Specific and High-Level Targeting of Radiolabeled Octreotide Analogues to Human Medulloblastoma Xenografts

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ABSTRACT

Purpose: The objective of this study was to determine the feasibility of exploiting the overexpression of somatostatin subtype-2 receptors (sstr2) on human medulloblastoma cells to develop targeted radiodiagnostics and radiotherapeutics for this disease.

Experimental Design: The following radiolabeled peptides were prepared using chloramine-T and evaluated: [131I]-Tyr3octreotide ([131I]TOC), [131I]-Tyr3octreotate ([131I]TOCA), involving substitution of Thr(ol)8 in TOC with Thr8, and glucose-[131I]-Tyr3octreotide ([131I]GlucTOC) and glucose-[131I]-Tyr3octreotate ([131I]GlucTOCA), prepared by conjugation of glucose to the peptide NH2-terminus. Specific internalization of the peptides by sstr2-expressing AR42J rat pancreatic carcinoma cells in vitro was evaluated in paired-label assays. The tissue distribution of i.v. administered [125I]TOC, [131I]TOCA, [131I]GlucTOC, and [131I]GlucTOCA was evaluated in athymic mice bearing s.c. D341 Med human medulloblastoma xenografts.

Results: Compared with [125I]TOC, internalized radioiodine levels were higher for the other three peptides. For example, internalized counts were 1.9 ± 0.2, 2.0 ± 0.3, and 5.7 ± 1.9 times higher for [131I]GlucTOC, [131I]TOCA, and [131I]GlucTOCA after a 3-h incubation, respectively, demonstrating that carbohydrate and COOH-terminus modification significantly improved the retention of radioiodine activity in sstr2-expressing tumor cells. COOH-terminus modification significantly increased [131I]localization in D341 Med medulloblastoma xenografts ([131I]TOCA, 8.1 ± 2.2% of injected dose/g (1% ID/g); [131I]TOC, 3.9 ± 0.5% ID/g at 1 h), whereas carbohydrate of the NH2-terminus resulted in even higher gains in tumor accumulation ([131I]GlucTOC, 11.1 ± 1.8% ID/g; [131I]GlucTOCA, 21.4 ± 7.3% ID/g). In addition, the three modified peptides exhibited liver activity levels that were less than half those of [131I]TOC. Uptake of the two glucose-peptide conjugates in this human medulloblastoma xenograft was blocked by coinjection of 100 μg of octreotide, demonstrating that it was receptor-specific. Tumor:normal tissue uptake ratios for [131I]GlucTOCA generally were higher that those for [131I]GlucTOC. At 1 h, tumor:normal tissue ratios for [131I]GlucTOCA were 29:1, 15:1, 8:1, 8:1, 240:1, and 82:1 for blood, liver, kidney, spleen, brain, and muscle, respectively.

Conclusions: Our findings suggest that additional investigation of radiolabeled GlucTOCA analogues for the imaging and targeted radiotherapy of medulloblastoma is warranted.

INTRODUCTION

Medulloblastoma is the most frequently occurring central nervous system tumor in children (1). Surgery and external beam radiation are curative in some patients; however, toxicities are often associated with conventional radiotherapy. Unfortunately, ~30% of children with standard-risk medulloblastoma and 40–60% of children with high-risk medulloblastoma fail therapy, with dismal outcome (2). Medulloblastoma exhibits a marked propensity for leptomeningeal dissemination and production of neoplastic meningitis, resulting in rapid mortality (3). About 5–30% of patients have neoplastic meningitis at the time of presentation with another 5–10% exhibiting this metastatic pattern during the course of their disease (4). Although some children treated with neuraxis external beam radiotherapy survive, long-term radiation-induced side effects are severe (5–7).

In vitro studies have shown that human medulloblastoma cells are relatively radiosensitive (8), suggesting that radiation might be effective in treating this disease if strategies that minimize normal tissue toxicity could be developed. Targeted radiotherapy is an appealing approach for medulloblastoma therapy because of the potential for selectively irradiating tumor cells while sparing normal central nervous system tissues. The potential utility of this strategy for treating tumors of the central nervous system has been demonstrated in gliomas (9, 10). Furthermore, neoplastic meningitis, in which tumor is present as thin sheets on the leptomeningeal surface and as free-floating cells within the subarachnoid space, is a setting well suited for the compartmental delivery of therapeutic agents such as radiolabeled monoclonal antibodies (11, 12).

A number of radiolabeled molecules have been evaluated as potential targeted radiotherapeutics for the treatment of medulloblastoma. Antibodies M340 and U181.4 directed against the developmentally associated antigen L1 present on...
primitive neuroectodermal tumors have been described (13). The median survival of 9 pediatric and 9 adult medulloblastoma patients treated with $^{131}$I-labeled M340 and U1J18.4 was 6 and 32 months, respectively (11). In vitro studies demonstrated that α-particle emitting $m^{211}$Atastatobenzylguanidine was highly cytotoxic for two human medulloblastoma cell lines; however, most cell lines failed to take up appreciable levels of the compound (14).

Recent studies suggest that the sstr2 is overexpressed on human medulloblastomas. In vitro analyses of the binding of $[125]$I TOC to 14 medulloblastoma biopsies revealed a very high level expression of sstr2 (15). Reverse transcription-PCR and Southern blot analysis demonstrated sstr2 expression in 78% of human medulloblastoma biopsies and multiple human medulloblastoma cell lines (16). In the later study, a $K_d$ of ~1 nM was measured for the binding of somatostatin to medulloblastoma tissue and cells.

The current study was undertaken to determine whether human medulloblastoma xenografts could be effectively targeted by radioiodinated somatostatin receptor-avid peptides. In addition to TOC, octreotide analogues with COOH-terminal oxidation and NH$_2$-terminal carboxylation were investigated based on prior studies suggesting that these modifications can improve tumor retention and tumor:normal tissue ratios (17, 18). TOC, TOCA, Gluc-TOC, and Gluc-TOCA were radioiodinated, their internalization was compared on the sstr2-expressing AR42J cell line, and their tissue distribution was evaluated in athymic mice with s.c. human D341 Med medulloblastoma xenografts.

**MATERIALS AND METHODS**

**Peptide Synthesis.** The procedures used for the preparation of Gluc-TOC, TOC, Gluc-TOCA, and TOCA, as well as their iodinated analogues, were based on manual synthetic methods reported previously (17, 18) with minor modifications. Briefly, F-moc-protected threonin (for TOC derivatives) was anchored to 3,4-dihydro-2H-pyranyl-2-ylmethoxymethylpolystyrene resin, and for TOCA derivatives threonine was anchored to trityl chloride polystyrene resin. The peptides were assembled using a standard F-moc protocol. Lys, with its side chain protected with a Dde group (versus Boc in Refs. 17 and 18), was used in the synthesis of the glucose-peptide conjugates. The peptides were cleaved from the resin using a 1:1 mixture of dichloromethane and a 95/2.5/2.5 (v/v/v) mixture of trifluoroacetic acid/water/tri-isobutylsilane. Disulfide bond formation was achieved by treatment of the peptide solution with H$_2$O$_2$ in a mixture of tetrahydrofuran/0.02 M ammonium acetate [buffered with saturated NaHCO$_3$ to (pH 7.0)]. A Maillard reaction and subsequent Amadori rearrangement was used to glucosylate the peptides (19). Peptides with Dde-protected Lys were treated with 10 equivalents of α-glucose at 60°C in methanol containing 5% HOAc. Finally, the Dde group was removed by treatment with 2% hydrazine (v/v) in dimethylformamide. Mass spectra were recorded using the liquid chromatography-mass spectrometry system LCQ from Finnigan (Bremen, Germany) using the Hewlett Packard Series 1100 HPLC System. Calculated monoisotopic mass for Gluc-TOC C$_{65}$H$_{77}$N$_{10}$O$_{12}$S$_2$ = 1196.5; found: $m/z =$ 1197.2 [M+H]+, $m/z =$ 1219.3 [M+Na]+. Calculated monoisotopic mass for Gluc-TOCA C$_{65}$H$_{76}$N$_{10}$O$_{12}$S$_2$ = 1210.5; found: $m/z =$ 1211.5 [M+H]+, $m/z =$ 1233.5 [M+Na]+. Calculated monoisotopic mass for iodo-Gluc-TOC C$_{65}$H$_{77}$N$_{10}$O$_{12}$S$_2$ = 1322.4; found: $m/z =$ 1323.1 [M+H]+, $m/z =$ 1345.2 [M+Na]+. Calculated monoisotopic mass for iodo-TOCA C$_{65}$H$_{76}$N$_{10}$O$_{12}$S$_2$ = 1174.3; found: $m/z =$ 1175.0 [M+H]+.

**Peptide Radioiodination.** To a solution of sodium $[125]$I iodide or sodium $[131]$I iodide (1–2 mCi in 3 μl of 0.1 ν NaOH; NEN Life Sciences, Billerica, MA) in a Reacti vial was added 20 μl of 0.05 μ phosphate buffer (pH 7.5) and a solution of peptide in 0.05 μ acetic acid (14 μg in 20 μl). After vortexing this mixture, a solution of Chloramine-T in the above phosphate buffer (1.6 μg in 20 μl) was added. After a 1-min reaction at room temperature, the labeled peptide was isolated from the reaction mixture by reversed-phase HPLC. HPLC was conducted using a Beckman System Gold system equipped with a Model 126 programmable solvent module, a Model 168 diode array detector, a Model 170 radiospectro detector, and a Model 406 analogue interface module. A Waters μ Bondapak C18 (10 μm, 3.9 × 300 mm) column was eluted with a linear gradient (10–40% B over a period of 30 min) consisting of solvents 0.1% trifluoroacetic acid in water (A) and 0.1% trifluoroacetic acid in acetonitrile (B) at a flow rate of 1 ml/min.

Radiochemical yields were ~70% for each of the four peptides. In each case, the HPLC system was able to fully resolve the radioiodinated peptide from the unlabeled peptide. Because no coeluting carrier peak was observed in the labeled peptide peak on the UV trace of the HPLC, the specific activity of the labeled peptides was assumed to be that of the radioiodine used in their preparation. The retention times of TOC, TOCA, Gluc-TOC, Gluc-TOCA, and their respective iodinated derivatives were 16.0 and 20.3, 17.9 and 21.8, 15.8 and 20.0, and 14.6, and 18.4 min, respectively. The HPLC fractions containing each labeled peptide were pooled, and most of the acetonitrile solvent was removed by evaporation with a gentle stream of argon. The resultant solution was passed through an activated solid-phase cartridge (tC18ENV; Waters). The cartridge was washed with 5 ml of water, and 2 × 0.5 ml HOAc, and then eluted with 5 × 0.25 ml ethanol. Most of the radioiodine activity eluted in ethanol fractions 2–4. These fractions were pooled, evaporated with a stream of argon at room temperature, and reconstituted in PBS (pH 7.14) for use in the cell culture and biodistribution experiments.

**Cells and Culture Conditions.** The sstr2-expressing AR42J rat pancreatic tumor cell line (20) was obtained from the American Type Culture Collection (Manassas, VA) and grown in DMEM containing 2 mM glutamine, 10% FCS, and 5 g/liter glucose. D341 Med is a continuous cell line derived from a tumor biopsy from a patient withAT a cerebellar medulloblastoma.
It was grown in 10% FCS and Zinc Option medium (21). Cells were maintained in a humidified atmosphere (37°C, 5% CO₂).

**Internalization Assay.** These studies were initially performed with AR42J cells, because this cell line has been used in the past to characterize the internalization behavior of a variety of radiolabeled octreotide conjugates (20, 22). Experiments were done in paired label format using a protocol reported earlier (23). To provide a common point of comparison, [¹²⁵I]TOC was included in each assay in tandem with either [¹³¹I]TOCA, [¹³¹I]Gluc-TOC, or [¹³¹I]Gluc-TOCA. About 200,000 cpm each of labeled peptide (≈90 pg/peptide based on peptide specificity activity) was incubated at 37°C in quadruplicate for 30 min, or 1, 2, 3, or 4 h with 5 × 10⁵ AR42J cells in tubes containing 1 ml of DMEM supplemented with 30 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, penicillin (10⁵ units/liter), fungizone (0.5 mg/liter), and 0.2% BSA adjusted to pH 7.4. In parallel, incubations were done in the presence of 1 μM octreotide to correct for nonspecific uptake. To determine the intracellular activity, the cells were incubated with 1 ml of 20 mM sodium acetate in HBSS (pH 5.0) for 10 min at 37°C. After the incubation, the cells were washed twice with ice-cold internalization medium. After removing the supernatant, the cells were washed once with HBSS (pH 5.0), solubilized in 1 N NaOH, and counted for radioactivity. An additional assay was performed to compare the internalization of [¹²⁵I]TOC and [¹³¹I]Gluc-TOC by the D341 human medulloblastoma cell line. The assay format was identical to that described above except that Zinc option medium was used.

**Receptor Binding Determination.** To determine the binding affinity of [¹²⁵I]Gluc-TOC and [¹²⁵I]Gluc-TOCA, an in vitro assay was done with AR42J cell membranes using a protocol adapted from that reported by Froidevaux et al. (24). In a 96-well plate, quadruplicates of 50 μg of membranes in 100 μl of buffer [20 mM Tris buffer (pH 7.5), containing 0.25 M sucrose, 1 mg/ml bacitracin, 0.1 mg/ml soybean trypsin inhibitor, and 0.125 mg/ml phenylmethylsulfonyl fluoride] per well, were incubated for 1 h at 37°C with 25–30 μCi of either [¹²⁵I]Gluc-TOC or [¹²⁵I]Gluc-TOCA and various concentrations of the corresponding cold iodinated peptide in binding medium (MEM supplemented with 25 mM HEPES, 0.4% BSA and 1 mM 1, 10-phenanthroline). The total volume per well was adjusted to 0.2 ml. At the end of the incubation, the plate was placed on a vacuum manifold until the wells were dry. Each well was washed with 0.25 ml of ice-cold buffer and dried as above. The filters were removed using a punch apparatus and counted using a gamma counter. Radioactivity bound to the membranes with coincubated with 50 nM unlabeled peptides was assumed to be nonspecific binding. Scatchard analysis was performed using the Kell program (Biosoft, Ferguson, MO) to calculate the Kd and the Bmax.

Additional assays were performed to evaluate the binding of [¹²⁵I]Gluc-TOCA to D341 Med cells. We were unsuccessful in obtaining reproducible results with membranes from this cell line so a live cell format was used. For these measurements, 1 × 10⁵ cells in 100 μl were added per well. The medium consisted of RPMI 1640 supplemented with 10% FCS, and was used for both the incubation and wash. The plates were vacuumed gently to avoid rupturing the cells and were dried overnight in a 37°C nonhumidified incubator. All of the other procedures were identical to those outlined above for the membrane assays.

**Tissue Distribution Measurements.** Athymic BALB/c-nu (SPF) mice weighing 20–25 g were obtained from the breeding colony maintained at the Duke University Comprehensive Cancer Center Isolation Facility. Details of the D341 Med human medulloblastoma model have been provided in a previous publication (21). Briefly, the D341 Med cell line was maintained in vivo by serial passage in athymic mice, with passage numbers 9–14 being used in these studies. Tumors were transplanted into the right flank with an inoculation volume of 50 μl. The biodistribution studies were initiated when tumors were about 200–300 mm³. Mice received 3–5 μCi (approximately 2–3 ng) of [¹³¹I]TOC, [¹³¹I]TOCA, [¹³¹I]Gluc-TOC, or [¹³¹I]Gluc-TOCA, and groups of 5–7 animals were killed 30 min and 1 h after injection of each labeled peptide. Additional groups of animals were studied at 4, 8, and 24 h for [¹³¹I]Gluc-TOCA and 4 and 24 h for [¹³¹I]Gluc-TOC. To determine the specificity of glucose-peptide conjugate uptake, unlabeled octreotide (100 μg; ∼4 mg/kg) was coinjected with [¹³¹I]Gluc-TOCA and [¹³¹I]Gluc-TOC in additional groups of mice, and the tissue distribution of [¹³¹I]activity was determined at 30 min after injection. Mice were killed with an overdose of halothane, dissected, and tissues of interest were isolated. Weights of blotted-dried tissues were determined, and the tissues were counted along with dose standards. The results were expressed as %ID/g unless otherwise stated. An unpaired Student’s t test was used to

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<tr>
<td>30 min</td>
<td>221 ± 87%</td>
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<td>3 h</td>
<td>204 ± 29%</td>
<td>186 ± 20%</td>
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<td>4 h</td>
<td>196 ± 18%</td>
<td>218 ± 59%</td>
<td>451 ± 55%</td>
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determine the statistical significance of differences in uptake between different peptides.

RESULTS

Peptide Radioiodination. Radiochemical yields were generally ~70% for the four peptides. After purification by reversed-phase HPLC, the radiochemical purity was >98%. No free iodide was detected in any of the preparations used for the in vitro or tissue distribution studies. Because the HPLC system was able to fully resolve the radioiodinated peptide from the unlabeled peptides, specific activities of the 125I-labeled and 131I-labeled products were estimated to be >2000 Ci/mmol.

Cell Retention and Internalization. The uptake of radiiodine activity in AR42J cells after incubation at 37°C with the four labeled octreotide analogues was measured as a function of time. Three paired-label experiments were done in which the percentage of the radioactivity in the incubation medium that was internalized for [125I]TOCA, [131I]Gluc-TOC, and [131I]Gluc-TOCA were compared with that determined for [125I]TOC. In all of the cases, nonspecific internalized activity, determined by coincubation of the labeled peptides with 1 μM octreotide, was between 5 and 10% of total internalized counts. The results described below were corrected for nonspecific binding. As shown in Fig. 1, the percentage of radiiodine activity internalized by AR42J cells was significantly higher for [131I]Gluc-TOC compared with [125I]TOC at all of the time points (P < 0.01). To facilitate comparison of the intracellular activity after internalization of the four peptides, the specific internalization percentages were normalized to the results obtained for [125I]TOC in each experiment. As summarized in Table 1, modification of the COOH terminus of octreotide by replacing Thr(ol)8 with Thr8 in TOCA resulted in a 196–18% to 278–59% increase in internalized activity over the 4-h observation period. Conjugation of glucose to the NH2 termini of TOC provided a similar increase in the percentage of internalized activity. Finally, the results for [131I]Gluc-TOCA indicate that modification of both the COOH- and NH2 termini of TOC provided a 4.0–5.7-fold increase in the retention of radioactivity in the intracellular compartment compared with TOC itself. An internalization assay also was performed in which the labeled peptides were incubated with D341 Med human medulloblastoma cells, and the results obtained were very similar to those described above for the AR42J cell line. Intracellular radioiodine activity in D341 Med cells for [131I]Gluc-TOC was 238–68%, 164–35%, 214–42%, 219–30%, and 222–52% higher than coincubated [125I]TOC at 30 min, 1, 2, 3, and 4 h, respectively. These values were not significantly different from those given in Table 1 for the intracellular uptake of radioiodine for these peptides by AR42J cells.

Receptor Binding. Both [125I]Gluc-TOC and [125I]Gluc-TOCA bound with subnanomolar affinity to AR42J cell membranes (Fig. 2). For [125I]Gluc-TOC, Scatchard analysis revealed an apparent equilibrium Kd of 0.37 ± 0.04 nM and a maximum binding Bmax of 693 ± 41 fmol/mg. Similar results were obtained for the binding of [125I]Gluc-TOCA to AR42J cell membranes (Kd, 0.47 ± 0.04 nM; Bmax, 872 ± 49 fmol/mg). The binding of [125I]Gluc-TOCA to D341 Med human medulloblastoma cells also was determined (Fig. 3). The Scatchard transformation of the binding data indicated a Kd of 1.9 ± 0.2 nM and a Bmax of (4.0 ± 0.4) × 104 receptors per cell.

Tissue Distribution of Radioiodinated Octreotide Analogues. The biodistribution of 131I activity after i.v. injection of the four labeled octreotide analogues was determined in athymic mice with s.c. D341 Med human medulloblastoma xenografts. The %ID/g recovered in tumor and liver 30 min and
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1 h after injection is shown in Fig. 4. Substitution of the Thr(ol)\(^8\) in TOC with Thr\(^8\) in TOCA resulted in a significant increase in radiiodine accumulation in these medulloblastoma xenografts at 30 min and 1 h ([\(^{131}\)I]TOC, 3.9 ± 0.5% ID/g; [\(^{131}\)I]TOCA, 8.1 ± 2.2% ID/g at 1 h). Tumor uptake of the two glucose-peptide conjugates was even greater, reaching 11.1 ± 1.8% ID/g and 21.4 ± 7.3% ID/g at 1 h for [\(^{131}\)I]Gluc-TOC and [\(^{131}\)I]Gluc-TOCA, respectively. On the other hand, the liver accumulation of the three other peptides was significantly lower than that of [\(^{131}\)I]TOC. Reduction in liver activity was greatest for [\(^{131}\)I]Gluc-TOCA, which exhibited 4-fold lower levels of radiiodine activity in the liver compared with [\(^{131}\)I]TOC.

Because of their encouraging properties, the glucose-peptide conjugates were selected for more extensive evaluation as tracers for targeting medulloblastoma. The tissue distribution data for [\(^{131}\)I]Gluc-TOC and [\(^{131}\)I]Gluc-TOCA in athymic mice with D341 Med xenografts are shown in Tables 2 and 3, respectively. For both peptides, rapid clearance of radioactivity from both the blood pool and normal tissues known to not express sstr\(_2\) was observed. An exception was the thyroid, where levels of radiiodine increased with time, reaching levels of 2.27 ± 0.49% ID/g and 2.52 ± 0.35% ID/g at 24 h for [\(^{131}\)I]Gluc-TOC and [\(^{131}\)I]Gluc-TOCA, respectively. Thyroid levels for the two glycated octreotide analogues were not significantly different at 30 min and 24 h; however, ~2-fold higher radiiodine levels were observed for [\(^{131}\)I]Gluc-TOCA at 1 and 4 h. Levels of [\(^{131}\)I]Gluc-TOCA also were significantly higher than those of [\(^{131}\)I]Gluc-TOC in the lungs, stomach, and pancreas.

To determine the specificity of peptide accumulation, the tissue distribution of the two glycated peptides was also measured 30 min after coadministration with a blocking dose of 100 μg of octreotide. Under these conditions, levels of [\(^{131}\)I]Gluc-TOC and [\(^{131}\)I]Gluc-TOCA in the D341 Med xenografts were reduced to 15%–25% of control levels, with values of 29:1, 15:1, 1.8:1, 1.4:1, and 1.1:1 seen 1 h after injection, with values of 29:1, 15:1, 1.8:1, 1.4:1, and 1.1:1 observed for blood, liver, kidney, spleen, brain, and muscle, respectively.

**DISCUSSION**

There is currently considerable interest in the utilization of radiolabeled octreotide analogues for the diagnosis and treatment of malignancies that overexpress somatostatin receptors. Most of this work has been directed at pituitary tumors, pancreatic tumors, carcinoids, gastromas, and paragangliomas (9, 25–27). Results of recent studies suggest that it might be possible to also apply these peptides to the targeted radiotherapy of...
Medulloblastomas, a malignancy for which more specific treatment options are sorely needed. High-level expression of sstr2 in human medulloblastoma biopsies and cell lines has been observed both by reverse transcription-PCR, and receptor binding and autoradiography experiments using [125I]TOC as the radioligand (15, 16). Furthermore, higher accumulation of [111In-DOTA-Phe1]-octreotide in medulloblastoma lesions compared with nonspecific [99mTc]DTPA has been reported in a small series of patients (16).

The goal of this study was to investigate several somatostatin analogues with regard to their potential as targeted radiotherapeutics for medulloblastoma. As a point of reference, TOC was included because it is the radioligand shown to bind with high affinity in vitro to human medulloblastomas (15, 16). However, [125I]TOC is not considered to be a realistic option for patient studies because of its low degree of tumor retention and relatively high accumulation in the hepatobiliary system (28, 29). In general, tumor:nongenome organ radiation dose ratios reported for [125I]TOC were not favorable (25, 30) and certainly not suitable for use in a pediatric population where minimizing normal organ radiation dose is an even greater concern than in adults.

Maximizing the retention of radioactivity at the tumor site is an important consideration in the development of these labeled peptides as targeted radiotherapeutics. Receptor-mediated internalization can increase the residence time of the radiolabeled peptide in tumor cells, particularly if the labeled molecule is charged, because the lipopholic lysosomal and cell membranes provide a barrier for escape into the extracellular environment (31). In addition, decays occurring within the tumor cell have a higher geometric probability of traversing the cell nucleus (32), which has generally been considered to be the critical subcellular target for radiotherapy. Finally, radiounudioses that emit subcellular range Auger electrons such as 125I can only be effective when their decay site is intracellular.

The internalization of a variety of radiolabeled somatostatin analogues has been described, and a 100-fold variation in internalized activity has been observed (17, 18, 20, 22, 23, 33, 34). Whereas some of these differences appear to reflect variations in receptor affinity of the radioligands, this is not the only factor, because peptides with comparable binding affinity can have considerably different percentages of internalized and retained (22, 34). It appears that increasing the polarity of the COOH and NH2 terminus of the peptide can result in higher levels of internalized activity; however, the nature and position of the radiolabel can also play a role. For example, the internalization percentage of [DOTA0, 125I-Tyr3]octreotide was reported to be 5-fold higher than that of [DTPA0, 125I-Tyr3]octreotide (34) whereas an opposite effect was seen when the internalization of [111In-DOTA0, Tyr3]octreotide and [111In-DTPA0, Tyr3]octreotide were compared (22).

To facilitate the interpretation of our results, all of the peptides involved in the current study were labeled at Tyr3, and the effects of COOH and NH2 terminus modification on internalization and retention of activity were investigated. In all of the cases, the Phe residue at position 3 were replaced by Tyr to decrease lipophilicity and provide a convenient site for radioiodination. Replacement of the COOH-terminal threoninol with threonine provided an additional negative charge on the TOCA molecule, and this modification yielded a 2-fold increase in internalized activity in both sstr2-expressing AR42J rat pancreatic carcinoma and D341 Med human medulloblastoma cell lines. This may in part reflect the higher affinity of the modified peptide for sstr2 (IC50 = 1.3 ± 0.3 nM, I-TOC; 0.47 ± 0.2 nM, I-TOCA; Ref. 17). The increased internalization of TOCA compared with TOC is consistent with the higher internalization of [111In-DTPA0]TOCA compared with [111In-DTPA3]TOC; however, in this report (17), the affinity of [DTPA0]TOCA was lower than [DTPA3]TOC, suggesting that affinity may not be the only factor.

The receptor binding affinity of the iodinated Gluc-TOC and Gluc-TOCA conjugates to AR42J cell membranes was similar: 0.37 ± 0.04 nM and 0.47 ± 0.04 nM, respectively. These values are somewhat lower than the IC50 values reported (between 0.95 ± 0.30 nM and 1.2 ± 0.2 nM) for the binding of iodinated maltotriose and maltose conjugates of TOCA and TOC, respectively, to cells transfected to express sstr2 (17, 18). The Kd determined in the current study for the binding of iodinated Gluc-TOCA to D341 Med human medulloblastoma cells was identical to that reported by Frühwald et al. (16) for

Table 3  Tissue distribution of [131I]Gluc-TOCA in athymic mice with D341 Med human medulloblastoma xenografts

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<thead>
<tr>
<th>Tissue</th>
<th>%ID/g Tissuea</th>
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<tr>
<td>Liver</td>
<td>3.03 ± 0.83</td>
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<tr>
<td>Spleen</td>
<td>3.56 ± 1.25</td>
</tr>
<tr>
<td>Lungs</td>
<td>13.33 ± 2.63</td>
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<tr>
<td>Heart</td>
<td>0.84 ± 0.14</td>
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<tr>
<td>Kidneys</td>
<td>5.69 ± 0.78</td>
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<td>Stomach</td>
<td>20.99 ± 6.26</td>
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<tr>
<td>Thyroid</td>
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<tr>
<td>Muscle</td>
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<tr>
<td>Pancreas</td>
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<tr>
<td>Blood</td>
<td>1.49 ± 0.33</td>
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<tr>
<td>Brain</td>
<td>0.11 ± 0.02</td>
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<tr>
<td>Tumor</td>
<td>16.97 ± 4.06</td>
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a %ID/g tissue; mean ± SD for n = 6–7.

b % injected dose per organ.
the binding of octreotide to membranes from another human medulloblastoma cell line, D283 Med.

Our recent studies have shown that carbohydration of the NH₂ terminus can decrease the lipophilicity of TOC derivatives without compromising their affinity for sstr² (17, 18). The current study shows that conjugation of glucose to the NH₂ terminus of TOC yielded a 2-fold increase in internalized activity in sstr²-expressing cell lines. Finally, the internalization of [131I]Gluc-TOCA was compared with that of [125I]TOC, and 4–6-fold higher internalized counts were observed. These results suggest that it is possible to optimize both the COOH and NH₂ terminus of TOC, and achieve additive, and perhaps even synergistic, gains in internalization.

The relative degree of retention of internalized activity in sstr₂-expressing cells in vitro for the four radioiodinated peptides was predictive of their tumor localizing capacity in the D341 Med human medulloblastoma xenograft model. Internalization is clearly only one factor influencing the accumulation and retention of labeled octreotide analogues by somatostatin-receptor-expressing tumors. Another potential variable is receptor affinity. The lower affinity of I-TOC compared with the other three peptides is consistent with the observation that its tumor accumulation is relatively low. On the other hand, the tumor uptake of [131I]TOCA is significantly lower than that of [131I]Gluc-TOC and [131I]Gluc-TOCA, yet the affinity of these peptides is quite similar.

Previous investigations of the in vivo behavior of radiolabeled octreotide conjugated have been performed in rodents bearing tumors of either rat or murine origin (22, 35–37). Thus, the ability of these peptides to target rat or murine somatostatin receptors was measured. In contrast, the current biodistribution experiments were performed in the D 341 Med human medulloblastoma xenograft model because of our interest in developing targeted radiotherapeutics for patients with this malignancy. The current study is the one of the first reports of the specific uptake of a labeled octreotide analogue in a xenograft expressing somatostatin receptors of human origin. Although the homology between human somatostatin receptors and murine and rat receptors is about 85–95% (36), demonstration of specific uptake of these labeled peptides in a human tumor model is an important step in making a case for their use in patients with medulloblastoma. The biokinetics of an¹¹¹In-labeled octreotide analogue in a xenograft derived from a well-differentiated human midgut carcinoid also has been reported recently (38). That the accumulation of [131I]Gluc-TOC and [131I]Gluc-TOCA in the D-341 Med human medulloblastoma xenograft was receptor mediated was demonstrated by the reduction in tumor levels to 15% and 21% of controls, respectively, by coadministration with an excess of cold octreotide. A number of studies have reported reduction to 5–10% of controls in similar blocking protocols done in several somatostatin receptor-expressing rodent tumor lines (22, 35–37). This may be related to differences between human and rodent sstr₂ but also could reflect differences in the receptor subtype specificity of the different radioligands, as well as differences in subtype expression in the tumor models. On the other hand, the fact that the extent of blocking observed in murine pancreas and stomach generally
was lower than observed in this human tumor xenograft might be suggestive that differences in human and murine receptor may be a factor to be considered.

Cold octreotide was able to reduce $[1^{31}]$I[Gluc-TOC and $[1^{31}]$I[Gluc-TOCA accumulation in lungs to 44% and 28% of control levels, respectively, suggesting that uptake in this organ may be related in part to receptor binding. Generally, the extent of blocking of lung uptake has not been reported (22, 35, 36); however, Lewis et al. (35) observed receptor-mediated lung accumulation for $^{64}$Cu-labeled-TETA TOCA in the mouse but not the rat lung. Expression of sstr2, but not sstr2 or sstr3, receptors in the rat lung has been described (39, 40). In contrast, expression of the sstr3, gene in the mouse lung has been reported (41), consistent with our observations and those of Lewis et al. (35).

The potential utility of these glycated octreotide analogues for the imaging and treatment of medulloblastoma depends not only on their tumor uptake but on their clearance from normal tissues. One of the primary limitations of TOC is its high degree of hepatobiliary excretion (28, 29), which reflects the lipophility of this peptide. We have shown previously that COOH-terminal oxidation and NH$_2$-terminal carbohydrate were effective approaches for decreasing liver and intestine accumulation of TOC analogues (17, 18). In the current study, we again observed significantly lower uptake of radiiodine activity in normal organs, particularly when these strategies were combined. It should be noted that thyroid uptake of radiiodine increased with time, indicating that $[1^{31}]$I[Gluc-TOC and $[1^{31}]$I[Gluc-TOCA were susceptible to deiodination, a limitation that could be minimized by labeling these peptides with radioiodinated templates that are not degraded by deiodinases (42, 43).

In conclusion, $[1^{31}]$I[Gluc-TOC, and particularly $[1^{31}]$I[Gluc-TOCA, exhibited excellent uptake in this human medulloblastoma xenograft model, and yielded favorable tumor:normal tissue ratios. We believe that these results provide a reasonable basis for the development of radiolabeled carbohydrate octreotides analogues as diagnostic and therapeutic agents for medulloblastoma. Because its 7.2-h physical half life is well matched to peptide pharmacokinetics and its $\alpha$-particles might be ideal for leptomeningeal disease, efforts are under way to synthesize carbohydrate octreotides labeled with the radiohalogens $^{[211]}$At (44), with the goal of using this compound for the targeted radiotherapy of medulloblastoma neoplastic meningitis.

ACKNOWLEDGMENTS
We thank Phil Welsh and Susan Slade for excellent technical assistance.

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