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CHARACTERIZATION OF A GAS-PURGE METHOD TO ACCESS ^{11}C-CARBON-DIOXIDE RADIOACTIVITY IN BLOOD

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ABSTRACT

Carbon-11 labeled radiotracers, such as ^{11}C-acetate and ^{11}C-palmitate are widely used in positron emission tomography (PET) for noninvasive evaluation of myocardial metabolism under varied physiological conditions.These tracers are attractive probes of tissue physiology, because they are simply radiolabled versions of the native biochemical substrates. One of the major metabolites generated by these tracers upon the administration is $^{11}CO_2$ produced via the citric acid cycle. In quantitative modeling of ^{11}C-acetate and ^{11}C-palmitate PET data, the fraction of blood ^{11}C radioactivity present as $^{11}CO_2$ needs to be measured to obtain a correct radiotracer arterial input function. Accordingly, the literature describes a method whereby the total blood ^{11}C-activity is counted in blood samples treated with base solution, while the fraction of $^{11}CO_2$ is measured after the blood is treated with acid followed by a 10 minutes gas-purge. However, a detailed description of the experimental validation of this method was not provided. The goal of this study was to test the reliability of a 10 minute gas purging method used to assay $^{11}CO_2$ radioactivity in blood.

Keywords: Assay, blood, $^{11}CO_2$, gas-purge, radioactivity, radiolable

INTRODUCTION

Simple carboxylic acids radiolabeled with carbon-11 ($t_{1/2}$: 20.4 min), especially [^{11}C]-acetate and [^{11}C]-palmitate, have been used widely as tools for imaging with positron emission tomography (PET) to evaluate the rate at which their endogenous counterparts are being oxidatively metabolized by myocardium under various physiological conditions. One of the major radiolabeled metabolites produced by these compounds is [^{11}C]CO_2, a product of the tricarboxylic acid cycle. Models for quantification of myocardial metabolic rate from the resulting PET data require knowledge of both the concentration of radioactivity in myocardium as a function of time, as well as knowledge of the concentration of radiopharmaceutical in the arterial blood supplying that tissue.

While the PET images readily quantify radioactivity in the myocardium, as well as the arterial blood radioactivity (based on a region of interest in the cavity of the left ventricle or left atrium), those PET data simply define regional radionuclide concentrations without information on the chemical form of the radionuclide. Thus, for tracer kinetic modeling to quantify metabolic rates, it is necessary to independently measure the fraction of total blood ^{11}C activity present in blood as [^{11}C]CO_2 to generate a correct radiopharmaceutical arterial input function.

The technique most commonly used to determine the radiolabeled CO_2 concentration of blood is simple *in vitro* acidification of a blood sample, followed by purging with an inert gas to drive off

CO_2 (Fox et al., 1985). The fraction of total blood ^{11}C activity that remains after the gas purge represents ^{11}C species other than $^{11}CO_2$. This method has been used in a number of preclinical and clinical PET studies yielding important insights into myocardial metabolism (Kisrieva-Ware et al., 1989; Kisrieva-Ware et al., 2009; Walsh et al., 1989). Here we report experimental validation of the selectivity of the gas-purge technique for removing $^{11}CO_2$ from blood.

MATERIALS AND METHODS

Radioactive sample handling was carried out behind lead-shielding in a chemical fume hood. A 50 - 100 μCi sample (20 - 100 μL) of aqueous $[^{11}C]CO_2$ or [^{11}C]-acetate or [^{11}C]-palmitate/HSA was added to 3 mL of blood *in vitro* and gently mixed. A 1 mL aliquot of the blood was then transferred to a counting vial containing 1 mL sodium hydroxide (0.1 N) and 3 mL isopropanol, which was then immediately capped to retain any $^{11}CO_2$ as bicarbonate. This base-treated blood contained all ^{11}C radioactivity (Vial A). A second 1 mL aliquot of the blood containing radiotracer was added to a counting vial containing 1 mL sodium bicarbonate (0.5 N) and 3 mL isopropanol, and the mixture was vortex mixed (Vial B). Then, 1 mL 6.0 N hydrochloric acid was added followed by 10-20 seconds of vortex mixing. Next, the acid-treated blood sample was purged with air, bubbled into the blood via a 4-inch flexible filter straw (B Braun Medical, Inc., Bethlehem, PA), for 10 and 15 minutes to drive off the $^{11}CO_2$ (vial B). The activity remaining in "Vial B" represents for total non-$^{11}CO_2$ radioactivity. After decay correction of the radioactivity measurements to a common time point, the percentage of total radioactivity released as $^{11}CO_2$ was calculated as:

$$\textit{Radioactivity present as } CO_2 = \frac{\textit{Vial A radioactivity} - \textit{Vial B radioactivity}}{\textit{Vial A radioactivity}} \times 100\%$$

RESULTS AND DISCUSSION

To verify the suitability of the gas purge method for determining the level of blood-borne $[^{11}C]CO_2$, analysis was carried out after directly mixing $[^{11}C]CO_2$ with whole blood *in vitro*. After acidification and air bubbling through the sample for 10-minutes, more than 99% of the ^{11}C was released from the $[^{11}C]CO_2$-spiked whole blood (Table 1). Continuation of the gas purge for an additional 5minutes did not appreciably alter the residual blood ^{11}C level. As expected, blood samples similarly spiked *ex-vivo* with [^{11}C]-acetate or [^{11}C]-palmitate showed no loss of radioactivity, even after 15 min of air purging (Table 1). These results are similar to previous reported findings, where 95% of [^{11}C]-acetate was retained, and 98% of [^{11}C]-labeled bicarbonate was eliminated, in acid-treated blood samples after 10-minutes of inert gas bubbling (Brown et al., 1988; Fox et al., 1985). This validation study confirmed that the acidification/gas purge method was quite effective for selectively releasing $[^{11}C]CO_2$ from acid-treated whole blood. Additionally, the analysis method is technically straightforward, simple to implement, and can readily be completed on a time frame compatible with the 20 minute ^{11}C half-life.

Table 1: Effectiveness of the gas-purge method for selectively removing [^{11}C]CO_2 from acidified whole blood

Radiopharmaceutical Added to Blood	**Percentage of Initial ^{11}C Lost from Blood Sample after 10 minute Air Purge**
$^{11}CO_2$	99.8 ± 0.3% (n = 4)
1-[^{11}C]acetate	-0.1 ± 0.5% (n = 3)
1-[^{11}C]palmitate	-2.4 ± 1.9% (n = 3)

CONCLUSIONS

The 10 minute gas purge method is quite reliable for removing $^{11}CO_2$ from acidified blood. This method is now being implemented in Indiana Universtiy School of Medicine in order to assay $^{11}CO_2$ radioactivity in animal and human blood after the intravenous administration of either ^{11}C-acetate or ^{11}C-palmitate radiopharmaceuticals.

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