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Hepatic Gluconeogenesis Influences ^{13}C Enrichment in Lactate in Human Brain Tumors During Metabolism of $[1,2-^{13}\text{C}]$ acetate

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Abstract

^{13}C -enriched compounds are readily metabolized in human malignancies. Fragments of the tumor, acquired by biopsy or surgical resection, may be acid-extracted and ^{13}C NMR spectroscopy of metabolites such as glutamate, glutamine, 2-hydroxyglutarate, lactate and others provide a rich source of information about tumor metabolism *in situ*. Recently we observed ^{13}C - ^{13}C spin-spin coupling in ^{13}C NMR spectra of lactate in brain tumors removed from patients who were infused with $[1,2-^{13}\text{C}]$ acetate prior to the surgery. We found, in four patients, that infusion of ^{13}C -enriched acetate was associated with synthesis of ^{13}C -enriched glucose, detectable in plasma. ^{13}C labeled glucose derived from $[1,2-^{13}\text{C}]$ acetate metabolism in the liver and the brain pyruvate recycling in

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the tumor together lead to the production of the ^{13}C labeled lactate pool in the brain tumor. Their combined contribution to acetate metabolism in the brain tumors was less than 4.0%, significantly lower than the direct oxidation of acetate in the citric acid cycle in tumors.

Keywords

Glioblastoma; Brain metastases; isotopomers; metabolism; pyruvate recycling; [1,2- ^{13}C]acetate

1. Introduction

Metabolic pathways in human tumors *in situ* may be examined by infusion of ^{13}C -enriched compounds followed by ^{13}C NMR spectroscopy of an aqueous extract of the tissue. This approach is readily incorporated into the work flow of the operating room because of the absence of ionizing radiation and simple substrates involved, such as acetate or glucose. Recently we found that glioblastomas and brain metastases have the capacity to oxidize acetate in the citric acid cycle based on an analysis of ^{13}C - ^{13}C spin-spin coupling in glutamate [1]. ^{13}C - ^{13}C coupling due to $J_{1,2}$ and $J_{2,3}$ was also observed in the ^{13}C NMR spectra of lactate in the tumors. This finding was unexpected because there is no simple pathway for [1,2- ^{13}C]acetate to enter the lactate or pyruvate pool [1-6]. There have been a few reports that discuss the possibility of ^{13}C -enriched pyruvate or lactate arising from ^{13}C -enriched acetate through ^{13}C -labeled acetyl-CoA contributing to ^{13}C enrichment in oxaloacetate (OAA) followed by decarboxylation and generation of ^{13}C -enriched pyruvate, known as pyruvate recycling [3-8]. However, systemic acetate may also enter the citric acid cycle of the liver. Although net synthesis of glucose from acetyl groups does not occur in mammalian liver, ^{13}C from acetate may mix in the oxaloacetate pool and enter gluconeogenesis. Exported ^{13}C -glucose may be metabolized in the brain tumor. Lactate could also be produced due to the metabolism of acetate elsewhere in the body and made available to the tumor through plasma. Here, we demonstrate that ^1H and ^{13}C NMR of plasma is a simple and powerful method to study hepatic gluconeogenesis during infusion of [1,2- ^{13}C]acetate.

2. Material and methods

Two patients with glioblastomas and two patients with brain metastases (breast cancer and lung cancer) were enrolled in a protocol approved by the University of Texas Southwestern Institutional Research Board. Subjects were infused with [1,2- ^{13}C]acetate at 6 mg/kg/min for 5 minutes, followed by 3 mg/kg/min for 2 hours. Methods for sampling tumor tissue and plasma for NMR spectroscopy have been described previously [1,2,9]. Proton decoupled ^{13}C spectra of tumor extracts and plasma were acquired at 150 MHz (for ^{13}C) on an Avance Bruker NMR Spectrometer equipped with 10-mm broadband cryogenically-cooled probe (Bruker Biospin, Billerica, MA). The lactate C3 carbon signal at 20.8 ppm was used as internal chemical shift reference. Examples of ^{13}C NMR spectra of these brain tumors have been published previously [1]. Signal areas were measured using ACD (Advanced Chemistry Development, Toronto, Canada) [1,9,10]. Amounts of ^{13}C - ^{13}C doublet peak areas are reported as a fraction of its total carbon resonance area.

3. Results and Discussion

The simple metabolic pathway for metabolism of [1,2- ^{13}C]acetate in a brain tumor is its conversion to acetyl-CoA and oxidation to CO_2 in the citric acid cycle, and thus providing no direct mechanism for ^{13}C entry into the lactate pool of the tumor. Nevertheless, ^{13}C NMR spectra of tumors demonstrated both [1,2- ^{13}C]- and [2,3- ^{13}C]lactate through the appearance of ^{13}C - ^{13}C doublets C2D12 and C3D23 respectively (Figure 1). To test whether ^{13}C label originating from infused [1,2- ^{13}C]acetate could enter hepatic gluconeogenesis, plasma from the patient was obtained at the time of tumor excision. A typical ^{13}C NMR spectrum of the plasma is shown in Figure 2. [1,2- ^{13}C] and [2,3- ^{13}C] isotopomers of glucose in the plasma would generate [2,3- ^{13}C]pyruvate and [1,2- ^{13}C]pyruvate, respectively, in the tumor which would yield the same ^{13}C labeled isotopomers of lactate. Amounts of [1,2- ^{13}C] (C2D12), [2,3- ^{13}C] (C3D23) isotopomers of lactate in the tumor ($n=4$) are 0.30 ± 0.07 , 0.17 ± 0.02 respectively and [2,3- ^{13}C] (C2D23), [1,2- ^{13}C] (C1D12) in plasma glucose are 0.23 ± 0.05 , 0.17 ± 0.04 respectively. Similar values of C3D23, C2D12 of tumor lactate and C1D12, C2D23 plasma glucose suggest that [1,2- ^{13}C]acetate leads to the production of ^{13}C enriched glucose generated in the liver through gluconeogenesis, which then enters the brain to generate corresponding ^{13}C enriched lactate isotopomers through glycolysis. C3D23 tumor lactate value (0.17 ± 0.02) is same to the value of C1D12 of plasma glucose (0.17 ± 0.04), which suggests that ^{13}C labeled glucose from [1,2- ^{13}C]acetate metabolism in the liver is the main source of [2,3- ^{13}C]lactate in the brain tumor. However, C1D12 tumor lactate value (0.30 ± 0.07) is slightly higher than C2D23 of plasma glucose value of (0.23 ± 0.05). This small difference in the [1,2- ^{13}C] isotopomer in the tumor lactate pool could be due to the effect of other metabolic pathways within the tumor. Pyruvate recycling has been reported in rat brain following infusion of [1,2- ^{13}C]acetate [3,7]. As shown in Figure 3, pyruvate recycling produces equimolar mixture of [1,2- ^{13}C] and [3- ^{13}C] pyruvate isotopomers from [1,2- ^{13}C] and [3,4- ^{13}C] OAA isotopomers through the activity of either malic enzyme or combined activity of the enzymes phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate kinase (PK). [1,2- ^{13}C]pyruvate isotopomer produced from the pyruvate recycling pathway could also contribute to the production of [1,2- ^{13}C]lactate in the brain tumor lactate pool. Also, [1,2- ^{13}C] pyruvate isotopomers could produce mono-labeled [1- ^{13}C]acetyl-CoA (Fc1; fraction of acetyl-CoA ^{13}C enriched only in its carbonyl carbon, C1) through the enzymatic action of pyruvate dehydrogenase (PDH), which was determined from C5 glutamate ^{13}C resonance signal through the ratio between C5 glutamate singlet and doublet C5D multiplied by the total [1,2- ^{13}C]acetyl-CoA, which in this case was $3.1\%\pm0.9\%$. Without the contribution from pyruvate recycling and the hepatic gluconeogenesis contributions, Fc1 should be at the ^{13}C isotope natural abundance value of 1.1%. ^{13}C fractional enrichment of plasma glucose is $4.0\%\pm1.0\%$ (from ^1H NMR spectra of plasma glucose, data not shown) and its contribution to the total Fc1 cannot exceed more than 0.8%. Therefore, the rest of the Fc1 contributions ($\sim 2\%$) are likely due to the activity of pyruvate recycling in the brain tumor. Based on the C1D12 value of plasma glucose of 0.17 ± 0.04 , its contribution to the [1,2- ^{13}C]acetyl-CoA pool in the brain tumor from peripheral metabolism of acetate cannot be more than 0.7%, which further confirms our earlier report of [1,2- ^{13}C]acetate being directly oxidized in human GBM and brain metastasis, contributing up to $48\%\pm4\%$ of [1,2- ^{13}C]acetyl-CoA production in the tumor [1]. Additionally, ^{13}C -

labeled lactate isotopomers (C2D12 and C3D23) were also detected in plasma, which could be a source of the ^{13}C -labeling observed in the lactate pool in the tumor [11]. Similar values of ^{13}C fractional enrichments of plasma glucose (C1 carbon) and C3 lactate in the tumor observed from ^1H NMR spectroscopy may suggest that ^{13}C labeled plasma glucose will be one of the main source for the lactate ^{13}C labeling detected in the tumors reported in this study.

4. Conclusion

In summary, we have presented ^{13}C isotopomer analysis of contributions from peripheral metabolism and brain pyruvate recycling in human GBM and brain metastasis patients infused with [1,2- ^{13}C]acetate. Together, they account for less than 4.0% to the ^{13}C enriched acetyl-CoA pool (both [1- ^{13}C] and [1,2- ^{13}C]acetyl-CoA) in the brain tumor. The present analysis will be applicable to study [1,2- ^{13}C] acetate metabolism in vivo in other organ systems.

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Figure 1. Tumor lactate ^{13}C -labeling in a patient with GBM (A and C) and a patient with lung metastasis to brain (B, D). ^{13}C - ^{13}C doublets C2D12 and C3D23 correspond to $[1,2-^{13}\text{C}]$ and $[2,3-^{13}\text{C}]$ isotopomers of ^{13}C labeled lactate respectively. C2S and C3S are singlet signals from C2 and C3 carbons of lactate respectively.

Figure 2.

Plasma glucose ^{13}C -labeling from the patient presented in Figure 1A, C. ^{13}C - ^{13}C doublets C1D12 and C2D23 are due to [1,2- ^{13}C] and [2,3- ^{13}C] isotopomers of ^{13}C labeled glucose respectively. C1S and C2S are singlet ^{13}C signals from C1 and C2 carbons of glucose respectively.

Figure 3.

Schematic diagram showing the sources of ^{13}C labeling in the tumor lactate pool during the $[\text{1,2-}^{13}\text{C}]\text{acetate}$ as infusion substrate. Filled (green) circles represent ^{13}C labeled carbons. Infused $[\text{1,2-}^{13}\text{C}]\text{acetate}$ can only lead to the production of $[\text{1,2-}^{13}\text{C}]\text{acetyl-CoA}$. Numbers represent carbon atom positions. Abbreviations: LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase; PEPCK, phosphoenolpyruvate carboxykinase; PK, pyruvate kinase; $\alpha\text{-KG}$, α -ketoglutarate; OAA, oxaloacetate.