Synthesis and Evaluation of $^{18}$F-labeled Choline as an Oncologic Tracer for Positron Emission Tomography: Initial Findings in Prostate Cancer

Timothy R. DeGrado, R. Edward Coleman, Shuyan Wang, Steven W. Baldwin, Matthew D. Orr, Cary N. Robertson, Thomas J. Polascik, and David T. Price

Departments of Radiology [T. R. D., R. E. C., S. W.] and Surgery [C. N. R., T. J. P., D. T. P.], Duke University Medical Center, Durham, North Carolina 27710, and Department of Chemistry, Duke University, Durham, North Carolina 27708 [S. W. B., M. D. O.]

ABSTRACT

The up-regulation of rates of choline uptake and phosphorylation in certain malignancies has motivated the development of positron-labeled choline analogues for noninvasive detection of cancer using positron emission tomography (PET). The choline analogue, no-carrier-added $^{[18}$F]$^{[1}$fluoromethyl-$^{2}$hydroxyethyl-ammonium) (FCH), was synthesized through the intermediate $^{[18}$F]$^{[1}$fluorobromomethane. FCH was evaluated in relationship to $^{[18}$F]$^{[1}$fluoro-2-deoxyglucose (FDG) as an oncological probe in cultured PC-3 human prostate cancer cells, a murine PC-3 human prostate cancer xenograft model, and in PET imaging studies of patients with prostate cancer. FCH was synthesized in 20–40% radiochemical yield and >98% radiochemical purity. Accumulation of FCH and FDG were comparable in cultured prostate cancer cells, whereas only FCH was inhibited (90%) by hemicholinium-3, a specific inhibitor of choline transport and phosphorylation. FCH showed similar biodistribution to $^{[14}$C]$^{[1}$choline in the tumor-bearing mouse, with prominent renal and hepatic uptake. Tumor uptake of FCH was similar to choline and FDG in the mouse model, although tumor:blood ratios were moderately higher for FCH. Initial PET imaging studies in prostate cancer patients showed high uptake of FCH in advanced prostate carcinoma and detection of osseous and soft tissue metastases. FCH uptake by tumors was markedly reduced in patients rescaned during androgen deprivation therapy. It is concluded that FCH closely mimics choline uptake by normal tissues and prostate cancer neoplasms. FCH is potentially useful as a PET tracer for detection and localization of prostate cancer and monitoring effects of therapy.

INTRODUCTION

PET$^2$ is uniquely suited to evaluate metabolic activity in human neoplasms for diagnostic imaging purposes. The glucose analogue FDG has proven successful as a PET imaging agent for detection and evaluation of oncologic probe in cultured PC-3 human prostate cancer cells, a murine PC-3 human prostate cancer xenograft model, and PET imaging studies of patients with prostate cancer. FCH was synthesized in 20–40% radiochemical yield and >98% radiochemical purity. Accumulation of FCH and FDG were comparable in cultured prostate cancer cells, whereas only FCH was inhibited (90%) by hemicholinium-3, a specific inhibitor of choline transport and phosphorylation. FCH showed similar biodistribution to $^{[14}$C]$^{[1}$choline in the tumor-bearing mouse, with prominent renal and hepatic uptake. Tumor uptake of FCH was similar to choline and FDG in the mouse model, although tumor:blood ratios were moderately higher for FCH. Initial PET imaging studies in prostate cancer patients showed high uptake of FCH in advanced prostate carcinoma and detection of osseous and soft tissue metastases. FCH uptake by tumors was markedly reduced in patients rescaned during androgen deprivation therapy. It is concluded that FCH closely mimics choline uptake by normal tissues and prostate cancer neoplasms. FCH is potentially useful as a PET tracer for detection and localization of prostate cancer and monitoring effects of therapy.

MATERIALS AND METHODS

Equipment. Nuclear magnetic resonance spectra were recorded on a Varian INOVA 400 MHz spectrometer. High-resolution fast atom bombardment mass measurements were made using a JEOL JMS-SX102A mass spectrometer operating at 10k resolution.

$N,N$-Dimethyl-$N$-Fluoromethylethanolamine (Fluorocholine, $^{[18}$F]$^{[1}$FCH). To a 50-m1 pressure tube containing 20 ml of dry tetrahydrafuran at ~78°C was added 5 ml of (0.0498 mole) $N,N$-dimethylmethanol (Aldrich Chemical Co.). Chlorofluoromethane (Synquest Labs, Alachua, FL) was bubbled through the solution for 15 min upon which the tube was sealed with a teflon screw cap. The mixture was allowed to warm to room temperature over 18 h, during which time a white solid precipitated. The solid was isolated by filtration, washed several times with cold tetrahydrafuran, and dried under vacuum. $N,N$-dimethyl-$N$-fluoromethylethanolamine was isolated as a hygroscopic, amorphous white solid (1.386 g, 17.7%); mp 184–185°C (dec.); $^1$H NMR (400 MHz, D$_2$O), δ 3.08 (d, $^2$J = 2.1 Hz, 6 H), 3.45–3.48 (m, 2 H), 3.90–3.93 (m, 2 H), 5.28 (d, $^2$J = 44.9 Hz, 2 H); $^1$C NMR (100 MHz, D$_2$O), δ 47.18, 55.28, 63.09, 95.77, 97.97; $^{18}$F NMR (376.5 MHz, D$_2$O) δ 106.45 (nt, J = 45.2 Hz); HRMS (FAB) Calcd for (M-H)$^+$ C$_8$H$_{13}$ONF: 122.0981, Found 122.0984.

Synthesis of $^{[18}$F$]FCH$. FCH was synthesized via the intermediate FBM (Fig. 1). The synthesis of FBM was essentially that of Eskola et al. (13), which was modified from Coenen et al. (14). The alkylation with FBM of dimethylamine, isolation of the resultant FCH, and performance of quality control HPLC were modified from the techniques used by Hara et al. (4) for synthesis and quality control of $^{[14}$C]choliniodide. FBM was produced by reaction of dibromomethane (0.05 ml) with no-carrier-added $^{[18}$F]$^{[1}$fluoride assisted by (Kryptofix 2.2.2.Ki),Co$_3$(10 μmol) in dry acetonitrile (0.7 ml). FBM was isolated by gas chromatography (Poropak Q, 80/100 mesh, 7.8 × 700 mm, 100°C, helium flow = 75 ml/min, retention time = 6 min) and trapped in a solution of 0.1 ml of dimethylethanolamine in acetone (1.5 ml) within a 2.5 ml conical glass vial kept at ~5°C using a Peltier cooling/heatng device (15). The vial was sealed and heated to 100°C for 10 min. The solvent was evaporated under a stream of helium, and the residue taken up in sterile water (5 ml) and transferred to a cation exchange SEP-PAK cartridge (Accell Plus CM Light; Waters). After further washing of the cartridge with sterile water (10 ml), the product was eluted with sterile isotonic saline (>2 ml) and passed through a 0.22-μm sterile filter (Millex GS; Millipore). Radiochemical purity of FCH was measured by analytical HPLC (C-18 250 × 4.6 mm, 0.05

Received 4/6/00; accepted 11/1/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Department of Radiology, Duke University Medical Center, Box 3949, Durham, NC 27710. Phone: (919) 684-7727; Fax: (919) 684-7130; E-mail: trd@petsparc.mc.duke.edu.

2 The abbreviations used are: PET, positron emission tomography; FDG, 2-[$^{18}$F]$^{[1}$fluoro-2-deoxyglucose; CH, trimethyl-2-hydroxyethyl-ammonium); FEC, 2-[$^{18}$F]$^{[1}$fluoromethyl-2-hydroxyethyl-ammonium; FBM, 1[$^{18}$F]$^{[1}$fluorobromomethane; HPLC, high-performance liquid chromatography; HC-3, hemicholinium-3; EGF, epidermal growth factor; FCH, 1[$^{18}$F]$^{[1}$fluoromethyl-2-hydroxyethyl-ammonium; PSA, prostate-specific antigen; CT, computed tomography; MRI, magnetic resonance imaging; SUV, standardized uptake value; PC, phosphocholine; CK, choline kinase; PI-3 kinase, phosphatidylinositol 3-kinase.

110
**18**F-Labeled Choline for Imaging Prostate Cancer

![Reaction sequence for synthesis of FCH.](Image)

M phosphoric acid and 1 mm 2-naphthalenesulfonic acid in 80% water/20% methanol, 0.5 ml/min, retention time = 4.4 min) using nonradioactive fluorocholine as a reference standard. The sample was doped with 0.1 mg of choline chloride before administration on the HPLC to avoid variable retention of the high specific activity \([^{18}\text{F}]\)FCH on the column.

**Accumulation of Radiotracers by PC-3 Human Prostate Cancer Cells.** Cells (2.5 \(\times\) 10^6/well) were seeded on 6-well plates and incubated for 2 days at which time >90% confluency was reached. The incubation medium was DMEM supplemented with 10% calf serum. The medium contained 15 mM glucose. On the day of the study, the medium was refreshed using a volume of 1 ml in each well. Cells were incubated in control conditions or with the addition of metabolic and growth factor receptor inhibitors to test the specificity of uptake of the radiotracers to specific inhibitions. The inhibitor of choline uptake and phosphorylation, HC-3 (Research Biochemicals, Natick, MA), was added to give a concentration of 5 mM. The PI-3 kinase inhibitor LY294002 (Calbiochem, San Diego, CA) was used at a concentration of 15 \(\mu\)M. The EGF receptor kinase inhibitor AG1478 (Calbiochem) was added at a concentration of 50 nM. The concentrations of the inhibitors were \(\pm\)10 times their respective literature in vivo IC_{50} values for choline phosphorylation (HC-3; Ref. 16), PI-3 kinase inhibition (LY294002; Ref. 17), and EGF receptor kinase inhibition (AG1478; Ref. 18). Following a 30-min incubation period, the radiotracers, FDG or FCH, were added (\(-2\muCi/well\)) and the cells were incubated for 2 h. The cells were washed three times with PBS solution, released from the plates by briefly incubating with 0.05% trypsin in DMEM, transferred to test tubes, and counted for F-18 radioactivity in a gamma counter. The amount of radioactivity in the cells was normalized by the dose administered to each well.

**Biodistribution Studies in a Murine PC-3 Human Prostate Cancer Xenograft Model.** All animal experiments were conducted under a protocol approved by the Duke University Institutional Animal Care and Use Committee. Androgen-independent prostate cancer cells (PC-3) suspended in matrigel (Collaborative Research, Bedford, MA) at a concentration of 1 \(\times\) 10^6 cells/100 \(\mu\)l were injected s.c. into the flank of male athymic mice (BALB/c nu/nu), 4–6 weeks of age. The mice were maintained in pathogen-free conditions as described previously (19). Body weight and tumor volume were measured weekly, and tumor volume (mm^3) was calculated using the formula \(S^2 \times L/2\), where \(S\) and \(L\) represent the large and small diameters of the tumor, respectively.

After the tumor volume had surpassed 0.5 cm^3, the mice were anesthesitized with pentobarbital (75 mg/kg) before injection of the radiotracer and remained anesthetized throughout the study. \([^{18}\text{F}]\)FCH (20–40 \(\mu\)Ci) and \([^{14}\text{C}]\)choline (4 \(\mu\)Ci) were simultaneously injected into a tail vein. A prescribed duration of time was allowed before procurement of heart, liver, lung, blood, kidney, bone (femur), brain (whole), prostate gland, tumor, bladder, and skeletal muscle. The tissues were weighed, and counted for \(^{18}\text{F}\) in a gamma counter, then dissolved in Solvable (DuPont, Boston, MA) and counted for \(^{14}\text{C}\) in a liquid scintillation counter. For the bladder, the percentage of the injected dose in the urine was determined. For all other tissues, radiotracer uptake was calculated as:

\[
\text{Uptake (\%dose/kg)} = \frac{\text{CPM(tissue)} \times \text{Body Wt. (kg) \times 100}}{\text{Tissue Wt. (g) \times CPM (dose)}}
\]

where cpm = counts per min.

In a separate experiment, the biodistribution of \([^{18}\text{F}]\)FDG was determined in the same animal model with a time of sacrifice of 45 min after injection.

**PET Imaging Studies.** The biodistribution of FCH was investigated in four patients with prostate cancer. The FCH-PET studies were approved by the Duke University Medical Center Investigational Review Board and Cancer Research Committee. The supportive data on toxicity and radiation dosimetry of FCH will be published elsewhere. The subjects were informed of all risks associated with the study, and written informed consent was obtained. Table 1 shows clinical data on the patients evaluated with FCH-PET. Patients 1 and 4, having hormone naive prostate cancer, underwent FCH-PET scanning before and after initiation of androgen deprivation therapy. Imaging was performed using the Advance PET scanner (GE Medical Systems, Milwaukee, WI), having an intrinsic spatial resolution of \(-5\) mm in all directions (20). A transmission scan of the pelvic region was obtained before administration of radiotracer. \([^{18}\text{F}]\)FCH (2.5–5 mCi) was administered i.v. in the first three patients, dynamic imaging of the lower pelvis region was commenced for 20 min (frame sequence = 12 \(\times\) 10 s, 2 \(\times\) 30 s, 1 \(\times\) 2 min, 3 \(\times\) 5 min). After the dynamic scan, a whole-body emission scan (4 min per bed position) was performed without attenuation correction. During image reconstruction, the emission data in the pelvic region were corrected for photon attenuation using the transmission scan. In the fourth patient, both transmission and emission data were collected over the entire thorax to provide an attenuation-corrected whole-body scan. The emission imaging was commenced over the lower pelvis at 5 min after injection to obtain an image of this region before the arrival of radioactive urine at the bladder. The images were reconstructed using an Ordered Subset Expectation Maximum algorithm. Regions of interest were drawn manually on the attenuation-corrected images for evaluation of FCH kinetics in tissues. Standardized uptake values of FCH uptake in tissues were calculated using the attenuation-corrected images according to the equation:

\[
\text{SUV} = \frac{\text{Body Wt. (g) \times C_{\text{FCH}}(nCi/ml)}}{\text{Dose(nCi)}}
\]

where \(C_{\text{FCH}}\) is the concentration of FCH in the tumor region of interest. Patient 3 also underwent an attenuation-corrected whole-body FDG-PET study within 4 days of the FCH-PET study for comparison of image quality and SUV values. Emission imaging was commenced 45 min after i.v. administration of \([^{18}\text{F}]\)FDG (9.7 mCi).

**RESULTS**

**Synthesis of \([^{18}\text{F}]\)FCH.** \([^{18}\text{F}]\)FCH was synthesized in 20–40% radiochemical yield (not decay corrected) in a synthesis time of under 40 min. The radiochemical yield was determined primarily by the yield of the intermediate synthon, \([^{18}\text{F}]\)FBM, because the yield of the alklylation reaction of FBM with dimethylmethylamine was \(>90\%\). In a subset of samples, the final preparation was subjected to mass spectrometry for detection of impurities. No organic material was detected in the samples, consistent with the high specific activity of the no-carrier-added F-18-labeled tracer and adequate isolation of FCH from dimethylmethylamine by the cation exchange SEP-PAK method. Radiochemical purity of \(>98\%\) of FCH was verified by analytical HPLC (Fig. 2).

**Accumulation of FCH and FDG by Cultured PC-3 Human Prostate Cancer Cells.** Under control conditions, cultured PC-3 human prostate cells accumulated FCH at a slightly lower rate in comparison with FDG (Fig. 3). Specific inhibition of choline transport and phosphorylation by HC-3 resulted in a 90% (\(P < 0.001\)) decrease in FCH uptake, whereas FDG uptake was unchanged, demonstrating the specificity of uptake of FCH to the choline processing pathway.

**Table 1 Clinical data on patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Clinical stage</th>
<th>Gleason grade</th>
<th>Serum PSA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>T3NXM1b</td>
<td>4.4</td>
<td>22.1</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>T2NXM0</td>
<td>4.4</td>
<td>7.6</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>T3NXM1b</td>
<td>4.4</td>
<td>4172</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>T3NXM1b</td>
<td>4.4</td>
<td>25.4*</td>
</tr>
</tbody>
</table>

* Patients status after radical prostatectomy and bilateral scrotal orchiectomy with hormone refractory disease.
  * Patients status after radical prostatectomy with hormone naïve disease.
  * Measured 8 months before initial PET study.
Inhibition of PI-3 kinase by LY294002 reduced uptake of FDG and FCH by 26% and 15%, respectively. The EGF receptor kinase inhibitor AG1478 had no affect on either FCH or FDG uptake.

**Biodistribution of FCH in a Murine PC-3 Xenograft Model.**

Table 2 and Fig. 4 show the biodistribution of [18F]FCH, [14C]CH (DuPont NEN Research Products, Boston, MA), and [18F]FDG in mice. The kidneys and liver were found to be the primary tracers. Uptake of FCH by normal brain was one-tenth that for FDG. Biodistribution of FCH in a Murine PC-3 Xenograft Model. **Fig. 2.** Reverse phase HPLC radiochromatogram of FCH. The sample was doped with 0.1 mg of choline chloride.

**PET Imaging in Patients.** Patient 1: FCH-PET imaging of a 59-year-old male with untreated locally advanced clinical stage T3 prostate cancer (serum PSA, 22 ng/ml; Gleason grade, 4.4) demonstrated accumulation of FCH in the prostate and metastasis and reached a plateau by 5 min after injection. Radioactivity concentration rose rapidly in the prostate and metastasis and reached a plateau by 5 min after injection. Radioactivity began to arrive in the bladder at about 8 min after injection, and the concentration increased rapidly over the next 20 min. The SUV of both the primary tumor and the metastasis was 7.7 after 5 min. Radioactivity concentration in the brain of the patient was measured to be <2% that measured in the prostate gland or metastasis. However, the pituitary gland and choroid plexus, which do not have a blood-brain barrier, showed relatively high uptake as previously noted in [11C]CH scans (4). Also in agreement with CH distribution (4), kidneys, liver, scalp tissue and salivary glands showed notable uptake of tracer. Uptake in these normal tissues were observed in all patients and considered normal sites of FCH localization in mice.

**Fig. 3.** Effect on metabolic and growth factor inhibitors on FDG and FCH uptake by cultured PC-3 human prostate cancer cells. Cells were preincubated in control conditions (□) or with 5 mM HC-3 (high-affinity choline transporter and CK inhibitor; ■), 15 μM LY294002 (PI-3 kinase inhibitor; □), or 50 nM AG1478 (EGF receptor kinase inhibitor; ■). Cells were incubated with radiotracers for 2 h. Values represent mean and SD of three wells in each condition. The asterisk denotes statistical significance (P < 0.01) relative to control conditions. A 90% decrease in accumulation of FCH was observed in HC-3-treated cells. Inhibition of PI-3 kinase by LY294002 reduced uptake of FDG and FCH by 26% and 15%, respectively.

**Biodistribution of FCH in a Murine PC-3 Xenograft Model.**

Table 2 and Fig. 4 show the biodistribution of [18F]FCH, [methyl-14C]CH (DuPont NEN Research Products, Boston, MA), and [18F]FDG in mice. The kidneys and liver were found to be the primary sites of uptake for both FCH and CH, similar to previous findings with radiolabeled choline (4, 21). Tumor uptake of the choline analogues and FDG were comparable at 45–60 min after injection. However, the tumor-blood ratio, a diagnostically important parameter, was higher (P < 0.05) at 60 min for FCH (5.3 ± 2.4) than for the other two tracers. Uptake of FCH by normal brain was one-tenth that for FDG (P < 0.0001) and one-half that of CH (P < 0.05). At 30 min, there was ~1% and ~10% of the injected dose in the urinary bladder for CH and FCH, respectively. Together with the observed slower renal clearance of radioactivity from the kidneys and lower blood radioactivity concentrations for FCH relative to CH, these findings are consistent with less reabsorption and excretion of radioactivity from the renal proximal tubular filtrate into the circulation for FCH. Liver uptake was lower (P < 0.05) for FCH than for CH.

**Table 2.** Uptake (% dose kg/100 g) of radiotracers in tissues of mice with PC-3 xenografts.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>FCH (n = 5)</th>
<th>FCH (n = 3)</th>
<th>FCH (n = 5)</th>
<th>CH (n = 5)</th>
<th>CH (n = 3)</th>
<th>CH (n = 5)</th>
<th>FDG (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
<td>30 min</td>
<td>60 min</td>
<td>10 min</td>
<td>30 min</td>
<td>60 min</td>
<td>45 min</td>
</tr>
<tr>
<td>Tumor</td>
<td>3.6 ± 0.6</td>
<td>7.1 ± 2.1</td>
<td>7.9 ± 5.0</td>
<td>3.2 ± 1.8</td>
<td>4.8 ± 1.6</td>
<td>6.7 ± 2.5</td>
<td>8.9 ± 0.7</td>
</tr>
<tr>
<td>Blood</td>
<td>2.7 ± 0.9</td>
<td>3.3 ± 0.2</td>
<td>1.5 ± 0.6</td>
<td>2.1 ± 0.3</td>
<td>1.5 ± 0.9</td>
<td>2.2 ± 1.0</td>
<td>2.8 ± 0.009</td>
</tr>
<tr>
<td>Heart</td>
<td>15.5 ± 5.9</td>
<td>13.2 ± 2.6</td>
<td>12.7 ± 3.2</td>
<td>20.3 ± 7.2</td>
<td>9.7 ± 1.9</td>
<td>9.1 ± 2.5</td>
<td>48.2 ± 17.9</td>
</tr>
<tr>
<td>Brain</td>
<td>0.8 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>1.4 ± 0.7</td>
<td>1.0 ± 0.8</td>
<td>1.7 ± 0.6</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>Lung</td>
<td>18.0 ± 5.3</td>
<td>17.1 ± 1.4</td>
<td>21 ± 4.4</td>
<td>26.0 ± 9.6</td>
<td>9.6 ± 6.6</td>
<td>16.7 ± 3.7</td>
<td>7.4 ± 3.1</td>
</tr>
<tr>
<td>Liver</td>
<td>50.7 ± 15.3</td>
<td>56.7 ± 13.2</td>
<td>58.4 ± 40.6</td>
<td>52.3 ± 11.9</td>
<td>65.2 ± 19.4</td>
<td>67.1 ± 49.7</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>127.7 ± 27.6</td>
<td>116 ± 17</td>
<td>94.3 ± 31.0</td>
<td>99.0 ± 12.9</td>
<td>53.4 ± 7.3</td>
<td>41.5 ± 15.0</td>
<td>5.3 ± 1.9</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>4.4 ± 1.8</td>
<td>1.1 ± 1.0</td>
<td>4.1 ± 0.6</td>
<td>4.7 ± 1.7</td>
<td>2.5 ± 1.5</td>
<td>2.8 ± 1.7</td>
<td>8.3 ± 0.4</td>
</tr>
<tr>
<td>Prostate</td>
<td>6.6 ± 2.2</td>
<td>7.1 ± 2.1</td>
<td>7.1 ± 3.0</td>
<td>9.0 ± 6.8</td>
<td>5.9 ± 2.5</td>
<td>5.5 ± 2.6</td>
<td>2.3 ± 0.4</td>
</tr>
</tbody>
</table>

Fig. 4. Kinetics of FCH and [14C]choline in PC-3 human prostate cancer tumor-bearing mice.
correspondence with choline uptake by tissues. The patient was rescanned with FCH at 2 months after initiating androgen deprivation therapy, at which time his PSA level had decreased to 0.9 ng/ml. Both the primary tumor and osseous metastasis were visualized in the follow-up study; however, the SUVs for FCH uptake by the tumors were substantially lower than in the initial study [SUV, 3.0 in primary tumor (61% decrease); SUV, 2.4 in ischial metastasis (68% decrease)].

Patient 2: Transmission and FCH emission scans of a 79-year-old male with hormone naïve clinical stage T2 prostate cancer were commenced over the lower pelvis, but the prostate was not in the field-of-view as revealed by the later whole-body scan. Radioactivity uptake was demonstrated in the prostate gland in the whole-body scan, but it was not quantifiable due to lack of attenuation correction in this region. No metastatic lesions were detected in the PET images. A recent radionuclide bone scan of the patient also showed no evidence of osseous metastases. Positioning of the prostate gland within the field-of-view of the PET scanner in the initial attenuation-corrected scans was made difficult by the obesity of the patient (height, 1.8 m; weight, 147 kg).

Patient 3: FCH-PET imaging of an 80-year-old male status after radical retropubic prostatectomy and bilateral scrotal orchiectomy with progressive hormone refractory prostate cancer (PSA, 4172) demonstrated extensive uptake of tracer in both bones and soft tissue lesions (Fig. 6). SUV values in osseous lesions of the pelvis region ranged between 3.8 and 8.0. SUV values for soft tissue lesions could not be quantified due to the lack of attenuation correction in the corresponding regions, but their signal intensities were similar to those of nearby osseous metastases. The same patient was scanned with FDG within the same week (Fig. 6). The FDG-PET images demonstrated fewer lesions and less pronounced uptake in the detected lesions. SUV values for FDG were approximately one-half those observed for FCH in the same lesions. No radioactivity was observed in the urinary bladder on the FCH whole-body scan obtained at ~25–29 min after injection. Following the PET scan (at ~1 h after injection), the patient produced 70 ml of urine that was measured to contain 1.3% of the injected dose of radioactivity. The experience in the first three patients led us to modify the scanning protocol to allow for imaging of the prostate gland with minimal chance of confounding activity in the urinary bladder: a single whole-body scan would be acquired, commencing over the pelvis 4–5 min after injection of FCH.

Patient 4: A 65-year-old man with a history of clinical stage T3b prostate cancer treated by radical retropubic prostatectomy was diagnosed with metastatic disease by bone scan. FCH-PET imaging was performed before and after initiating androgen deprivation therapy. In the first study, whole-body emission scanning was started 5 min after injection, beginning at the pelvic region to image the pelvis and prostatic bed before arrival of urinary radioactivity. The images (Fig. 7) demonstrated FCH uptake (SUVs, >8) in several locations presumed to be local recurrence in the prostatic bed and metastatic prostate cancer in the pelvic lymph nodes and bone. The osseous metastases seen on FCH-PET images were corroborated by recent bone scan results. The patient was rescanned 2 weeks after initiating androgen deprivation therapy, at which time his serum PSA was 121 ng/ml. The repeat scan showed 35–40% decreases of SUVs in the various tumors (Fig. 7). Of particular note, a positive single lesion in the left pelvis presumed to be prostate carcinoma within a pelvic lymph node was not visualized on the repeat scan.

DISCUSSION

PET is used to detect and stage cancer because of its unique strength in providing noninvasive assessment of metabolic and physiological rates through tracer techniques. The present results with
FCH confirm the potential use of positron-labeled choline analogues (7) for detection of prostate cancer. Differentiation of malignant cancer tissue from neighboring nonmalignant tissues may be accomplished by exploiting changes in choline processing that may occur in response to metabolic, genetic, or microstructural changes in malignant cells.

Choline is transported into cells and used for synthesis of phospholipids and sphingomyelin. Intracellular choline is rapidly metabolized to PC or oxidized by choline dehydrogenase and betaine-aldehyde dehydrogenase to betaine (mainly in liver and kidneys). Phosphorylation of choline, catalyzed by CK, is an obligatory step for incorporation of choline into phosphatidylcholine. Once phosphorylated, the polar PC molecule is trapped within the cell. Extensive studies using magnetic resonance spectroscopy (22) and biochemical analyses (23–26) have revealed elevated levels of choline, PC, and phosphoethanolamine in many types of cancer cells. The activity of CK has been found to be up-regulated in malignant cells (24–26), providing a potential mechanism for the enhanced accumulation of radiolabeled choline analogues by neoplasms. These findings have motivated the development of magnetic resonance spectroscopic imaging techniques.
for mapping of choline levels in prostate cancer (27, 28) and brain tumors (29, 30). For localization of prostate cancer in a sextant of the prostate (27) and diagnosis of extracapsular extension of prostate cancer (28) using proton MR imaging, the addition of magnetic resonance spectroscopy imaging data on the ratio of choline plus creatine to citrate was found to improve diagnostic sensitivity and accuracy, particularly for less experienced readers (28).

The present results indicate that FCH closely mimics choline in its uptake and sequestration by prostate cancer cells. Pretreatment of cultured PC-3 human prostate cancer cells with 5 mM HC-3, a specific inhibitor of choline uptake and phosphorylation (16, 31), resulted in a 90% decrease in FCH accumulation. However, because the biochemical form of F-18 radioactivity was not determined in our studies and HC-3 acts as an inhibitor of both choline transport and CK-mediated phosphorylation (16, 31), we cannot strictly conclude that FCH uptake by the cells was dependent on CK activity. The inhibitory effects of PI-3 kinase and EGF receptor kinase inhibitions on FCH accumulation were less pronounced than for choline transport/phosphorylation inhibition by HC-3, suggesting the existence of other signaling pathways that are able to activate choline transport and phosphorylation within malignant cells.

FCH showed accumulation in PC-3 prostate cancer xenografts in mouse similar to radiolabeled choline. By 1 h after injection the tumor-blood ratio had reached about 5:1, suggesting a trapping of tracer in the tumor cells consistent with metabolic trapping via phosphorylation by CK. However, the accumulation of tracer in tumor tissue was low relative to the normal uptake by kidneys, liver, heart, and lung. The human imaging data also showed high normal uptake of FCH in kidneys and liver but relatively low uptake in lung and heart in comparison with the mouse data. The prostate cancer metastases in the ribs were well differentiated from normal lung in the images. These data suggest a species difference in the lung and heart uptake of FCH.

Consistent with previous data with [11C]CH (4, 7), blood clearance for FCH is very rapid in the human, and excellent tumor to background contrast is obtained in PET images by 3 min after injection. The rapidity of the uptake process reveals the avidity of the choline transport system in prostate cancer as well as certain normal tissues. Tissue perfusion, transporter density, and CK activity are presumably important determinants of tracer uptake and sequestration by tissues. The short residence time of FCH in the blood may limit the diffusion of tracer into areas that are poorly perfused. The potential effects of perfusion, particularly during interventions that may affect tumor vascularity and vasomuscular tension, should be carefully considered when interpreting the uptake data. Also, there is potential for poor sensitivity of this technique to detect malignant tissue that is poorly perfused. On the other hand, the rapid and extensive clearance of tracer from the bloodstream minimizes the effects of any potential radiolabeled metabolites on the tissue kinetics. It also makes the SUV parameter more useful than for a tracer that has a slow (and, therefore, more variable) blood clearance at the time of measurement of tissue uptake. Finally, the rapid blood clearance allows PET imaging to be commenced as early as 4–5 min after injection.

FCH differs from a previously reported (11) analogue, FEC, by a single methylene group. It is not clear from the limited data provided in abstract form on FEC (11) what differences may exist between the tracer kinetic properties of FCH and FEC. Our finding that FCH and CH have similar biodistributions in mice suggest that electronegativity effects of the α-fluorine atom are well tolerated by the processes (presumably CK catalyzed phosphorylation) that are responsible for the trapping of choline analogues in tissues. The notably higher urinary clearance of FCH relative to choline was also seen with FEC (11). Choline is efficiently reabsorbed by renal proximal tubular cells under normal conditions. The primary route of clearance of choline from the body depends on oxidation via choline dehydrogenase and betaine-aldehyde dehydrogenase (primarily in the kidney) to betaine. Betaine is then excreted in the urine. The more pronounced early urinary clearance of the radiofluorinated choline analogues could be explained by incomplete tubular reabsorption of intact tracer or enhanced excretion of oxidative metabolites.

Of high interest for future investigations is determining the metabolic fate of FCH in the cancer cell in relationship to choline processing in normal and transformed (malignant) cells. The finding of avid uptake of FCH in prostate cancer with prolonged retention strongly suggests metabolic sequestration of the tracer consistent with trapping in the cell subsequent to phosphorylation in analogy to the known processing of choline. However, detailed biochemical studies are required to confirm this indication. It is known that transformation is accompanied by increases in both choline and PC levels in a variety of cancer types (22–26). The activation of the choline uptake and phosphorylation seems to be a later event involved in a cascade of intracellular signal transduction events that result in transformation, including activation of ras-GTPase-activating protein (32), PI-3 kinase (33), and various protein and tyrosine kinases (33–35). Cuadrado et al. (36) showed that PC triggered DNA synthesis in quiescent NIH3T3 fibroblasts, whereas choline, phosphorylserine, and phosphoethanolamine had no effect. The CK inhibitor, HC-3, was found to block proliferation induced by growth factors, but the blockade was bypassed by PC addition. Thus, PC seemed to act as a prerequisite second messenger for mitogenic activity, implicating CK activity as a critical step during regulation of cell proliferation by growth factors (36). If, as suspected, CK-mediated phosphorylation of radiolabeled choline analogues determines their retention in tissues, these radiotracers may not only be useful as cancer detection probes, but may also be used in experimental studies as unique tools to allow noninvasive monitoring of the regulation of an important signal transduction pathway.

**Implications for Clinical PET Studies.** There is keen interest in the development of a sensitive and accurate noninvasive imaging technique for prostate cancer. The existing imaging technologies, including CT (37, 38), MRI (38, 39), ultrasound (39), nuclear medicine scanning with 111In-capromab pendetide (40), and PET imaging with FDG (3, 8, 41–44) are not sufficiently sensitive to obviate the need for surgical staging pelvic lymphadenectomy. Radioisotope bone scans are highly sensitive in detection of osteoblastic bone metastases, but false positive readings may occur due to tracer uptake associated with degenerative joint disease and related benign bone diseases and abnormalities. Furthermore, bone scans cannot detect metastases in lymph nodes and other soft tissue. Hara et al. (7) reported superior sensitivity of [11C]CH-PET relative to FDG for the detection of metastatic prostate cancer. Initial findings reported herein with FCH in prostate cancer patients seems to agree with the data on [11C]CH. The longer half-life of fluorine-18 (110 min) is better suited for the demands of clinical PET and may allow off-site production and distribution of the radiotracer. The rapid uptake kinetics of the positron-labeled choline analogues allow transmission scanning before emission scanning while maintaining efficient use of the PET scanner. The trapping of FCH in neoplasms makes PET scanning a straightforward process uncomplicated by the effects of redistribution of tracer over time. Furthermore, the simple kinetics of FCH may support the usefulness of the ratio of tumor to reference tissue (e.g., lung) concentrations as a quantitative index, rather than the SUV parameter. However, the presence of radioactivity in the urine encourages early imaging of the pelvic region before arrival of radioactivity at the urinary bladder.

Preliminary data obtained in four patients with prostate cancer...
suggest that further clinical research is warranted to determine the diagnostic accuracy and sensitivity of 18F-labeled choline analogues in prostate cancer detection and staging. FCH-PET imaging of two patients with advanced disease demonstrated increased uptake in soft tissue lesions in the pelvis that may correlate with pelvic lymph nodes. Thus, FCH-PET imaging may represent a potential new imaging technique for identifying nodal metastases and/or local recurrences in patients with advanced disease; however, the validity of the technique must be correlated with histological specimens in future prospective studies before a definitive conclusion can be reached. Nevertheless, preliminary data demonstrating increased uptake of FCH in known metastatic lesions identified by bone scan in patients with advanced prostate cancer does provide a rationale for future prospective clinical investigations to evaluate the usefulness of this technique in prostate cancer detection and staging.

Fluorine-18-labeled choline analogues may be useful in other malignancies where elevated choline and PC levels have been demonstrated, such as tumors in the breast, lung, colon, and brain (22, 23, 26, 29, 30, 45, 46). FCH-PET imaging may also be useful for monitoring the therapeutic efficacy of traditional and novel chemo-therapeutic agents. The SUV index should be a fairly robust parameter to indicate tracer trapping in neoplasms, but the potential effects of therapy on tumor blood flow needs to be carefully considered since the uptake kinetics are likely to be highly dependent on tissue perfusion.


