhaled breath at various time intervals up to 180 min using IR spectroscopy (UBiT-IR300). DPD enzyme activity and DPYD genotype were determined by radioassay and denaturing high-performance liquid chromatography, respectively.

The mean ($\pm$ SE) C_{max}, T_{max}, δ over baseline values at 50 min (DOB_{50}) and cumulative percentage of ^{13}C dose recovered (PDR) for normal, partially, and profoundly

*Correspondence to: Anil S. Modak, Cambridge Isotope Laboratories, Med Products Development, 50 Frontage road, Andover, MA 01810, USA. E-mail: anilm@isotope.com
†Proceedings of the Ninth International Symposium on the Synthesis and Applications of Isotopically Labelled Compounds, Edinburgh, 16–20 July 2006.

Copyright © 2007 John Wiley & Sons, Ltd.

WILEY InterScience® DISCOVER SOMETHING GREAT

DPD-deficient individuals were 186.4 ± 3.9, 117.1 ± 9.8, and 3.6 DOB; 52 ± 2, 100 ± 18.4, and 120 min; 174.1 ± 4.6, 89.6 ± 11.6, and 0.9 DOB_{50}; and 53.8 ± 1.0, 36.9 ± 2.4, and <1 PDR, respectively. The differences between the normal and DPD-deficient individuals were highly significant (all P's < 0.001).

We demonstrated statistically significant differences in the 2-^{13}C-uracil breath test indices (C_{max}, T_{max}, DOB_{50}, and PDR) among healthy and DPD-deficient individuals. These data suggest that a single time-point determination (50 min) could rapidly identify DPD-deficient individuals with a less costly and time-consuming method that is applicable for most hospitals or physicians' offices.[2]

(^{13}C)sodium bicarbonate breath test (SBT)

Arterial partial pressure of carbon dioxide ($paCO_2$) is commonly evaluated by an invasive test, the arterial blood gas (ABG) analysis. The SBT can potentially estimate arterial $paCO_2$. We studied 55 subjects with respiratory disorders and performed the ABG and the SBT to determine if the SBT can predict hypercapnia. The percentage of $^{13}CO_2$ recovered in exhaled breath at 30 min (PDR_{30}) alone was able to discriminate hypercapnia (> 45 mmHg) with a sensitivity of 92% and specificity of 71% ($p < 0.001$). To evaluate the clinical utility of the SBT as a noninvasive substitute to repeated ABGs, we monitored the progress of seven COPD patients on therapy with both the ABG and the SBT. The PDR_{30} values from the SBT were able to correctly predict improvement or worsening of $paCO_2$ with 100% accuracy. In conclusion, the SBT is a simple test that can be used in clinical practice to detect clinically significant hypercapnia and monitor COPD patients before and after therapy.[3]

Phenylalanine-1-^{13}C breath test (PBT)

Although metabolic response after partial hepatectomy has been well studied in animal models, there are few studies examining restoration of metabolic capacity after right hepatectomy in humans. We used L-[1-^{13}C]-phenylalanine (^{13}C-Phe) administered orally or IV to adult living liver donors and resection patients and measured $^{13}CO_2$ to determine the extent of metabolic impairment and time course of its return. Patients given oral ^{13}C-Phe had approximately 70–90% reduction in $^{13}CO_2$ production compared with baseline 2–3 days after surgery. Patients given IV ^{13}C-Phe had only 40–50% reduction in $^{13}CO_2$ production and recovered their baseline ^{13}C-Phe metabolism much sooner than their oral ^{13}C-Phe metabolic capacity ($P < 0.05$). In some cases oral ^{13}C-Phe did not recover to baseline for as long as 56 days after surgery. Those recovering ^{13}C-Phe metabolism had significantly higher $^{13}CO_2$ recovery 60 min after ingestion by day 4 (0.97 vs 3.06, $P = 0.033$) and day 7 (1.50 vs 5.02, $P = 0.031$). We conclude that orally administered amino acids are not well absorbed and/or metabolized in some subjects for weeks after partial hepatectomy whereas intravenously delivered substrates are much better oxidized by the regenerating liver. These findings may be due to impaired gut motility or portal venous flow that reduces delivery of oral agents after liver surgery. These preliminary findings have wide implications for nutrition and drug delivery in the early recovery phase for living liver donors and liver resection.[4]

Breath tests for detection of enzyme activity of CYP polymorphic enzymes

The CYPs are quantitatively the most important Phase I drug biotransformation enzymes and genetic variation of several members of this gene superfamily results in dramatic interindividual differences in metabolism of substrate drugs. Stable isotope-labeled xenobiotics can be used to evaluate the clearance of drugs in the liver by polymorphic CYP (cytochrome P450) enzymes – 2D6, 2C9, 1A2, 2C19.[5]

^{13}C-levodopa breath test

Optimizing medication regimens for Parkinson's disease: Sinemet, a combination pill of carbidopa (CD) and levodopa (LD) has been the therapy of choice for Parkinson's disease. The dose of the inhibitor Carbidopa in Sinemet at 1:10 and 1:4 ratios of CD:LD is totally inadequate for the complete suppression of peripheral dopamine decarboxylase activity (DDC). ^{13}C-LD can be utilized to evaluate DDC activity and doses of CD optimized for each individual to deliver maximal doses of LD across the blood brain barrier (unpublished results).

REFERENCES

1. Modak AS. ^{13}C Breath tests: transition from research to clinical practice. In *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring*, Amann A, Smith D (eds). World Scientific: Singapore, 2005; 457–478.
2. Mattison LK, Ezzeldin H, Carpenter M, Modak AS, Johnson MR, Diasio RB. *Clin Cancer Res* 2004; **10**: 2652–2659.
3. Modak AS, Irie Y. Use of ^{13}C labelled substance for measuring lung function, US 6,890,305, May 10, 2005 WO 03/072144.

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr

4. Freeman RB, Melanson AM, Dixon M, Palladino MB, Horth B, Cooper J, Rohrer R, Reid JM, Modak AS. *Am J Transpl* 2004; **4**(Suppl. 8): P1051.

5. Modak AS, Reid JM, Kurogi Y. A rapid, non-invasive *in vivo* breath test to evaluate CYP2D6 enzyme activity. *13th North American ISSX Meeting/20th JSSX Meeting*, Maui, HI, October 23–27, 2005.

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr