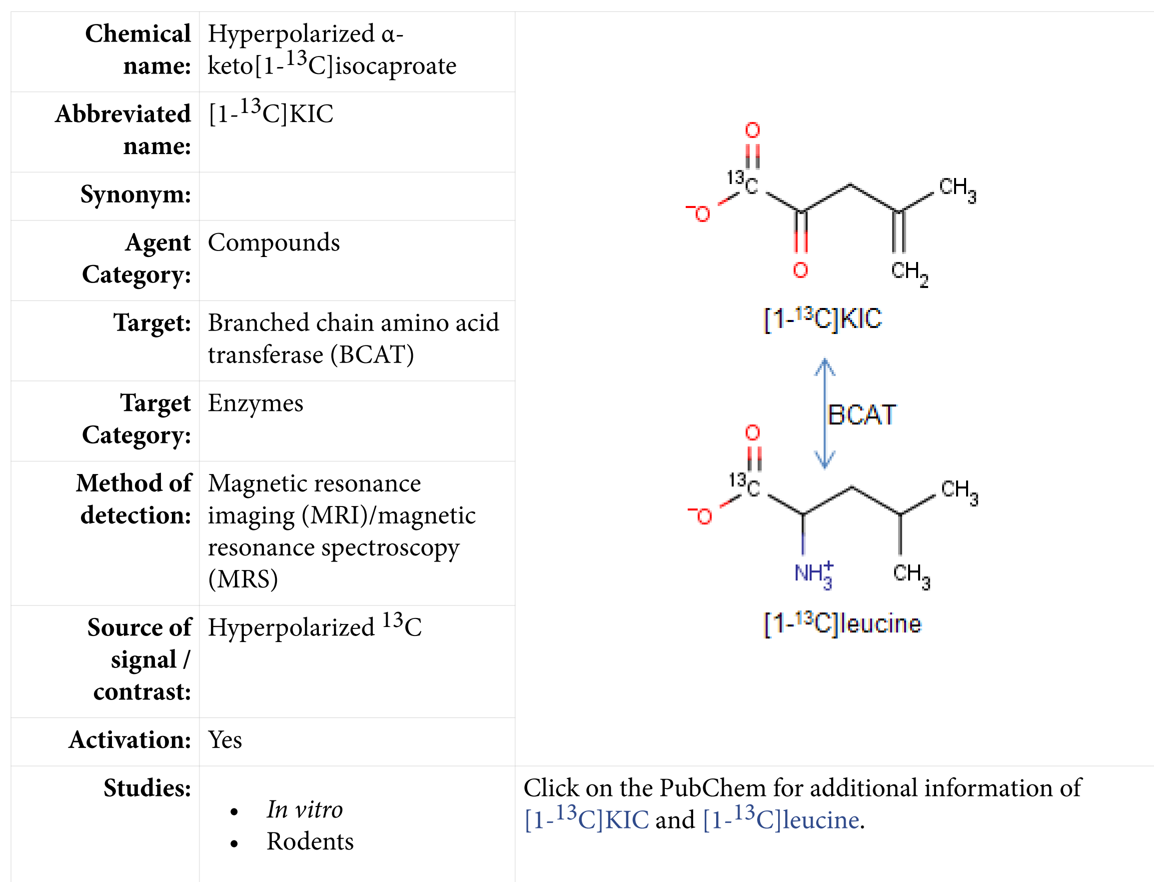
Hyperpolarized α-keto[1-^{13}C]isocaproate as a ^{13}C magnetic resonance spectroscopic agent for profiling branched chain amino acid metabolism in tumors

[1-^{13}C]KIC

Liang Shan, PhD[⊠1]

Created: January 15, 2010; Updated: May 19, 2010.



Chemical name:	Hyperpolarized α-keto[1-^{13}C]isocaproate	
Abbreviated name:	[1-^{13}C]KIC	
Synonym:		
Agent Category:	Compounds	
Target:	Branched chain amino acid transferase (BCAT)	
Target Category:	Enzymes	
Method of detection:	Magnetic resonance imaging (MRI)/magnetic resonance spectroscopy (MRS)	
Source of signal / contrast:	Hyperpolarized ^{13}C	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	Click on the PubChem for additional information of [1-^{13}C]KIC and [1-^{13}C]leucine.

Background

[PubMed]

[1] National Center for Biotechnology Information, NLM, NIH; Email: micad@ncbi.nlm.nih.gov.

⊠ Corresponding author.

Magnetic resonance spectroscopy (MRS) is a technique that allows us to non-invasively detect multiple small metabolites within cells or extracellular spaces *in vivo* (1, 2). The clinical use of MRS as an adjunct to magnetic resonance imaging (MRI), particularly in cancer detection and treatment response evaluation, has expanded dramatically over the past several years. Although MRS is theoretically applicable to any nucleus possessing spin, the most important and more frequently investigated applications are in proton (^{1}H) and carbon-13 (^{13}C) (1, 3, 4). ^{13}C MRS is superior to ^{1}H MRS in many respects (5-7). ^{13}C MRS can provide specific information about the identity and structure of biologically important compounds. The chemical shift range for carbon (~250 ppm) is much larger than that for proton (~15 ppm), allowing for improved resolution of metabolites. In addition, the T_1 relaxation time of ^{13}C in small molecules is much longer than that of ^{1}H (0.1–2.0 s in a magnetic field of 0.1–3.0 T), allowing the generation of hyperpolarized ^{13}C-labeled tracers outside the subject and the MRI scanner. Some of these tracers are endogenous and therefore have lower toxicity than drugs and exogenous contrast media (2). However, ^{13}C MRS is limited by the low natural abundance of ^{13}C (1.1%) and its low magnetogyric ratio (γ of ^{13}C is one quarter that of ^{1}H) (1, 6).

Several techniques have been used to overcome the ^{13}C MRS limitation through enhancing the polarization of nuclear spins. One technique is proton decoupling, which eliminates the coupling of ^{1}H with ^{13}C by irradiating the entire ^{1}H resonance absorption range and consequently collapsing ^{13}C resonances to singlets (8, 9). The signal/noise ratio of ^{13}C resonances has been shown to be significantly increased with proton decoupling. Another technique is known as dynamic nuclear polarization (DNP), which introduces one or more ^{13}C molecules into a metabolic substrate (3, 10, 11). DNP transfers high electron spin polarization to nuclear spins *via* microwave irradiation. Nearly 100% nuclear polarization for ^{1}H and 50% for ^{13}C can be achieved in various organic molecules when DNP is performed in a strong magnetic field and at cryogenic temperatures. Hyperpolarization of protons ex vivo is less interesting for medical applications, because most of the hyperpolarization would have vanished before the molecule reaches the target organ. However, the T_1 of ^{13}C in small molecules, in general, is much longer than the T_1 of protons. A large number of low molecular weight substances with ^{13}C T_1 in excess of 10 s are available. Replacing the ^{12}C isotope (98.9% natural abundance) with the ^{13}C isotope at a specific carbon or carbons in a metabolic substrate does not affect the substrate's biochemistry. With ^{13}C MRS, the body tissues are virtually invisible, and only regions where the hyperpolarized ^{13}C-labeled substance is present will appear in the generated images. Thus, ^{13}C-labeled substrates can provide >10,000-fold enhancement of the ^{13}C MRS signals from the substrate and its subsequent metabolic products, allowing

the assessment of changes in metabolic fluxes *in vivo* through glycolysis, citric acid cycle, and fatty acid synthesis (2, 11, 12). Vascular and perfusion imaging can also be performed without background signal from surrounding tissues (6, 13).

Karlsson et al. generated a hyperpolarized small molecule, α-keto[1-^{13}C]isocaproate ([1-^{13}C]KIC) as a ^{13}C MRS agent for imaging the molecular signature of branched chain amino acid metabolism, which is regulated by the branched chain amino acid transferase (BCAT) (5). There are two isoforms, BCAT1 and BCAT2, that code for mitochondrial and cytosolic BCAT, respectively (5, 14). BCAT is highly expressed in early embryogenesis, and it is also a target for MYC activity during oncogenesis. BCAT has been shown to be a useful marker for grading and genetic characterization of tumors. KIC is a substrate of BCAT and is metabolized to leucine *in vivo*. Karlsson et al. demonstrated that metabolism of the hyperpolarized [1-^{13}C]KIC yielded unprecedented MRI contrast between EL4 murine lymphoma and surrounding healthy tissue, but yielded no contrast between R3230AC rat mammary adenocarcinoma and its surrounding tissue. The [1-^{13}C]leucine signal detected in the two tumor models correlated well with *ex vivo* measurements of the BCAT activity in the two tumor tissues. The investigators concluded that the understanding of metabolic differences between tumors could be advanced with use of the hyperpolarized [1-^{13}C]KIC biomarker in assessing tissue BCAT activity *in vivo* by means of the [1-^{13}C]leucine signal (5).

Related Resource Links:

- Chapters of ^{13}C MRS in MICAD
- BCAT articles in OMIM
- Gene information of BCAT

Synthesis

[PubMed]

Karlsson et al. detailed the generation of hyperpolarized [1-^{13}C]KIC (5). The starting agent [1-^{13}C]KIC, as a sodium salt, was commercially available. [1-^{13}C]KIC was first converted to the acid form by acidifying an aqueous solution of the salt to pH < 1 with sulfuric acid, followed by an extraction of the acidified solution with diethylether. After drying the ether phase with anhydrous magnesium sulfate, the solvent was removed in vacuum. The DNP preparation of [1-^{13}C]KIC was established by dissolving the trityl radical OX063 (tris(8-carboxyl-2,2,6,6-tetra(2-(1hydroxyethyl))-benzo[1,2-d:4,5-d']bis(1,3)dithiole-4-yl)methyl sodium) in the acid, followed by adding trimeric gadolinium 2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododec-1-yl] acetic acid (Gd-DOTA) complex. Addition of the Gd-DOTA complex could enhance the solid state polarization, and the negative effect on the relaxation time constant of the hyperpolarized substance in solution was negligible at the concentrations used. This DNP preparation of [1-^{13}C]KIC was hyperpolarized in a polarizer, and the hyperpolarized sample was subsequently dissolved in an aqueous solution of sodium hydroxide and phosphate buffer

(40 mmol; osmolality was adjusted with NaCl to 210 mOsm) to provide a 20-mmol solution of hyperpolarized [1-^{13}C]KIC at pH 7.4 ± 0.1, an osmolality of 290 ± 10 mOsm, T_1 (9.4 T) of 55 ± 3 s, and a polarization of 32 ± 3% at the time of administration. A dose of 0.175 mmol/kg was infused into animals within 6 s. MRS was started at 20 s after start of the infusion. The total acquisition time was 12 s. A 30% polarization is equal to a signal enhancement on the order of 10^5 relative to the equilibrium ^{13}C spin polarization in the medical scanner of a 3-T magnetic field at 310 K (~0.0003%) (5).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Karlsson et al. measured the BCAT activity in cell cultures of EL4 and R3230AC lines with spectrophotometric assay (5). Significantly high BCAT activity was observed in EL4 (16.6 ± 1.7 U/g protein) as compared with R3230AC cell suspensions (3.7 ± 1.8 U/g protein), which is in agreement with the data from *in vivo* [1-^{13}C]KIC MRS and spectrophotometric assays of *ex vivo* tumor tissues (see below).

Animal Studies

Rodents

[PubMed]

Karlsson et al. first assessed the BCAT reaction with [1-^{13}C]KIC in mice bearing EL4 tumors (n = 5) (5). Upon [1-^{13}C]KIC injection, ample ^{13}C signal was detectable in mouse tissues within a few seconds, both for [1-^{13}C]KIC (δ^{13}C = 172.6 ppm) and its transamination product [1-^{13}C]leucine (δ^{13}C = 176.8 ppm). With the exception of [1-^{13}C]leucine, no other reaction product was quickly detectable. In EL4 tumor, the maximum [1-^{13}C]leucine signal was detected at ~20 s after injection of [1-^{13}C]KIC. The signal/noise ratio of [1-^{13}C]leucine was 13.3 ± 6.3 (mean ± SD) in tumor. The [1-^{13}C]leucine signal was 6.9 ± 1.0 times higher in tumor than the highest [1-^{13}C]leucine signal in surrounding tissues. Overlay of the ^{13}C chemical shift images with the anatomical ^{1}H images showed that [1-^{13}C]KIC migrated to the gastrointestinal tract and tumor within the first 20 s after injection. Both [1-^{13}C]KIC and [1-^{13}C]leucine signals lost the vast majority of their enhanced ^{13}C spin polarization after ~100 s.

To evaluate the influence of [1-^{13}C]KIC uptake on the contrast of [1-^{13}C]leucine signal between tumor and surrounding tissue, Karlsson et al. analyzed the KIC levels in tissues *ex vivo* with an optical test. Total KIC pools before injection were 0.05 ± 0.05 and 0.17 ± 0.05 µmol per gram tissue (µmol/g) for tumor and muscle, respectively, while at 25 s after injection these values increased to 0.67 ± 0.04 and 0.61 ± 0.05 µmol/g (n = 5 for all groups) for tumor and muscle, respectively. Muscle was chosen for comparison with tumor because it was among the tissues with the highest BCAT activity in healthy mammals and thus was a determinant of the tissue contrast of branched chain amino acid metabolism between tumor and healthy tissue. Because BCAT has a low Michaelis

constant (K_M = 0.14 mmol/l) for KIC, levels of the substrate after injection of hyperpolarized [1-^{13}C]KIC did not limit the BCAT catalyzed reaction either in tumor or muscle tissue, and substrate availability was not expected to contribute significantly to tissue contrast of branched chain amino acid metabolism between tumor and surrounding tissue.

To assess tumor-specific differences in branched chain amino acid metabolism, Karlsson et al. also performed imaging experiments in a rat R3230AC breast cancer model (5). As for the EL4 lymphoma model, the hyperpolarized [1-^{13}C]KIC signal was evenly distributed in the rat tissues. Turnover of [1-^{13}C]KIC to [1-^{13}C]leucine in the tumor was, however, minor. Substantial [1-^{13}C]KIC signal in the tumor indicated that [1-^{13}C]leucine synthesis was not limited by low tissue perfusion, which was further confirmed with KIC measurements *ex vivo*. KIC uptake above the BCAT K_M was found both for muscle (0.07 ± 0.003 µmol/g before KIC infusion, 151 ± 14 µmol/g after KIC infusion) and tumor (0.05 ± 0.01 µmol/g before KIC infusion, 199 ± 56 µmol/g after KIC infusion). BCAT activities were comparable in rat muscle, R3230AC tumor, and mouse muscle, but were substantially increased in EL4 tumors.

Tissue perfusion in both tumor models was also evaluated with hyperpolarized [1-^{13}C]pyruvate. Hyperpolarized [1-^{13}C]pyruvate is a well established tumor marker because of increased levels of anaerobic glycolysis, and the resultant high lactate dehydrogenase catalyzes flux of ^{13}C signal between [1-^{13}C]pyruvate and [1-^{13}C]lactate in neoplastic tissues. Therefore, [1-^{13}C]pyruvate was used as a reference substrate both in EL4 and R3230AC tumors. The expected high [1-^{13}C]lactate formation was observed in both tumor models, suggesting high tissue perfusion and efficient cellular monocarboxylic acid uptake in both models. The high [1-^{13}C]lactate formation also indicated that the higher [1-^{13}C]leucine formation resulted from the higher BCAT activity or co-substrate availability in EL4 tumors *versus* R3230AC tumors rather than from higher tissue perfusion and marker uptake. This indicates the use of hyperpolarized [1-^{13}C]pyruvate as a general *in vivo* tumor marker, whereas hyperpolarized [1-^{13}C]KIC provides a discriminative marker for the non-invasive profiling of branched chain amino acid metabolism in tumors.

Karlsson et al. concluded that [1-^{13}C]KIC MRS is sufficiently sensitive for *in vivo* assay of BCAT activity (5). Information on the distribution and metabolism of [1-^{13}C]KIC and [1-^{13}C]leucine can be sensitively obtained within the first minute after injection. Hyperpolarized [1-^{13}C]KIC is a biomarker that specifically reports on metabolism by BCATs *in vivo* in small animals (5).

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

References

1. Kurhanewicz J., Bok R., Nelson S.J., Vigneron D.B. *Current and potential applications of clinical 13C MR spectroscopy.* J Nucl Med. 2008;49(3):341–4. PubMed PMID: 18322118.
2. Golman K., Ardenkjaer-Larsen J.H., Petersson J.S., Mansson S., Leunbach I. *Molecular imaging with endogenous substances.* Proc Natl Acad Sci U S A. 2003;100(18):10435–9. PubMed PMID: 12930896.
3. Hu S., Lustig M., Chen A.P., Crane J., Kerr A., Kelley D.A., Hurd R., Kurhanewicz J., Nelson S.J., Pauly J.M., Vigneron D.B. *Compressed sensing for resolution enhancement of hyperpolarized 13C flyback 3D-MRSI.* J Magn Reson. 2008;192(2):258–64. PubMed PMID: 18367420.
4. Larson P.E., Kerr A.B., Chen A.P., Lustig M.S., Zierhut M.L., Hu S., Cunningham C.H., Pauly J.M., Kurhanewicz J., Vigneron D.B. *Multiband excitation pulses for hyperpolarized 13C dynamic chemical-shift imaging.* J Magn Reson. 2008;194(1):121–7. PubMed PMID: 18619875.
5. Karlsson M., Jensen P.R., Zandt R.I., Gisselsson A., Hansson G., Duus J.O., Meier S., Lerche M.H. *Imaging of branched chain amino acid metabolism in tumors with hyperpolarized (13)C ketoisocaproate.* Int J Cancer. 2010;127(3):729–36. PubMed PMID: 19960440.
6. Mansson S., Johansson E., Magnusson P., Chai C.M., Hansson G., Petersson J.S., Stahlberg F., Golman K. *13C imaging-a new diagnostic platform.* Eur Radiol. 2006;16(1):57–67. PubMed PMID: 16402256.
7. Golman K., Olsson L.E., Axelsson O., Mansson S., Karlsson M., Petersson J.S. *Molecular imaging using hyperpolarized 13C.* Br J Radiol. 2003;76(Spec No 2):S118–27. PubMed PMID: 15572334.
8. Chen A.P., Tropp J., Hurd R.E., Van Criekinge M., Carvajal L.G., Xu D., Kurhanewicz J., Vigneron D.B. *In vivo hyperpolarized 13C MR spectroscopic imaging with 1H decoupling.* J Magn Reson. 2009;197(1):100–6. PubMed PMID: 19112035.
9. Ardenkjaer-Larsen J.H., Fridlund B., Gram A., Hansson G., Hansson L., Lerche M.H., Servin R., Thaning M., Golman K. *Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR.* Proc Natl Acad Sci U S A. 2003;100(18):10158–63. PubMed PMID: 12930897.

10. Chen A.P., Kurhanewicz J., Bok R., Xu D., Joun D., Zhang V., Nelson S.J., Hurd R.E., Vigneron D.B. *Feasibility of using hyperpolarized [1-13C]lactate as a substrate for in vivo metabolic 13C MRSI studies.* Magn Reson Imaging. 2008;26(6):721–6. PubMed PMID: 18479878.
11. Albers M.J., Bok R., Chen A.P., Cunningham C.H., Zierhut M.L., Zhang V.Y., Kohler S.J., Tropp J., Hurd R.E., Yen Y.F., Nelson S.J., Vigneron D.B., Kurhanewicz J. *Hyperpolarized 13C lactate, pyruvate, and alanine: noninvasive biomarkers for prostate cancer detection and grading.* Cancer Res. 2008;68(20):8607–15. PubMed PMID: 18922937.
12. Gallagher F.A., Kettunen M.I., Day S.E., Hu D.E., Ardenkjaer-Larsen J.H., Zandt R., Jensen P.R., Karlsson M., Golman K., Lerche M.H., Brindle K.M. *Magnetic resonance imaging of pH in vivo using hyperpolarized 13C-labelled bicarbonate.* Nature. 2008;453(7197):940–3. PubMed PMID: 18509335.
13. Olsson L.E., Chai C.M., Axelsson O., Karlsson M., Golman K., Petersson J.S. *MR coronary angiography in pigs with intraarterial injections of a hyperpolarized 13C substance.* Magn Reson Med. 2006;55(4):731–7. PubMed PMID: 16538605.
14. Jensen P.R., Karlsson M., Meier S., Duus J.O., Lerche M.H. *Hyperpolarized amino acids for in vivo assays of transaminase activity.* Chemistry. 2009;15(39):10010–2. PubMed PMID: 19714690.