Contribution of Labeled Carbon Dioxide to PET Imaging of Carbon-11-Labeled Compounds

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$^{11}CO_2$ is one of the major metabolites of many [^{11}C]-labeled radiopharmaceuticals, including glucose, thymidine, acetate, amino acids, and fatty acids. Our data contradict the notion that the contribution of labeled CO_2 to PET images can be disregarded because of its rapid elimination through the lungs. We have measured the retention and excretion of $^{11}CO_2$ in dogs after the intravenous injection of labeled CO_2/HCO_3^-, which had been equilibrated ex vivo with blood. Only 58% of the label was exhaled as CO_2 over the first 60 min after injection, with the rest retained in the body. The injection of [^{11}C]thymidine labeled in the ring-2 position or [^{11}C]acetate labeled in the carboxylate position resulted in the production of large amounts of labeled CO_2 with the exhalation of about 47% and 23%, respectively, of the injected label over 60 min. At 10 min after injection of either [^{11}C]thymidine and [^{11}C] acetate, approximately 60% to 70% of total blood activity was in labeled CO_2 or bicarbonate. On the other hand, the use of [1-^{11}C]glucose only resulted in exhalation of 5% of the injected dose and CO_2/HCO_3^- made up <10% of blood activity at 10 min. Our results indicate that retention and distribution of labeled CO_2 needs to be considered when interpreting PET data obtained from ^{11}C-labeled compounds.

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Carbon-11 has been used to produce a number of compounds useful in PET, including labeled glucose, thymidine, acetate, amino acids, fatty acids, and carbon dioxide. PET measures the total radioactivity retained in each tissue at any point in time, regardless of the chemical species with which the isotope is associated. Thus, to interpret the images obtained with PET, one must have a detailed knowledge of the metabolism of the compound being studied and know the proportion of the activity found in the various chemical species. When many ^{11}C-labeled compounds are used, CO_2 is generated as one of the major metabolites. Our results, as well as previously published work (*1,2*), indicate that labeled CO_2 is cleared relatively slowly from the body, and must be taken into account when interpreting PET images.

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MATERIALS AND METHODS

Radiochemistry

$^{11}CO_2$ was produced by proton irradiation of nitrogen gas containing trace amounts of oxygen. The product $^{11}CO_2$ was cold trapped using liquid argon and then bubbled through whole blood with about 50 ml/min of nitrogen gas for less than 5 min prior to injecting. The equilibration of $^{11}CO_2$ in the blood results in a mixture of labeled CO_2 and bicarbonate, which was injected intravenously; for simplicity's sake, we will henceforth indicate that labeled CO_2 was utilized. Radiochemical purity is routinely checked using gas chromatography with a CTRI column (Altech Associates, Deerfield, IL) and is >99%.

Carbon-11-thymidine labeled in the ring-2 position was prepared just prior to use according to a modification of the method of Vander Borght et al. (*3*). The radiochemical purity of HPLC isolated material was >99% and the specific activity was 8–30 Ci/mmol at the time of injection. The entire preparation of [^{11}C] thymidine from $^{11}CO_2$ required 60–80 min.

1-[^{11}C]-D-glucose was synthesized according to the method of Shiue and Wolf (*4*). Radiochemical purity ranged from 95% to >99% with ^{11}C-labeled mannose as the contaminant, as measured by HPLC using an Aminex 87P column (BioRad Inc., Richmond, CA). The specific activity of the product was >30 Ci/mole at the end of the synthesis.

1-[^{11}C]acetate was made by reacting $^{11}CO_2$ with dry methyl magnesium bromide in ether as described by Pike et al. (*5*). After 1 min, the reaction was quenched by addition of 1 ml of water. The resulting solution was then purified by passage through an AG50WX8 [H^+] cation exchange column (BioRad Inc.). The product was used as recovered and not further analyzed. When the synthesis was developed the product was analyzed by gas chromatography on Chrome WH-P (Alltech Assoc.) and no impurities were detected.

Subject Preparation

Five normal dogs weighing from 13 kg to 23 kg were the main study group. Two additional dogs with spontaneous lymphoma were also utilized. Dogs were fasted overnight, initially anesthetized with Brevitol, intubated, and maintained on methoxyflurane. The animals were spontaneously breathing 100% oxygen through a rebreathing system. Arterial and venous catheters were placed in the groin. Each of the isotopes was diluted to 20 ml with physiologic saline and infused intravenously over 60 sec

using a syringe pump. Ninety minutes were allowed between injections to collect data and allow for radioactive decay.

Blood samples were also obtained from three humans undergoing metabolic imaging of their brain tumors (gliomas) using [1-^{11}C]glucose. The time course of ^{11}C-labeled CO_2/HCO_3^- was measured in blood samples obtained from a radial artery catheter.

Blood Sampling

Blood samples (1.0 ml) were drawn from the arterial line using an automated blood sampler (*6*). Sampling was performed at 10-sec intervals over the first minute after injection, then 20-sec intervals over the second minute, then at 3, 4, 5, 7, 9, 11, 13, 15, 18, 21, 24, 27, 30, 35, 40, 45, 50, 55, and 60 min. Aliquots (0.2 ml) of blood were immediately transferred to test tubes containing 0.8 ml of 0.5 N NaOH and then capped to fix all labeled CO_2 as bicarbonate. Radioactivity was measured by gamma spectroscopy (COBRA, Packard Instrument Co., Meriden, CT). Another 0.2 ml aliquot of blood was processed to remove CO_2 and bicarbonate by adding the blood to 0.6 ml of isopropanol, followed by the addition of 0.2 ml of 0.5 *M* HCl (*7*). The sample was vortexed after each addition to prevent gelation. Nitrogen gas was then bubbled through each sample for 10 min before determining the radionuclide content. Tests demonstrated that this technique removed more than >99% of $^{11}CO_2$ and bicarbonate from the blood. Results were decay-corrected to the time of injection and expressed as percent of injected dose/(%ID/g).

Collection of Exhaled Gas

To measure the rate of $^{11}CO_2$ excreted by exhalation we collected a portion of the exhaled gas by pumping about 100 ml/min through a 2-m inline filter (Milton-Roy, Riviera Beach, FL) into 5 ml of 0.5 *M* NaOH. Samples were obtained for 1.0-min intervals for the first 10 min and then 5-min intervals for the next 50 min. Radioactivity in aliquots of the NaOH containing $^{11}CO_2$ was then quantitated in a gamma spectrometer. To measure the total labeled CO_2 exhaled during the course of the experiment, we placed a cylinder with 250 ml of soda lime (Sodasorb, W.R. Grace & Co., Lexington, MA) on the exhalation side of the anesthesia machine. This cylinder was removed 60 min after injection and the activity measured in a dose calibrator along with the remaining NaOH samples and used to calculate the total excreted dose. This was compared with the injected dose measured in the same dose calibrator. In control experiments, using two canisters in series, we found that the first soda-lime-containing cylinder trapped >99.5% of labeled CO_2.

RESULTS

In order to better understand the excretion of labeled CO_2, three normal dogs were infused with ^{11}C-labeled glucose, acetate, thymidine, and CO_2 (the latter in equilibrium with bicarbonate). Exhaled gas was collected and measured in a gamma-ray spectrometer. The percent of total injected label excreted as $^{11}CO_2$ over the 60 min after injection was 5% from glucose, 23% from acetate, 47% from thymidine, and 58% from CO_2 (Fig. 1). While the peak of $^{11}CO_2$ excretion occurred at about 3 min after the injection of labeled CO_2, it took several minutes longer for thymidine and acetate to reach their maximal rates of excretion. On the other hand, the rate of $^{11}CO_2$ excretion from labeled glucose was still rising at 60 min, the end of the study (Fig. 2).

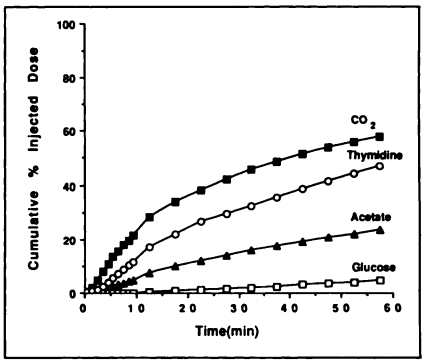

FIGURE 1. Cumulative excretion of $^{11}CO_2$ by exhalation after the injection of ^{11}C-labeled glucose, acetate, thymidine, and carbon dioxide. The data are presented as the mean of three dog studies.

After the infusion of labeled CO_2, as expected, all of the blood activity was initially volatile (Figs. 3A and 4). By 60 min, however, about 5%–18% of the blood activity was fixed in compounds other than CO_2 or bicarbonate and could no longer be driven off. After the infusion of labeled acetate and thymidine all the activity was initially nonvolatile, but by 10 min about 60%–70% of the blood activity was in CO_2 and bicarbonate (Figs. 3C–D, 4). After the infusion of labeled glucose, almost all the activity was nonvolatile, even by 60 min after injection <10% of the activity was in CO_2/HCO_3^- (Figs. 3B, 4). The labeled CO_2/HCO_3^- content of the blood was also measured after the infusion of [1-^{11}C]glucose into three patients with gliomas (Fig. 5). The results were similar to those obtained in dogs in that we found little volatile activity.

Analysis of images obtained after the infusion of labeled CO_2 demonstrated marked differences in the tissue distribution seen within a few minutes of the infusion (Fig. 6). By 30 to 60 min after injection, there had been diffusion of tracer and all tissues began to approach a uniform distribution.

DISCUSSION

Carbon-11 has been used to produce a number of labeled compounds to image brain, heart, and tumor metabolism; these include glucose, thymidine, acetate, amino acids, and CO_2. One must thoroughly understand the metabolism of these compounds in order to interpret the information obtained from PET imaging. One postulated

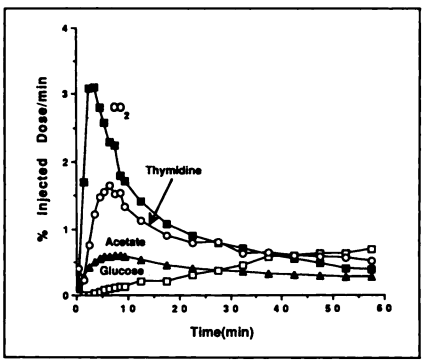

FIGURE 2. Rate of excretion of $^{11}CO_2$ after injection of the indicated ^{11}C-labeled compounds in dogs.

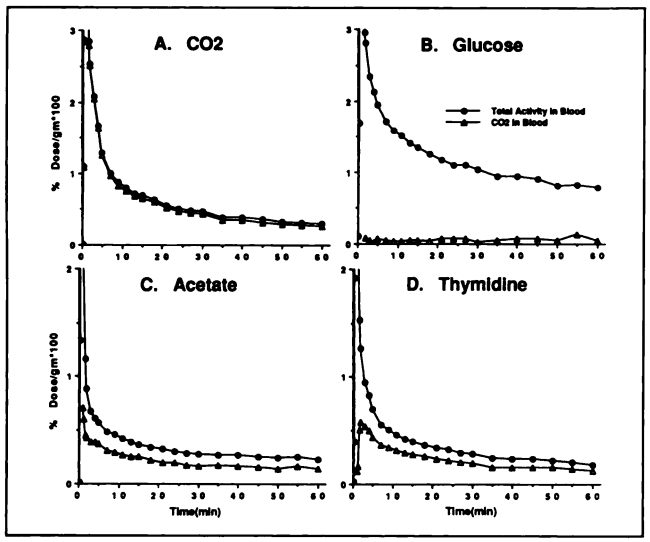

FIGURE 3. Time course of total ^{11}C activity and volatile activity (as CO_2/HCO_3^-) in the blood after injection of the indicated compounds. The data are plotted as the mean, with n=3 for acetate, n=4 for glucose and thymidine, and n=5 for CO_2.

advantage of using ^{11}C is that CO_2, one of the major metabolites of these compounds, would be rapidly cleared from the body by exhalation. Our results demonstrate a need for caution; assuming that $^{11}CO_2$ will be cleared in a few minutes is too simplistic. After the injection of labeled CO_2/HCO_3^- in dogs, we found that only 33% of the injected dose was excreted by exhalation in the first 20 min and 56% over the first hour. This is consistent with results in humans where we calculated that the two subjects studied by Fowle et al. (*2*) excreted 49% and 69% of the injected activity over 45 min. It is also of note that over the 60 min after injection of ^{11}C-labeled CO_2/HCO_3^- approximately 10% of the blood activity is converted to a nonvolatile form. Hetenyi et al. (*8*) demonstrated that the injection of $NaH^{14}CO_3$ in dogs generated appreciable amounts of labeled urea, as well as lactate and glucose. In rats, 30% of the label was metabolically trapped in the brain 30 min after inhalation of $^{11}CO_2$ (*9*).

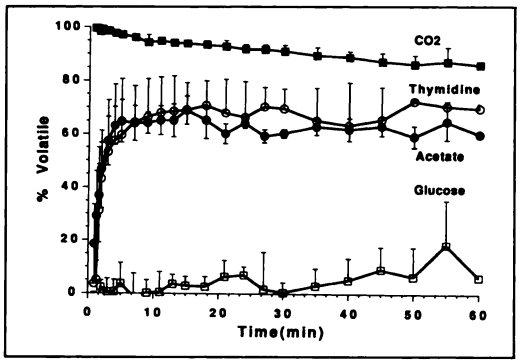

FIGURE 4. Percentage of blood volatile activity as CO_2/HCO_3^- after injection of the indicated compounds.

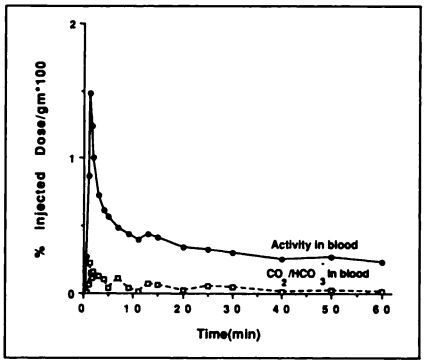

FIGURE 5. Time course of total blood activity and ^{11}C activity in CO_2/HCO_3^- after injection of [1-^{11}C]glucose in patients with gliomas. The data are presented as the mean percentage of activity in the indicated fractions (n=3).

The injection of ^{11}C-acetate results in its metabolism to CO_2/HCO_3^- as reflected in the excretion of 23% of the activity in the exhaled gas. Labeled CO_2/HCO_3^- also makes up about 70% of the blood activity as early as 10 min after injection. This is consistent with the work of Brown et al. (*7*) and Buck et al. (*10*). Therefore, one must take the distribution of CO_2/HCO_3^- into account when interpreting the images obtained with PET. Similarly, studies using [1-^{14}C]palmitate in humans have demonstrated that it is rapidly cleared from the blood and that the exhalation of labeled CO_2 reaches a maximum in less than 30 min (*11*). Therefore, it is likely that labeled CO_2/HCO_3^- and its metabolites also make a significant contribution to the images obtained with ^{11}C-labeled palmitate and might dominate late images (*12*).

Carbon-11-thymidine labeled in either the ring-2 or methyl positions has been developed for use with PET (*3*, *13*). The generation of CO_2 will vary with the position of the label as well as the actual molecule labeled. Methyl-labeled thymidine is degraded to numerous small metabolites rather than CO_2 (*14*,*15*). In contrast, ring-2 labeled thymidine is either incorporated into DNA or rapidly degraded to CO_2. Our data using ring-2 labeled ^{11}C-thymidine demonstrated that 47% of the label is excreted within one hour of injection, but a substantial amount of labeled CO_2/HCO_3^- is still retained in the body and contributes to the images obtained with PET. The contribution of labeled CO_2/HCO_3^- to the images must be taken into account when fitting the results with kinetic models.

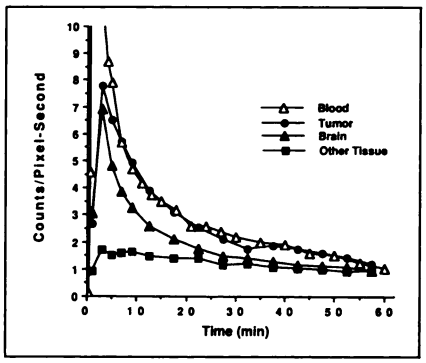

FIGURE 6. Time course of activity in the blood, tumor, brain, and other tissue from a dog with lymphoma in the submandibular lymph nodes.

Such models also need to consider the trapping of labeled biochemicals generated from $^{11}CO_2/HCO_3^-$ (*9*).

[1-^{11}C]glucose was different from the other compounds we studied in that there was little generation of labeled CO_2 in the course of the 60-min study. Only 5% of the injected activity was excreted as CO_2 over 60 min. Our results are in agreement with Blomqvist et al. (*16*). Furthermore, while the rate of CO_2 excretion peaked at 10 min for acetate and thymidine, it was still rising at 60 min for glucose. Studies in humans have demonstrated that the maximum rate of labeled CO_2 excretion occurred at 90 to 120 min after the injection of [1-^{14}C]glucose (*17*). Unlike thymidine and acetate, labeled CO_2/HCO_3^- made up less than 10% of the blood activity, reflecting the slow production of CO_2. The slow production of CO_2 from glucose is in part a reflection of its high blood level (about 5 m*M* in humans) and low uptake in a single passage, versus the lower levels of acetate (87 μM) and thymidine (<1 μM) and much higher single-pass extraction (*7,18*). When using labeled glucose, it is important to remember that the generation of labeled CO_2 will depend on the position of the label. For example, uniformly labeled glucose generates labeled CO_2 faster than [1-C]-labeled glucose, which has a faster rate of labeled CO_2 production than [6-C]-labeled glucose (*17*).

The use of ^{11}C-labeled compounds with PET requires a thorough understanding of their metabolism, especially the contribution of labeled CO_2 and bicarbonate to the images. For compounds that produce large amounts of labeled CO_2, one must incorporate this information into the biochemical and kinetic models needed to quantitatively interpret the images obtained with PET. The distribution of $^{11}CO_2$ in the body is in part dependent on the relative pH of the blood and the tissues. Investigators have taken advantage of this to develop techniques and models for measuring tissue pH using the continuous inhalation of $^{11}CO_2$ (*19–21*). PET investigators employing other ^{11}C-labeled compounds which are metabolized to $^{11}CO_2$ should also consider using these models. For example, in the models of tracer metabolism one might include separate compartments to take into account the distribution of $^{11}CO_2$. Then rate constants for the distribution of $^{11}CO_2$ and its metabolites would be most accurately measured by performing a separate injection of $^{11}CO_2$ and fitting the results to a model. Such a model needs to be produced and validated and would have to take into account the blood time-activity curves of each isotope and calculate the tissue contribution of $^{11}CO_2$ and its fixed metabolites. Alternatively average values for the rate constants for the distribution of $^{11}CO_2$ could be employed if they were found to be relatively stable in a given tissue. Finally, the tracer kinetic model could attempt to simultaneously fit the tracer metabolic rates of the parent compound in parallel with the $^{11}CO_2$ distribution.

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