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One-pot enzymatic synthesis of L-[3-^{11}C]lactate for pharmacokinetic analysis of lactate metabolism in rat brain

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ABSTRACT

Introduction: Lactate could serve as an energy source and signaling molecule in the brain, although there is insufficient *in vivo* evidence to support this possibility. Here we aimed to use a one-pot enzymatic synthetic procedure to synthesize L-[3-^{11}C]lactate that can be used to evaluate chemical forms in the blood after intravenous administration, and as a probe for pharmacokinetic analysis of lactate metabolism in *in vivo* positron emission tomography (PET) scans with normal and fasted rats.
Methods: Racemic [3-^{11}C]alanine obtained from ^{11}C-methylation of a precursor and deprotection was reacted with an enzyme mixture consisting of alanine racemase, D-amino acid oxidase, catalase, and lactate dehydrogenase to yield L-[3-^{11}C]lactate *via* [3-^{11}C]pyruvate. The optical purity was measured by HPLC. Radioactive chemical forms in the arterial blood of Sprague Dawley rats with or without insulin pretreatment were evaluated by HPLC 10 min after bolus intravenous injection of L-[3-^{11}C]lactate. PET scans were performed on normal and fasted rats administered with L-[3-^{11}C]lactate.
Results: L-[3-^{11}C]Lactate was synthesized within 50 min and had decay corrected radiochemical yield, radiochemical purity, and optical purity of 13.4%, >95%, and >99%, respectively. The blood radioactivity peaked immediately after L-[3-^{11}C]lactate injection, rapidly decreased to the minimum value within 90 s, and slowly cleared thereafter. HPLC analysis of blood samples revealed the presence of [^{11}C]glucose (78.9%) and L-[3-^{11}C]lactate (12.1%) 10 min after administration of L-[3-^{11}C]lactate. Insulin pretreatment partly inhibited glyconeogenesis conversion leading to 55.4% as [^{11}C]glucose and 38.9% as L-[3-^{11}C]lactate simultaneously. PET analysis showed a higher SUV in the brain tissue of fasted rats relative to non-fasted rats.
Conclusions: We successfully synthesized L-[3-^{11}C]lactate in a one-pot enzymatic synthetic procedure and showed rapid metabolic conversion of L-[3-^{11}C]lactate to [^{11}C]glucose in the blood. PET analysis of L-[3-^{11}C]lactate indicated the possible presence of active lactate usage in rat brains *in vivo*.

1. Introduction

Living brain tissues carry out needed functions using ATP molecules produced *via* oxidative phosphorylation of D-glucose and oxygen under normal physiological conditions. In the astrocyte-neuron lactate shuttle hypothesis, glycolysis mainly occurs in astrocytes located between neurons and capillary vessels. Monocarboxylic acid transporters move lactate produced through glycolysis to neighboring neurons that use lactate as an energy source in mitochondrial oxidative phosphorylation [1,2]. However, this hypothesis remains controversial [3,4]. Recent studies also reported that lactate could be used as an energy source under certain conditions [5,6] and as a signaling molecule [7] in living brain tissue.

Nuclear medical techniques such as Positron Emission Tomography (PET) are useful for noninvasive measurement of living functions and rely on the detection of radioactivity emitted from radiolabeled tracers administered in advance of the test. For instance, PET scans with a ^{18}F-labeled glucose derivative, 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG), are useful not only for *in vivo* quantitative analysis of glucose metabolism in the brain and the heart [8,9], but also for noninvasive detection of disperse tumor sites (distant metastasis), atherosclerosis, and inflammation [10-12]. To clarify the role of lactate in living systems by PET, a previous study reported a synthetic procedure for L-[3-^{11}C]lactate [13]. In that report, racemic [3-^{11}C]alanine was first synthesized using a

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standard ^{11}C-methylation method with [^{11}C]CH_3I and the corresponding precursor, which was then reacted with an enzyme mixture consisting of alanine transaminase (ALT), D-amino acid oxidase (DAAO), and catalase. ALT and DAAO converted L-[3-^{11}C]alanine and D-[3-^{11}C]alanine, respectively, to [3-^{11}C]pyruvate, whereas catalase eliminated hydrogen peroxide generated as a byproduct. [3-^{11}C]Pyruvate was next reacted with lactate dehydrogenase (LDH) to selectively produce L-[3-^{11}C]lactate.

The usefulness of L-[3-^{11}C]lactate has only been evaluated by PET in animal models of heart disease [14]. In this study, we aimed to use a novel enzyme reaction to synthesize L-[3-^{11}C]lactate in a one-pot synthetic procedure. We used the resulting L-[3-^{11}C]lactate to evaluate radioactive chemical forms in the blood after tracer administration, and performed *in vivo* brain PET scans of normal and fasted rats.

2. Materials and methods

2.1. Radiochemical synthesis (Scheme 1)

All reagents were obtained commercially and used without further purification. The tracer L-[3-^{11}C]lactate was synthesized following a 2-step enzymatic reaction as previously described [13,14] except that alanine racemase was used instead of ALT [15]. An enzyme mixture was prepared by mixing 1.0 ml 0.1 M phosphate buffer (pH 8) containing 500 U rabbit muscle LDH (Oriental Yeast Co., Ltd., Tokyo, Japan), 0.017 mM flavin adenine dinucleotide (FAD, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), 0.1 mM pyridoxal-5′-phosphate (PLP, Tokyo Chemical Industry), and 5 mM nicotinamide adenine dinucleotide (NADH, Sigma-Aldrich Japan, Tokyo, Japan) with 0.1 ml pyruvate synthase solution (PYR, Ikeda Shokken, Hiroshima, Japan) consisting of 680 U alanine racemase, 45 U DAAO, and 3600 U catalase. Carbon-11 was produced *via* the ^{14}N(p,α)^{11}C nuclear reaction with a 0.1% O_2/N_2 gas target and a 20 μA current of protons accelerated by an in-house CYPRIS-HM18 cyclotron (Sumitomo Heavy Industries, Ltd., Tokyo, Japan) for 60 min. The [^{11}C]CO_2 was transported to an automated C-11-labeled compound synthesis system (CUPID C-11-BII, Sumitomo Heavy Industries) followed by lithium aluminum hydride reduction and iodination to synthesize [^{11}C]CH_3I using a conventional method. The resulting [^{11}C]CH_3I was reacted with 3 mg *N*-(diphenylmethylene)glycine *t*-butyl ester in 300 μl *N*,*N*-dimethylformamide (DMF) with 10 μl 5 M KOH at 85 °C for 3 min. The solution was applied to a Sep-Pak Plus short cartridge tC18 column (Nihon Waters, Tokyo, Japan) followed by a water wash and recovery with 2 ml acetone. After solvent evaporation, 1.3 ml 3 M HCl solution was added and reacted at 130 °C for 5 min. The solvent was evaporated to dryness, cooled to approximately 50 °C, and then mixed with the whole enzyme mixture followed by incubation at 45 °C for 5 min. The solution was applied to a strong cation exchange resin (AG 50W-X8 Cation Exchange Resin, biotechnology grade, 100–200 mesh, Bio-Rad Laboratories, Inc., Hercules, CA) for both deproteinization and adsorption of L-[3-^{11}C]lactate, which was eluted with 3 ml water. The water solution was passed through a Sep-Pak C18 column and a Millex-GS filter (0.22 μm, Merck Japan, Tokyo, Japan) to obtain an injection solution of L-[3-^{11}C]lactate in a final vial containing 100 mg sodium bicarbonate to modulate the pH. The radiochemical purity was measured by a high performance liquid chromatography (HPLC) method using a Cosmosil HILIC column (4.6 × 250 mm, Nacalai Tesque, Inc., Kyoto, Japan). The mobile phase (acetonitrile/20 mM phosphate buffer (pH = 3.5) = 50/50) was delivered at a flow rate of 1.0 ml/min. The optical purity was measured by HPLC using a Shodex ORpak CRX-853 column (8.0 × 50 mm, Showa Denko, Tokyo, Japan). The mobile phase (0.5 mM $CuSO_4$ aqueous solution) was delivered at a flow rate of 1.0 ml/min.

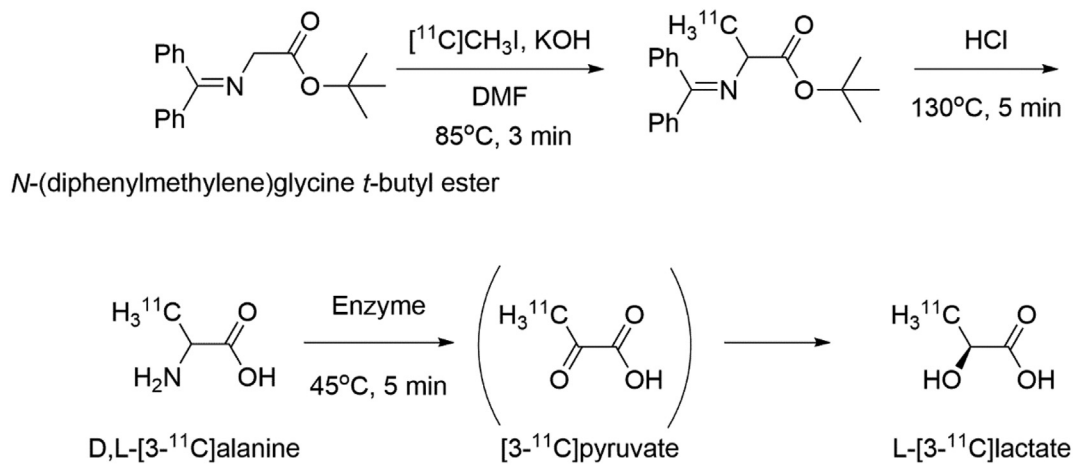
2.2. Animal experiments, general

The animal experiments in this study were conducted in accordance with guidelines for animal research on Human Care and Use of Laboratory Animals (Rockville, National Institute of Health/Office for Protection from Research Risks, 1996). The study protocol was approved by the Sub-committee for Laboratory Animal Welfare, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan (Permit Number: 14028 and 15063). Animal allocation for each experiment is shown in Table 1.

2.3. Metabolite analysis

Male Sprague Dawley (SD) rats (n = 16, 5–6 weeks old) were purchased from Japan SLC (Shizuoka, Japan) and housed under a 12 h light/12 h dark cycle with free access to food and water. Seven rats were fasted for 12 h before the experiment. The minimal number of rats (n = 2) were intraperitoneally treated with insulin (20 IU/kg) at 150 min prior to L-[3-^{11}C]lactate administration.

Determination of the [^{11}C]CO_2 fraction in the blood after intravenous injection of L-[3-^{11}C]lactate was based on the loss of [^{11}C]CO_2 under acidic conditions as described in a previous report [14]. Normal male SD rats (183.3 ± 10.7 g, n = 6) were anesthetized under isoflurane on a heating pad to maintain the body temperature. A cannula was placed in the unilateral femoral artery. After intravenous administration of L-[3-^{11}C]lactate (134.0 ± 69.7 MBq) *via* a tail vein, 10 μl of arterial blood was collected from the cannula at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, and 110 s, and 5, 15, 30, and 60 min. The radioactivity in the blood samples was determined with an NaI well-type scintillation counter (1470 Wizard; PerkinElmer, Kanagawa, Japan). In addition, 250 μl of



Scheme 1. Three-step radiosynthesis of L-[3-^{11}C]lactate from *N*-(diphenylmethylene)glycine *t*-butyl ester as a precursor compound.