MIBG molecular imaging for evaluating response to chemotherapy in patients with malignant pheochromocytoma: preliminary results

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Abstract

Malignant pheochromocytomas respond to chemotherapy with a reduction in tumor size and catecholamine secretion. We investigated the usefulness of molecular imaging with meta-iodobenzylguanidine (MIBG) for evaluating the effects of chemotherapy in patients with malignant pheochromocytoma. Six patients were studied before and after 6 ± 4 months of combination chemotherapy with cyclophosphamide, vincristine, and dacarbazine. Urinary catecholamines, metanephrines, and vanillylmandelic acid (VMA) levels were measured before and after chemotherapy. [131I]MIBG uptake was calculated for each tumor lesion on images before and after chemotherapy. An intensity ratio (IR) of abnormal to normal tissue count density was used to evaluate the change in lesion activity with therapy. Urinary catecholamines, metanephrines, and VMA significantly decreased with chemotherapy. MIBG uptake decreased in most lesions and the reduction in overall IR correlated with the reduction in urinary VMA. However, the change in individual lesions was variable and MIBG IR did not change or increased in a number of lesions. In conclusion, MIBG imaging is useful in the evaluation of patients with malignant pheochromocytoma who are receiving chemotherapy. It can provide not only a measure of overall effectiveness of treatment but also allows a lesion-by-lesion evaluation of the heterogeneity of response to chemotherapy.

Keywords: Neuroendocrine tumor; treatment monitoring; molecular imaging.

Introduction

Pheochromocytomas are catecholamine-secreting tumors of chromaffin tissue originating in the adrenal medulla or sympathetic paraganglia. About 10% of these tumors are malignant and because the diagnosis of malignancy can only be made when they are located in non-chromaffin tissue sites, malignant pheochromocytomas are, by definition, metastatic. Despite this, surgery is the preferred treatment for both benign and malignant pheochromocytomas. However, with unresectable disease, other types of therapy are usually needed, such as antihypertensive treatment with pharmacologic agents or inhibitors of catecholamine excess and radiotherapy to palliate painful bone metastases or radiometabolic treatment. Previous studies have demonstrated, by measuring urinary catecholamine and metanephrine levels or RECIST (Response Evaluation Criteria in Solid Tumors) criteria, that combination chemotherapy can be effective for advanced malignant pheochromocytomas.

Meta-iodobenzylguanidine (MIBG) is a physiologic analogue of norepinephrine and guanethidine that is taken up by pheochromocytoma cells. This agent is not taken up in fibrotic and/or necrotic tumor sites, but is concentrated only in viable neoplastic cells. Imaging with radiolabeled MIBG is able to detect adrenal and extra-adrenal tumor masses, such as pheochromocytoma.
and paraganglioma; in particular, tumor lesions that concentrate MIBG can be benign or malignant and the ability to detect tumors on MIBG scans depends on both tumor size and differentiation\cite{7,15,19}. We previously demonstrated that qualitative analysis using MIBG imaging allows lesion-by-lesion evaluation of the heterogeneity of neuroblastoma response to chemotherapy\cite{10}.

The aim of this study was to investigate the usefulness of molecular imaging with MIBG for evaluating the effects of combination chemotherapy on individual tumor lesions by monitoring MIBG uptake changes after treatment in patients with malignant pheochromocytoma.

**Materials and methods**

**Study group**

Six patients (5 men and 1 woman) ranging in age from 28 to 64 years (mean age 43 years), with sporadic malignant pheochromocytoma were evaluated with \([^{131}I]\)MIBG imaging and measurement of 24-h urinary total catecholamine, metanephrine, and vanillylmandelic acid (VMA) levels before and after (mean 6 ± 4 months) the last cycle of chemotherapy. Four patients had previous surgical treatment for the disease. In 2 others, the diagnosis of pheochromocytoma was made by biopsy (Table 1). None of the patients had undergone MIBG treatment previously. In all patients, the malignant pheochromocytoma was located in non-chromaffin tissue. The patients received intravenous chemotherapy with cyclophosphamide (750 mg/m² body surface area), vincristine (1.4 mg/m² body surface area), vincristine (1.4 mg/m² body surface area) on day 1 and dacarbazine (600 mg/m² body surface area) on days 1 and 2 of 21- to 28-day cycles\cite{3,15,19}. The institutional review board approved the chemotherapy and MIBG imaging protocols and informed written consent was obtained from all patients.

**Urinary measurements**

The urinary total free catecholamines were determined by quantitative fluorometric analysis (SmithKline Bioscience Laboratories, Philadelphia, PA) (normal range up to 0.68 µmol/day); the urinary metanephrines and VMA were determined using a spectrophotometric assay (normal range: metanephrines up to 6.6 µmol/day, VMA up to 55 µmol/day)\cite{1}.

**MIBG imaging**

Thyroid uptake of unbound \([^{131}I]\) was blocked with a saturated solution of potassium iodide (200 mg iodide/day by mouth), beginning 1 day before tracer administration and continuing for 8 days. Medications that could potentially interfere with the tumor uptake of MIBG, such as reserpine, monoamine oxidase inhibitors, and tricyclic antidepressants, were discontinued for 30 days or longer before the study. Anterior and posterior whole-body scans and spot images were obtained 24 and 48 h after the intravenous administration of 0.5 mCi (18 MBq) of \([^{131}I]\)MIBG using a whole-body scanner (E.CAM 180 dual detector gamma-camera, Siemens Medical Systems, Hoffman Estates, IL) equipped with a high-energy collimator and connected to a dedicated computer system. We used a 20% window centered at the 364 keV photopeak.

MIBG images were considered positive when adrenal or extra-adrenal foci of increased uptake were seen on each of the 24- and 48-h scans. For quantitative analysis, MIBG uptake was measured on 48-h images in lesions and in adjacent or contralateral normal tissue using region of interest analysis. To obtain background correction, the intensity ratio (IR) of MIBG tumor uptake (tumor lesion counts/normal tissue counts) was calculated for each lesion. A lesion-by-lesion comparison of the pre- and post-chemotherapy IR values was made. In addition, the sum of IR values for all lesions in a scan

| Patient | Age (years) | Sex | Primary site | Sites of metastases | Surgical procedure | Biopsy | Pathology | No. of cycles of chemotherapy |
|---------|-------------|-----|--------------|---------------------|-------------------|--------|-----------|-----------------------------|
| 1       | 28          | Female | Right adrenal | Liver, lungs, lymph nodes, multiple bones | Right adrenalectomy, liver metastases resected | Not performed | + | 3 |
| 2       | 45          | Male | Left adrenal | Liver, lungs, lymph nodes, multiple bones | Left adrenalectomy | Not performed | + | 4 |
| 3       | 44          | Male | Pelvic ganglia | Bone | Presacral mass resected, T7 laminectomy | Not performed | + | 4 |
| 4       | 39          | Male | Right adrenal | Multiple bones | Right para-renal mass, involved manubrium resected | Not performed | + | 8 |
| 5       | 64          | Male | Right adrenal | Liver, Lymph nodes, multiple bones | Not performed | Liver | + | 4 |
| 6       | 38          | Male | Left adrenal | Not performed | Supraclavicular lymph node | Not performed | + | 13 |
gave a total scan IR value for each patient. This summed value represented the total abnormal MIBG uptake and was used to compare pre- and post-chemotherapy MIBG scans in each patient. In preliminary studies, the precision of the measurements was assessed by repeatedly (5 times) measuring the MIBG uptake in 10 lesions with activity ranging from 0.39 to 2.21. The average coefficient of variation for measurement of the activity of a lesion was 3.3%.

Statistical analysis
Data are presented as mean ± standard deviation. For statistical analysis, we used the Wilcoxon signed-rank test for pairwise comparison and the Pearson coefficient of correlation. All data were collected in an Excel database and analyzed by SPSS 18.0. A P value <0.05 was considered statistically significant.

Results

Urine measurements
Pre- and post-chemotherapy laboratory data for each patient are illustrated in Fig. 1. After chemotherapy, a significant decrease in total urinary catecholamines (1.8 ± 2.2 µmol/24 h vs 3.95 ± 2.1 µmol/24 h; P < 0.005), metanephrines (22.6 ± 17.5 µmol/24 h vs 96.3 ± 77.9 µmol/24 h; P < 0.05), and VMA (64.1 ± 12.8 µmol/24 h vs 173.3 ± 120.3 µmol/24 h; P < 0.05) was observed compared with baseline.

MIBG imaging
Pre-chemotherapy, a total of 73 tumor lesions with abnormal MIBG uptake were detected in all patients. Post-chemotherapy, 30 lesions showed no MIBG uptake and 43 lesions persisted (26 showed decreased MIBG uptake, 3 had unchanged MIBG uptake, and 14 had increased MIBG uptake). The results of pre- and post-chemotherapy MIBG studies for each patient lesion by lesion are illustrated in Fig. 2. In detail, patient 1 had 22 abnormal foci at baseline and after chemotherapy 14 of them disappeared, 5 had decreased and 3 had increased MIBG uptake; patient 2 had 37 lesions at baseline and 12 disappeared, 13 had decreased and 10 had increased MIBG uptake after therapy, and in 2 lesions MIBG uptake remained unchanged; patient 3 had a single lesion in the left parietal skull at baseline and its IR value decreased from 4.8 to 2.1 after therapy; at baseline, patient 4 had 7 lesions, after therapy the IR value decreased in 6 lesions and increased in 1; a single baseline lesion in patient 5 was unchanged after therapy; in patient 6, 4 of 5 lesions at baseline disappeared with therapy and the IR of the 5th lesion decreased from 11.6 to 5.8. Representative magnetic resonance (MR) images with MIBG in a patient with mixed response are presented in Fig. 3.

Discussion
The initial evaluation of patients with malignant pheochromocytoma included a physical examination, serum and urine laboratory tests, as well as computed tomography (CT), MR imaging and radionuclide procedures.[1,2] Tumor lesions detected by anatomic imaging can be characterized as pheochromocytoma by functional imaging agents that target the catecholamine synthesis, storage, and secretion pathways of chromaffin tumor cells.[3] These techniques include [123I or 131I] MIBG scintigraphy, [18F]fluoro-dihydroxyphenylalanine (DOPA)-positron emission tomography (PET), and [18F]fluorodopamine (FDA)-PET. [18F]Fluorodeoxyglucose (FDG)-PET provides another modality for localization of metastatic pheochromocytoma, although with less tissue specificity than the other functional approaches targeting the catecholamine biosynthetic and storage pathways. Timmers et al.[11] recommend the use of FDA-PET/CT in patients with a biochemically established diagnosis of pheochromocytoma and paraganglioma when the aim is to localize the primary tumors and rule out metastases. However, when FDA is unavailable, DOPA-PET or MIBG scintigraphy can be used as valid alternatives.[11]

After chemotherapy, the same diagnostic approach might be used to evaluate both primary and metastatic disease.[1–5,12,13] Physical examination is useful for assessing lymph node changes and should always be included in follow-up evaluations. Urinary catecholamines and their metabolites are useful markers of the biochemical response of malignant pheochromocytoma. However, these urinary tests reflect the function of the entire tumor mass and are not helpful in assessment of individual sites of disease. Individual lesions, their anatomic and functional status and their response to chemotherapy are best evaluated with diagnostic imaging studies. CT and MR imaging are excellent for detecting mass lesions and for following their size during therapy. Chemotherapy, however, may leave a residual of necrotic tumor and fibrous tissue. In the follow-up evaluation of neoplastic disease, differentiation of viable tumor from fibrosis is of fundamental importance and often requires needle biopsy; for this purpose, another way to non-invasively monitor the effects of chemotherapy is to assess the viability of a tumor using molecular imaging modalities; in
**Figure 1** Individual values of 24-h urinary catecholamines (A), metanephrines (B) and VMA (C) levels before and after chemotherapy.

**Figure 2** Individual values of MIBG summed IR before and after chemotherapy.
Figure 3  MIBG scan in the anterior view of the pelvis before chemotherapy (A) shows increased tracer uptake in the right iliac wing and left acetabulum (black arrows), faint uptake in multiple lymph nodes of the right and left iliac chains and in proximal femurs; after chemotherapy (B) there is reduction of uptake in the right iliac wing (arrow), no uptake in the left acetabulum and femur, unchanged uptake in the right femur, and increased uptake in multiple lymph nodes of the right and left iliac chains (white arrows). Sagittal MR T2-weighted turbo spin echo image of the right iliac wing before chemotherapy (C) shows a 2-cm hyperintense lesion (white arrow); after chemotherapy (D), there is a reduction in size to 1 cm (white arrow). Sagittal MR T2-weighted turbo spin echo image of the left pelvis before chemotherapy (E) shows no abnormalities; after chemotherapy (F), part of a large iliac lymphadenopathy measuring more than 3 cm (white arrow) is detectable.
In this regard, the role of nuclear medicine techniques has been previously suggested. FDG-PET combined with CT imaging has been used in monitoring the response to therapy of several oncologic diseases, such as brain tumors, lymphoma, and colon carcinoma. More recently, Nakazawa et al. demonstrated that FDG-PET is a useful method for evaluating the effect of [131I]MIBG therapy in patients with malignant pheochromocytoma. Although we previously demonstrated that MIBG imaging might be useful in monitoring the response to chemotherapy in patients with neuroblastoma, no studies have been performed to assess chemotherapy efficacy in patients with malignant pheochromocytoma by MIBG or PET imaging.

In the present study, we propose that serial MIBG imaging can be useful in monitoring patients with malignant pheochromocytoma who are receiving chemotherapy. We found that the quantification method described in this study is useful to follow lesion activity. First, the overall MIBG results were consistent with the biochemical response of tumor. Second, quantification of individual lesions confirmed the visual impression that change of individual disease sites was variable, disappearing in some cases, remaining unchanged or increasing in others. It is well known that MIBG uptake in pheochromocytoma cells is a complex mechanism related to tumor differentiation. The heterogeneity of response to chemotherapy might reflect a different grade of tumor metabolic activity. In this regard, patient 2 provides an example in which MIBG imaging is particularly useful in clinical management evaluating the heterogeneity of tumor response to treatment. In particular, this patient had 37 sites of disease identified by MIBG imaging; after 4.1 months of chemotherapy, MIBG uptake decreased in 13 lesions and disappeared in another 12. However, MIBG uptake increased in 10 tumor lesions and remained unchanged in the remaining 2 lesions. Similar findings were also observed in 2 other patients in our population, showing 3 (patient 1) and 1 (patient 4) lesions with increased MIBG uptake after chemotherapy. These tumor sites with increased MIBG activity were further evaluated by radiography and were found to be expanding lesions requiring radiation therapy. Thus, MIBG findings after chemotherapy may guide additional treatment options and the heterogeneity of malignant pheochromocytoma response to chemotherapy might have important clinical implications, particularly when alternative therapeutic approaches are contemplated and/or prognostic observations are requested.

We also found that the overall change in MIBG uptake paralleled the change in urinary VMA excretion and in clinical status. It is unclear why we did not also find a correlation with changes in total urinary catecholamine...
or metanephrine excretion. This may have been due to the limited number of patients studied or to the fact that VMA excretion represented a large percentage of the total excretion of catecholamine metabolites.

A major limitation of our study is the small number of patients included. Therefore, larger studies are needed to confirm our preliminary results. Another limitation is that pathologic data were not available to compare with the changes evident on MIBG imaging. Furthermore, CT and MR studies were not available in all patients for systematic comparison with the MIBG findings. \( [123I] \text{MIBG} \) is preferable to \( [131I] \text{MIBG} \) because it provides higher-quality images and the lower radiation burden of \( 123I \) allows a higher permissible dose, resulting in a higher count rate. However, \( [123I] \text{MIBG} \) is less available and more expensive than \( 131I \text{MIBG} \).

**Conclusion**

The results of the present study show that successful combination chemotherapy of malignant pheochromocytoma leads to a reduction in catecholamine production and MIBG uptake by these tumors. Changes in overall MIBG uptake mirrored the change in urinary VMA excretion. However, laboratory measurements reflect only the global functional status of the disease; they are not helpful in defining the response of individual tumor lesions to the treatment. Conversely, MIBG imaging allows a lesion-by-lesion evaluation of the heterogeneity of response to chemotherapy.

**Conflict of interest**

The authors have no conflicts of interest to declare.

**References**

[1] Chrissoulidou A, Kaltas G, Ilias I, Grossman AB. The diagnosis and management of malignant pheochromocytoma and paraganglioma. Endocr Relat Cancer 2007; 14: 569–585. doi:10.1677/ERC-07-0074. PMid:17914089.

[2] Andersen KF, Alnaf R, Krarup-Hansen A, et al. Malignant pheochromocytomas and paragangliomas – the importance of a multidisciplinary approach. Cancer Treat Rev 2011; 37: 111–119. doi:10.1016/j.ctrv.2010.07.002. PMid:20675056.

[3] Averbuch SD, Steakley CS, Young RC, et al. Malignant pheochromocytoma: effective treatment with a combination of cyclophosphamide, vincristine, and dacarbazine. Ann Intern Med 1988; 109: 267–273. PMid:3395037.

[4] Scholz T, Eisenhofer G, Pacak K, Drahle H, Lehnhert H. Clinical review: current treatment of malignant pheochromocytoma. J Clin Endocrinol Metab 2007; 92: 1217–1225. doi:10.1210/jc.2006-1544. PMid:17284633.

[5] Huang H, Abraham J, Hung E, et al. Treatment of malignant pheochromocytoma/paranglioma with cyclophosphamide, vincristine, and dacarbazine: recommendation from a 22-year follow-up of 18 patients. Cancer 2008; 113: 2020–2028. doi:10.1002/cncr.23812. PMid:18780317.

[6] Jacques S, Tobes MC, Sisson JC. Sodium dependency of uptake of nor-epinephrine and m-iodobenzylguanidine into cultured human pheochromocytoma cells: evidence for uptake-one. Cancer Res 1987; 47: 3920–3928.

[7] Ilias I, Sahdev A, Reznek RH, Grossman AB, Pacak K. The optimal imaging of adrenal tumours: a comparison of different methods. Endocr Relat Cancer 2007; 14: 587–599. doi:10.1677/ERC-07-0045. PMid:17914090.

[8] Nguyen HH, Proye CA, Carnaille B, Combemale F, Pattou FN, Huglo D. Tumor size: the only predictive factor for \( 131I \) MIBG uptake in phaeochromocytoma and paraganglioma. Aust N Z J Surg 1999; 69: 350–353. doi:10.1111/j.1440-1622.1999.01570.x.

[9] Maurea S, Cuocolo A, Imbriaco M, et al. Imaging characterization of benign and malignant pheochromocytoma or paraganglioma: comparison between MIBG uptake and MR signal intensity ratio. Ann Nucl Med 2012; 26: 670–675. doi:10.1007/s12149-012-0624-1. PMid:22752959.

[10] Maurea S, Lastoria S, Caracò C, et al. Iodine-131-MIBG imaging to monitor chemotherapy response in advanced neuroblastoma: comparison with laboratory analysis. J Nucl Med 1994; 35: 1429–1435. PMid:8071687.

[11] Timmers HJ, Chen CC, Carrasquillo JA, et al. Comparison of \( 11 \text{F} \)-fluoro-L-DOPA, \( 11 \text{F} \)-fluoro-deoxyglucose, and \( 18 \text{F} \)-fluorodopa-MIBG scintigraphy in the localization of pheochromocytoma and paraganglioma. J Clin Endocrinol Metab 2009; 94: 4757–4767. doi:10.1210/jc.2009-1248. PMid:19864450.

[12] Eisenhofer G, Bornstein SR, Brouwers FM, et al. Malignant pheochromocytoma: current status and initiatives for future progress. Endoc Relat Cancer 2004; 11: 423–436. doi:10.1677/erc.1.00829.

[13] Adler JT, Meyer-Rochow GY, Chen H, et al. Pheochromocytoma: current approaches and future directions. Oncologist 2008; 13: 779–793. doi:10.1634/theoncologist.2008-0043. PMid:18617683.

[14] Kim EE, Haynie TP. Role of nuclear medicine in chemotherapy of malignant lesions. Semin Nucl Med 1985; 15: 12–20. PMid:3885397.

[15] Cook GJR, Fogelman I. The role of nuclear medicine in monitoring treatment in skeletal malignancy. Semin Nucl Med 2001; 31: 206–211. PMid:11430527.

[16] Ramanna L, Waxman A, Binney G, Waxman S, Mirra J, Rosen G. Thallium-201 scintigraphy in bone sarcoma: comparison with gallium-67 and technetium-MDP in the evaluation of chemotherapeutic response. J Nucl Med 1990; 31: 567–572. PMid:2341892.

[17] Weber WA, Figlin R. Monitoring cancer treatment with PET/CT: Does it make a difference? J Nucl Med 2007; 48: 36S–44S. doi:10.2967/jnumed.106.034206. PMid:17024719.

[18] Nakazawa A, Higuchi T, Oriuchi N, Arisaka Y, Endo K. Clinical significance of 2-[\( 18 \text{F} \)]fluoro-2-deoxy-D-glucose positron emission tomography for the assessment of \( 131 \text{I} \)-iodolobenzylguanidine therapy in malignant pheochromocytoma. Eur J Nucl Med Mol Imaging 2011; 38: 1869–1875. doi:10.1007/s00259-011-1872-3. PMid:21732103.

[19] Intenzo CM, Jabbour S, Lin HC, et al. Scintigraphic imaging of body neuroendocrine tumors. Radiographics 2007; 27: 1355–1369. doi:10.1148/rg.275065729. PMid:17848696.