CLINICAL UTILITY OF SOMATOSTATIN RECEPTOR SCINTIGRAPHIC IMAGING (OCTREOSCAN) IN ESTHESIONEUROBLASTOMA: A CASE STUDY AND SURVEY OF SOMATOSTATIN RECEPTOR SUBTYPE EXPRESSION

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Accepted 22 July 2005
Published online 9 February 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/hed.20356

Abstract: Background. For tumors that express somatostatin receptors (SSTR), radiolabeled somatostatin analogs, such as 111In-pentetreotide, can demonstrate the presence of tumor by radioligand uptake using somatostatin receptor scintigraphy (SRS). The use of 111In-pentetreotide for SRS depends on the specific high affinity of octreotide for SSTR subtypes 2, 3, and 5. Of these, SSTR2 has the greatest affinity for octreotide and the greatest relevance for tumor detection with Octreoscan imaging. Discriminating between postoperative changes and residual or recurrent tumor after extensive skull base surgery is often difficult, but in a case of recurrent esthesioneuroblastoma (ENB) we found the use of Octreoscan imaging clinically useful. To better define the general relevance of this imaging technique in this setting, we analyzed SSTR subtype expression in a panel of ENB tumors.

Methods. The case history and correlations between MRI and 111In-pentetreotide SRS of a patient with recurrent ENB were reviewed. The expression pattern of the SSTR subtypes in a panel of ENB tumors was then analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) to better define the potential of more general use of Octreoscan for imaging ENB. To correlate SSTR2 protein expression with 111In-pentetreotide uptake, immunohistochemistry to detect SSTR2 was performed on tumor samples from regions of increased uptake on Octreoscan.

Results. The SSTR2 message was expressed at high levels in all five ENB tumor samples, and either SSTR2 protein or histologic findings typical for ENB were found in all tumor tissue obtained from regions of increased 111In-pentetreotide uptake. Furthermore, Octreoscan imaging in this case proved useful in clinical decision making.

Conclusion. The expression pattern of SSTR2 and the specificity of the Octreoscan for regions of active tumor growth support further investigation of the utility of Octreoscan imaging in...
The interpretation of CT scans or MR images of skull base tumors after surgical treatment can be confounded by the presence of postoperative changes and treatment complications such as infection that produce contrast-enhancing granulation tissue and scar. In the case of such a patient with a previously treated esthesioneuroblastoma (ENB), the use of somatostatin receptor scintigraphy (SRS) provided valuable information regarding the interpretation of postsurgical changes seen on MR images that helped direct clinical decision making. This prompted us to then analyze somatostatin receptor (SSTR) expression in a panel of ENBs to assess the potential general usefulness of this imaging technique in these tumors.

Imaging with an Octreoscan is based on the binding of the radiolabeled somatostatin analog, $^{111}$In-pentetreotide ($^{111}$In-DTPA-D-Pheoctreo-
tide), to a subset of SSTRs. The SSTR family (SSTR1–5) is divided into two classes based on affinity for somatostatin analogs such as octreotide. Class I receptors (SSTR2, 3, and 5) avidly bind $^{111}$In-pentetreotide, whereas class II receptors (SSTR1, 4) have negligible affinity for $^{111}$In-pentetreotide. Of the three class I receptors, SSTR2 has the highest affinity for $^{111}$In-pentetreotide, with binding affinities approximately 10-fold and 90-fold greater than SST5 or SST3, respectively.

Scintigraphic imaging of tumors that express SSTRs has been used extensively with $^{111}$In-pentetreotide and other SSTR ligands such as $^{111}$In-lanreotide, and promising therapeutic approaches have been devised using the properties of SSTR binding and ligand-receptor internalization of modified SSTR ligands. Thus, radiolabeled SSTR ligands provide the unique possibilities to both image and treat SSTR-positive tumors.

Because of the clinical value of the Octreoscan in the preceding case, we investigated whether Octreoscan imaging might be of more general application for diagnosis, staging, and surveillance of patients with ENB. Octreoscan imaging of two patients with ENB has been previously reported, but the clinical relevance and potential general application of this imaging technique for detection or treatment of ENB is not known. The frequency of class 1 SSTR gene expression, and in particular SSTR2 (which has the highest affinity for the octreotide ligand), is expected to reflect the general usefulness of Octreoscan for imaging ENB, but to our knowledge, no studies of SSTR expression in ENB have been reported. Therefore, we examined the expression of the SSTR subtypes in a panel of ENB tumors and in samples corresponding to regions of $^{111}$In-pentetreotide uptake on a preoperative Octreoscan.

**MATERIALS AND METHODS**

**Clinical Data.** Clinical information described in this case report was obtained from a retrospective chart review including operative, imaging, and pathology reports, as well as primary review of imaging studies. All data were obtained with patient consent for a University of Washington Human Subjects Institutional Review Board–approved protocol.

**Octreoscan Imaging.** Somatostatin receptor imaging was performed using $^{111}$In-pentetreotide prepared according to the manufacturer’s specifications (Octreoscan, Mallinckrodt Medical, St. Louis, MO) as per clinical routine. Initial imaging was performed on a dual-headed gamma-camera system (VG, GE Medical Systems, Waukesha, WI) using a medium-energy collimator and two 20% energy windows centered on 173 and 245 keV. For the first study, after the intravenous administration of 263 MBq (7.1 mCi) of radiopharmaceutical, 4- and 24-hour anterior and posterior survey images were obtained using a sweep from head to thighs. Tomographic (single photon emission computed tomography [SPECT]) imaging of the head was performed at 24 hours using 120 views with a 23-second acquisition per view. Images were reconstructed onto a $128 \times 128 \times 128$ image matrix using filtered backprojection. Tomographic images were viewed using display software that allows three-dimensional triangulation of foci of tracer uptake. A second scan was performed at the time of cranial reconstruction, 17 months after the initial scan. The same protocol was followed with the exception that combined SPECT/CT imaging was performed using a combined SPECT/CT imaging device (Hawkeye, GE Medical Systems, Waukesha, WI) to provide additional anatomic detail regarding the sites of radiotracer uptake. Standard clinical MR images with and
without gadolinium and CT scans with and without contrast enhancement obtained during the routine course of the patient’s care were reviewed and compared with the findings on both Octreoscan images.

Archival Pathology Specimens. To corroborate the histologic appearance of tissue samples taken from areas of radioligand uptake on Octreoscan, corresponding archival hematoxylin–eosin (H&E) stained tumor sample sections were retrieved from the University of Washington Department of Pathology and photographed.

Reverse Transcriptase-Polymerase Chain Reaction Analysis of Somatostatin Receptor Subtype Expression in Human ENB Tumor Samples. Tumor samples ($n = 5$) were obtained with written consent and in compliance with a University of Washington Human Subjects protocol from patients being operated on for ENB. All samples were immediately snap frozen in liquid nitrogen and stored at $-80^\circ C$ before processing. Each frozen sample used for reverse transcriptase-polymerase chain reaction (RT-PCR) was simultaneously processed for H&E histology to document the presence of tumor.

Total RNA extracted from adjacent tumor tissue using Trizol (Invitrogen) was treated with DNase I (Invitrogen) and reverse transcribed with the Advantage RT for PCR kit (Clontech). Hot Start PCR (Qiagen) was performed for all samples with an annealing temperature of 60$^\circ C$ for 40 cycles with the following primers (written in 5'–3' orientation):

SSTR1 F/R: AGCCGGTTGACTATTACGCC/GCT-CTCACCTTCTACCATTTGTC
SSTR2 F/R: GGTGGAAGTCCTCTGGAATTCC/CCA-TTCCAGTGACAGAGAC
SSTR3 F/R: TCATCTGCCTCTGCTACCTG/GAG-CCCACAAGAACGACG
SSTR4 F/R: CGGCAGTCTTCTGGTGCTTAC/GCA-TCAAGGCCTGGTCAAGC
SSTR5 F/R: GGGAACACGCTGGTCATCTACGG/GCACAGGATGTGAATFC
GAPDH F/R: ACGGATTTGGTGCTATTTGGG/TGA-TTTGGAGGGATCTCGC

Primer sequences for SSTR 1–4 and SST were based on previously published methods, whereas SSTR5 primers were designed using Primer3 software. Controls for genomic or reagent contamination included reactions omitting RT or cDNA, respectively. Representative PCR products were directly sequenced to confirm specificity of cDNA amplification and visualized by electrophoresis in agarose gels containing ethidium bromide.

Immunohistochemistry. Sections of formalin-fixed, paraffin-embedded metastatic preauricular lymph node tumor with increased uptake on Octreoscan imaging were stained with H&E and processed for immunohistochemistry (IHC) by microwave, citrate buffer antigen retrieval. Primary SSSTR2a (Gramsch Laboratories #SS 800) and common leukocyte antigen, CD45 (DAKO #M0701) antibodies were used at 1:1000 and 1:400 dilutions, respectively. Antibody detection was performed using the avidin-biotin complex peroxidase method (Vectastain ABC kit) and visualized by incubation with diaminobenzidine hydrochloride. Human pancreas sections were used as positive controls, whereas negative controls were performed using both irrelevant immunoglobulin G (IgG) and by omission of the primary antibody from the staining protocol.

RESULTS

Case Report. A 53-year-old man was initially seen with nasal drainage and epistaxis 8 years after initial craniofacial resection of an extensive ENB with cranial extension. His initial treatment was complicated by a cerebrospinal fluid (CSF) leak and a brain abscess that required additional cranial procedures. Subsequent to identifying cervical metastasis at radical left neck dissection 4 years after the initial surgery and before presenting with epistaxis, his residual disease had been clinically stable. Direct nasal examination confirmed sinonasal tumor recurrence, and needle aspiration documented a new left preauricular lymph node metastasis. MR images revealed heterogeneously enhancing mass lesions in the ethmoid and left maxillary sinuses (Figure 1A,B), extensive changes in the frontal region (Figure 1A,C), two discrete preauricular lesions (Figure 1B, coronal image), and intracranial dural-based deposits in the right and left parietal regions (Figure 1C). Treatment options at this point included sinus surgery to alleviate his symptoms of obstruction versus a more radical craniofacial resection to debulk his cranial disease as well.

Because imaging changes from prior surgeries and brain abscess confounded the assessment of the extent of recurrent tumor, an $^{111}$In Octreo-
scan was performed in an attempt to better differentiate between tumor and postsurgical changes. Whole-body scans demonstrated $^{111}$In-pentetreotide uptake restricted to the craniofacial region, where $^{111}$In-pentetreotide uptake correlated closely with enhancing soft tissue on brain MR images, including the anterior frontal midline (Figure 2A,C), midline nasoethmoidal region and the left maxillary sinus (Figure 2A,B), biparietal dura (Figure 2C), and left preauricular enhancing subcutaneous nodules (Figure 2B). Because of the demonstration of diffuse unresectable disease and symptoms that were primarily related to his extracranial disease, a palliative resection of the sinonasal disease and superior enhancing preauricular nodule was performed. Histologic analysis of the tissue from the maxillary sinus and the superior preauricular lymph node was consistent with recurrent ENB (Figure 3).

At the time of an emergent optic nerve decompression 8 months later, biopsy specimens from the frontal region of $^{111}$In-pentetreotide uptake on prior scans revealed ENB. Nine months later (17 months from his original Octreoscan), a second Octreoscan demonstrated persistent and increased uptake in the inferior left preauricular lymph node (Figure 2A arrowheads) and the left maxillary sinus (Figure 2A,B arrows) and persistent bifrontal uptake (Figure 2B arrowhead) corresponding to gadolinium-enhanced tissue on MRI (not shown). A stable decrease in uptake in the previously debulked central sinonasal region was also noted (Figure 2B compared with Figure 1A).

**Somatostatin Receptor Expression in Esthesioneuroblastoma Tumor Samples.** To determine the potential general usefulness of Octreoscan imaging for detection of ENB, we assessed the expression patterns of all known SSTR subtypes in a panel of ENBs from five patients using RT-PCR. In all samples, there was high expression of SSTR2, the SSTR with the highest binding affinity for octreotide. The expression of the other SSTRs was variable. In particular, the other class II receptors,
SSTR3 and SSTR5, were not expressed in all tumors and at lower levels than SSTR2. The RT-PCR data are summarized in Figure 4. To demonstrate that $^{111}$In-pentetreotide uptake on Octreoscan correlates with SSTR2 protein expression, sections from the superior preauricular lymph node shown in Figure 3 were immunostained with an SSTR2-specific antibody. This revealed diffuse homogeneous membrane-associated expression of SSTR2 receptor in ENB tumor tissue (Figure 5). Thus, the ubiquitous expression in ENB samples of SSTR2 message and the demonstration of the SSTR2 protein in association with high $^{111}$In-pentetreotide uptake support future investigations of the general utility of Octreoscan imaging in diagnosis and surveillance imaging of ENB.

**DISCUSSION**

In this report, we describe an example of the clinical relevance of radioactive octreotide analog imaging for management of ENB and demonstrate its potential for more general use in the management of ENB on the basis of expression patterns of the high-affinity SSTR2 in ENB tumors. The discrimination of active tumor from postoperative change after craniofacial resections can be challenging because of the inevitable production of gadolinium-enhancing scar tissue on MR images. In the case reported here, the additional history of infection and multiple craniotomies further confounded the interpretation of the MR images. The Octreoscan provided confirmatory information regarding the extent of tumor involvement in this...
On repeat Octreoscan imaging after a 17-month interval, the tumor bulk in the debrided sinonasal region was significantly smaller, whereas the previously recognized cranial disease remained stable. The ability to detect cervical metastases in this report and widespread systemic disease in a previously reported case provides the rationale for use of Octreoscan for staging and surveillance imaging of patients with ENB.

SRS has been reported in only two cases of ENB but has been used extensively to detect a variety of tumors including neuroendocrine gastrointestinal tumors, lung cancer, neuroblastoma, meningioma, pituitary adenoma, medulloblastoma, and glioma. In most studies, Octreoscan imaging results correlate well with expression of SSTRs, and in particular SSTR2, which has the highest affinity for octreotide-based somatostatin analogs such as \(^{111}\)In-pentetreotide. Tumors are visualized because they express an increased number of SSTRs, and the radioactively labeled receptor-ligand complex is internalized and sequestered in the cell. The sensitivity of Octreoscan detection of tumor varies by type, but neuroendocrine tumors that are histologically similar to ENB have detection rates of 70% to 100% and, in many cases, detect disease not found on conventional staging procedures.

The specificity and sensitivity of the Octreoscan for ENB is not known. Because uptake on Octreoscan has been attributed to inflammatory tissue, neovascularization, or delivery to regions of blood–brain barrier disruption, further study is required to determine the clinical settings in which such effects may confound the interpretation of Octreoscan imaging and thus limit its usefulness. In this case, all tissue samples removed from areas of \(^{111}\)In-pentetreotide uptake showed either high levels of SSTR2 protein expression in tumor cells or histologic findings typical for ENB, thus providing rationale to further investigate the sensitivity and specificity of SRS for ENB tumor detection. In addition, all tumor samples expressed high levels of SSTR2 regardless of whether they were recurrent, previously treated by radiation or chemotherapy, or metastatic. Taken together, these data suggest that further study of SRS with Octreoscan imaging is warranted for the purposes of establishing its utility for ENB detection, diagnosis, staging, and surveillance in comparison with standard clinical MRI.

Of importance, such studies could provide the necessary demonstration of feasibility to consider the potential use of somatostatin receptor ligands for treatment of metastatic or recurrent ENB. A recent meta-analysis of ENB revealed overall and disease-free survivals of 45% and 41%, respectively. Thus, most patients with ENB will have a recurrence, and adjuvant treatment options, such as chemotherapy, are of limited value at the time of progression. In the MAURITIUS phase II trial, administration of the SSTR ligand, (90)Y-DOTA-lanreotide, which has affinity for SSTR2, 3, 4, and 5, demonstrated disease regression or stabilization in 60% of patients with a wide range of progressive cancer types, with no major systemic toxicity. Because octreotide analogs have significantly higher affinities for SSTR2 and more reliably detect neuroendocrine tumors, radioactive octreotide analogs may potentially have better response rates as salvage therapy for treating recurrent, previously treated ENB than the mixed

![FIGURE 4. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis of SSTR subtype and somatostatin expression in 5 esthesioneuroblastoma tumors. SSTR2, which has the greatest affinity for the octreotide ligand, was expressed at high levels in all samples. A negative RT control is included for each sample in the lane to the right of the designated sample. RT-PCR controls for RNA abundance and integrity using glyceraldehyde-3-phosphate dehydrogenase are also shown. The molecular weight for each product is listed to the right. Each product produced the appropriate-sized band, and direct sequence analysis of randomly selected products confirmed specific amplification of the intended target cDNA.](image-url)
group of tumors treated with (90)Y-DOTA-lanreotide in the MAURITIUS trial. Although application of SSTR-based therapeutic strategies to ENB is supported by the findings of this case study, additional investigation of SSTR expression patterns and SSTR ligand uptake is required in a larger number of ENB tumors.

Acknowledgments. The authors thank Janet Schukar and Paul Schwartz for their invaluable help with the preparation of the figures, and Rosemary Kimmel for her help in preparation and submission of the manuscript.

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