indication of I-131 MIBG therapy is usually determined based on the results of I-123 MIBG scintigraphy including SPECT/CT to identify accurate anatomic localization of the lesions, because image quality of I-123 MIBG scintigraphy is generally superior to diagnostic low-dose I-131 MIBG scintigraphy.[14,15] After I-131 MIBG therapy, I-131 MIBG imaging might be recommended in order to confirm the lesions with MIBG accumulation. In this study, more than 2 times lesions were detected in high-dose I-131 MIBG scintigraphy than in diagnostic I-123 MIBG scintigraphy. Even an I-123 MIBG SPECT/CT was inferior to planar high-dose I-131 MIBG image in lesion detectability (3.7 vs. 7.3 lesions/study, respectively). Since high-dose I-131 MIBG scintigraphy have great diagnostic value in the detection of the lesions, it is believed that I-131 MIBG scintigraphy after I-131 MIBG therapy is essential for the management of patients.

Recently, many studies investigated the incremental value of SPECT/CT over planar image in various tumors. Even-Sapir et al reported that SPECT/CT improved image interpretation by providing a better anatomic localization of SPECT-detected lesions in 41% of the patients with known or suspected endocrine tumor and detected unsuspected bone involvement in 15% of the patients.[21] Rozovsky et al investigated added value of SPECT/CT over the correlation of I-123 MIBG scintigraphy and diagnostic CT in neuroblastoma and pheochromocytoma and reported that SPECT/CT provided additional information in 53% of all cases.[26] In various type of tumor scans, Roach et al reported that SPECT/CT modified the interpretation with planar/SPECT alone in 56% of the cases.[32] Chen et al reported that, in patients with differentiated thyroid carcinoma, precise localization and characterization of I-131-avid foci were achieved through I-131 SPECT/CT over planar image in 69 (85.2%) and 67 (82.7%) of the 81 foci, respectively, and uncommon metastatic lesions were found in 9 (13.6%) of 66 patients with regard to SPECT/CT fusion images.[33] In our study, unknown lesions in planar images were detected by SPECT/CT images in 45.2% of studies and 68.8% of patients and anatomic locations of the lesions were modified after analysis of SPECT/CT in 45.2% of studies and 62.5% of patients in I-123 MIBG scintigraphy. In high-dose I-131 MIBG scintigraphy, unknown lesions in planar images were detected by SPECT/CT in 23.5% of studies and 33.3% of patients and anatomic locations of the lesions were altered after analysis of SPECT/CT in 47.1% of studies and 66.7% of patients. As a whole, SPECT/CT images provided additional diagnostic information in 80.6% of studies, 75.0% of patients and 52.9% of studies, 75.0% of patients over planar images in I-123 MIBG scintigraphy and high-dose I-131 MIBG scintigraphy, respectively. The detection rate of the new lesions by SPECT/CT was higher in I-123 MIBG scintigraphy than in high-dose I-131 MIBG scintigraphy. It is thought that signal-to-noise ratio is high enough to be identified in planar image when high dose is administered. There were no apparent differences in the rate of alteration of anatomic location of the lesions between diagnostic I-123 MIBG and high-dose I-131 MIBG images.

A few lesions found in planar images became undetectable in SPECT/CT images in both I-123 MIBG and I-131 MIBG. Because all lesions showed weak uptake, low lesion counts of each projection image might not permit to develop tomographic image.

CONCLUSIONS

Post-therapy high-dose I-131 MIBG scintigraphy is superior to diagnostic I-123 MIBG scintigraphy for lesion detectability even in comparison with I-123 MIBG SPECT/CT images and high-dose I-131 MIBG planar images in patients with malignant neuroendocrine tumor. SPECT/CT images are helpful for the detection of the new lesions and accurate identification of anatomic localization compared with planar images. SPECT/CT imaging is especially useful for the detection of the lesions near or overlapping physiological accumulation compared with planar images.

REFERENCES

1. Jaques S, Tobes MC, Sisson JC. Sodium dependency of uptake of norepinephrine and m-iodobenzylguanidine into cultured human pheochromocytoma cells: evidence for uptake-one. *Cancer Res.* 1987;47:3920–3928.
2. Gasnier B, Roisin MP, Scherman D, et al. Uptake of metaiodobenzylguanidine by bovine chromaffin granule membranes. *Mol Pharmacol.* 1986;29:275–280.
3. Edström Elder E, Hjelm Skog AL, Höög A, et al. The management of benign and malignant pheochromocytoma and abdominal paraganglioma. *Eur J Surg Oncol.* 2003;29:278–283.
4. Whalen RK, Althausen AF, Daniels GH. Extra-adrenal pheochromocytoma. *J Urol.* 1992;147:1–10.
5. Lonergan GF, Schwab CM, Suarez ES, et al. From the archives of the AFIP neuroblastoma, ganglioneuroblastoma, and ganglioneuroma: radiologic-pathologic correlation. *Radiographics.* 2002;29:911–934.
6. Papaioannou G, McHugh K. Neuroblastoma in childhood: review and radiological findings. *Cancer Imaging.* 2005;5:116–127.
7. Sisson JC, Frager MS, Valk TW, et al. Scintigraphic localization of pheochromocytoma. *N Engl J Med.* 1981;305:12–17.
8. Shapiro B, Copp JE, Sisson JC, et al. Iodine-131 metaiodobenzylguanidine for the locating of suspected pheochromocytoma: experience in 400 cases. *J Nucl Med.* 1985;26:576–585.
9. Boubaker A, Bischof Delaloye A. MIBG scintigraphy for the diagnosis and follow-up of children with neuroblastoma. *Q J Nucl Med Mol Imaging.* 2008;52:388–402.
10. van der Harst E, de Herder WW, Bruining HA, et al. [I-123] metaiodobenzylguanidine and [In-111]octreotide uptake in benign and malignant pheochromocytomas. *J Clin Endocrinol Metab.* 2001;86:685–693.
11. van der Horst-Schrivers AN, Jager PL, Boezen HM, et al. Iodine-123 metaiodobenzylguanidine scintigraphy in localising phaeochromocytomas—experience and meta-analysis. *Anticancer Res.* 2006;26:1599–1604.
12. Bhatia KS, Ismail MM, Sahdev A, et al. 123I-metaiodobenzylguanidine (MIBG) scintigraphy for the detection of adrenal and extra-adrenal phaeochromocytomas: CT and MRI correlation. *Clin Endocrinol (Oxf).* 2008;69:181–188.
13. Wiseman GA, Pacak K, O'Dorisio MS, et al. Usefulness of I-123 MIBG scintigraphy in the evaluation of patients with known or suspected primary or metastatic pheochromocytoma or paraganglioma: results from a prospective multicenter trial. *J Nucl Med.* 2009;50:1448–1454.
14. Shulkin BL, Shapiro B, Francis IR, et al. Primary extra-adrenal pheochromocytoma: positive I-123 MIBG imaging with negative I-131 MIBG imaging. *Clin Nucl Med.* 1986;11:851–854.
15. Lynn MD, Shapiro B, Sisson JC, et al. Pheochromocytoma and the normal adrenal medulla: improved visualization with I-123 MIBG scintigraphy. *Radiology.* 1985;156:789–792.
16. Campbell L, Mouratidis B, Sullivan P. Improved detection of disseminated pheochromocytoma using post therapy I-131 MIBG scanning. *Clin Nucl Med.* 1996;21:960–963.
17. Iwano S, Kato K, Nihashi T, et al. Comparisons of I-123 diagnostic and I-131 post-treatment scans for detecting residual thyroid tissue and metastases of differentiated thyroid cancer. *Ann Nucl Med.* 2009;23:777–782.
18. Weber DA, Ivanovic M. Correlative image registration. *Semin Nucl Med.* 1994;24:311–323.
19. Pérault C, Schvartz C, Wampach H, et al. Thoracic and abdominal SPECT-CT image fusion without external markers in endocrine carcinomas. *J Nucl Med.* 1997;38:1234–1242.
20. Hutton BF, Braun M, Thurfjell L, et al. Image registration: an essential tool for nuclear medicine. *Eur J Nucl Med.* 2002;29:559–577.
21. Even-Sapir E, Keidar Z, Sachs J, et al. The new technology of combined transmission and emission tomography in evaluation of endocrine neoplasms. *J Nucl Med.* 2001;42:998–1004.
22. Pfannenberg AC, Eschmann SM, Horger M, et al. Benefit of anatomical-functional image fusion in the diagnostic work-up of neuroendocrine neoplasms. *Eur J Nucl Med Mol Imaging.* 2003;30:835–843.
23. Schillaci O. Functional-anatomical image fusion in neuroendocrine tumors. *Cancer Biother Radiopharm.* 2004;19:129–134.
24. Morcila PP, Duarte LH, Vieira F, et al. Value of SPECT/CT image fusion in the assessment of neuroendocrine tumours with In-111 pentetreotide scintigraphy. *Rev Esp Med Nucl.* 2005;24:14–18.

25. Anthauer H, Denecke T, Rohlfing T, et al. Value of image fusion using single photon emission computed tomography with integrated low dose computed tomography in comparison with a retrospective voxel-based method in neuroendocrine tumours. *Eur Radiol.* 2005;15:1456–1462.
26. Rozovsky K, Koplewitz B, Krausz Y, et al. Added value of SPECT/CT for correlation of MIBG scintigraphy and diagnostic CT in neuroblastoma and pheochromocytoma. *AJR.* 2008;190:1085–1090.
27. Sisson JC, Shapiro B, Beierwaltes WH, et al. Radiopharmaceutical treatment of malignant pheochromocytoma. *J Nucl Med.* 1984;24:197–206.
28. Gedik GK, Hoefnagel CA, Bais E, et al. I-131 MIBG therapy in metastatic phaeochromocytoma and paraganglioma. *Eur J Nucl Med Mol Imaging.* 2008;35:725–733.
29. Gonias S, Goldsby R, Matthay KK, et al. Phase II study of high-dose [I-131]metaiodobenzylguanidine therapy for patients with metastatic pheochromocytoma and paraganglioma. *J Clin Oncol.* 2009;27:4162–4168.
30. DuBois SG, Matthay KK. Radiolabeled metaiodobenzylguanidine for the treatment of neuroblastoma. *Nucl Med Biol.* 2008;35:35–48.
31. Scholz T, Eisenhofer G, Pacak K, et al. Clinical review: current treatment of malignant pheochromocytoma. *J Clin Endocrinol Metab.* 2007;92:1217–1225.
32. Roach PJ, Schembri GP, Ho Shon IA, et al. SPECT/CT imaging using a spiral CT scanner for anatomical localization: impact on diagnostic accuracy and reporter confidence in clinical practice. *Nucl Med Commun.* 2006;27:977–987.
33. Chen L, Luo Q, Shen Y, et al. Incremental value of I-131 SPECT/CT in the management of patients with differentiated thyroid cancer. *J Nucl Med.* 2008;49:1952–1957.

Eur J Nucl Med Mol Imaging (2011) 38:1869–1875
DOI 10.1007/s00259-011-1872-3

ORIGINAL ARTICLE

Clinical significance of 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography for the assessment of ^{131}I-metaiodobenzylguanidine therapy in malignant phaeochromocytoma

Azusa Nakazawa · Tetsuya Higuchi · Noboru Oriuchi · Yukiko Arisaka · Keigo Endo

Received: 16 March 2011 / Accepted: 17 June 2011 / Published online: 6 July 2011

Abstract
Purpose The aim of this study was to evaluate the significance of 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) in the assessment of the therapeutic response to ^{131}I-metaiodobenzylguanidine (MIBG) in malignant phaeochromocytoma.
Methods We reviewed the records of 11 patients (7 men and 4 women) with malignant phaeochromocytoma who underwent ^{131}I-MIBG therapy (100–200 mCi). ^{18}F-FDG PET and serum catecholamine assays were performed 3 months before and after the first dose of ^{131}I-MIBG. FDG uptake was evaluated in the observed lesions using the maximum standardised uptake value (SUV_{max}). The average SUV_{max} of all lesions (ASUV) was calculated. If more than five lesions were identified, the average SUV_{max} of the five highest SUV_{max} (ASUV5) was calculated. The ratio of pre- and post-therapy values was calculated for the highest SUV_{max} (rMSUV), ASUV (rASUV), ASUV5 (rASUV5), CT diameter (rCT) and serum catecholamine (rCA). Responder (R) and non-responder (NR) groups were defined after a clinical follow-up of at least 6 months according to changes in symptoms, CT, magnetic resonance imaging (MRI) and ^{123}I-MIBG scan.
Results Post-therapy evaluation revealed five R and six NR patients. The size of the target lesions was not significantly different before and after therapy ($p>0.05$). However, ASUV and ASUV5 were significantly lower in the R group (rASUV 0.64±0.18, rASUV5 0.68±0.17) compared to the NR group (rASUV 1.40±0.54, rASUV5 1.37±0.61) ($p<0.05$).
Conclusion ^{18}F-FDG PET can be potentially used to evaluate the response of malignant phaeochromocytoma to ^{131}I-MIBG therapy.

Keywords Phaeochromocytoma · 2-[^{18}F]Fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) · ^{131}I-metaiodobenzylguanidine (MIBG) · Malignant phaeochromocytoma · Radionuclide therapy

A. Nakazawa (✉) · T. Higuchi · N. Oriuchi · Y. Arisaka · K. Endo
Department of Diagnostic Radiology and Nuclear Medicine,
Gunma University Hospital,
3-39-22 Showa-machi,
Maebashi, Gunma 371-8511, Japan
e-mail: azusa-n@sea.sannet.ne.jp

Introduction

Phaeochromocytoma is a rare, catecholamine-producing tumour. It originates from chromaffin cells, mainly located in the adrenal medulla [1]. Although most phaeochromocytomas develop from the adrenal medulla, they may also derive from the extra-adrenal organs such as sympathetic paraganglia [2]. Phaeochromocytoma is diagnosed on the basis of symptoms of catecholamine excess, such as severe hypertension, sweating, headache and anxiety attacks [3].

The annual incidence of phaeochromocytoma is 1–4/10^6 population [1]. About 10% of these tumours metastasise. Classically, only metastasised tumours identified in the extra chromaffin tissue are considered malignant, for it is almost impossible to differentiate a benign from a malignant tumour only by histological criteria [4]. As a consequence, malignant phaeochromocytoma is not surgically curable. Moreover, surgically unresectable malignant phaeochromocytoma tumours have limited therapeutic options. These cases are currently treated mainly with combination chemotherapy including cyclophosphamide,

vincristine and dacarbazine (CVD). However, the chemotherapy frequently produces adverse events [5]. The 5-year survival rate of metastatic phaeochromocytoma has been reported to be less than 50% [6, 7]. Metaiodobenzylguanidine (MIBG) is a guanethidine analogue that is structurally similar to norepinephrine and accumulates in sympathomedullary tissues. ^{131}I-MIBG and ^{123}I-MIBG are commonly used in the diagnosis of phaeochromocytoma. A high dose of ^{131}I-MIBG has anti-tumour efficacy by emitting β-particles [8]. ^{131}I-MIBG was initially used in the treatment of phaeochromocytoma in 1983 [9]. The advantages of ^{131}I-MIBG therapy are effectiveness to both primary and metastatic lesions and relatively mild adverse events. The application of a ^{131}I-MIBG therapeutic protocol in phaeochromocytoma was established at a consensus meeting in Rome in 1991 [10].

Nevertheless, the assessment method for treatment with ^{131}I-MIBG is still controversial. In a retrospective study with 33 patients, Safford et al. reported that symptomatic and hormonal response to ^{131}I-MIBG therapy, but not reduction in tumour volume, was the best predictor of prolonged survival [11].

In contrast, Gonias et al. reported that MIBG scan or computed tomography/magnetic resonance imaging (CT/MRI), but not hormonal response, was predictive of overall survival (OS) after high-dose ^{131}I-MIBG therapy [12].

2-[^{18}F]Fluoro-2-deoxy-D-glucose (FDG) is a radiolabelled glucose analogue used for imaging of malignant tumours with high glucose metabolism. FDG has been applied to the diagnosis of phaeochromocytoma. Its usefulness for monitoring the efficiency of ^{131}I-MIBG therapy in malignant phaeochromocytoma has also been reported [13]. A comparative study between MIBG scan and FDG positron emission tomography (PET) revealed that FDG PET is able to detect a greater number of phaeochromocytoma lesions and metastases and that it may be used for the assessment of therapeutic effects [14]. In another comparative study by Menzel et al. [13], FDG PET revealed a significant decrease in standardised uptake value (SUV) after ^{131}I-MIBG therapy, while no significant volume reduction was detected by X-ray CT. Thus, determination of the SUV by FDG PET is expected to be a good alternative therapeutic assessment of the tumour which shows little change in size by CT/MRI [13]. Although several approaches have been performed, the appropriate procedure for therapeutic evaluation of ^{131}I-MIBG therapy in malignant phaeochromocytoma is still controversial.

In this study, we evaluated the efficacy of FDG PET for assessment of ^{131}I-MIBG therapy compared with X-ray CT and serum catecholamine levels in order to identify biomarkers for the treatment of malignant phaeochromocytoma.

Materials and methods

Patients and ^{131}I-MIBG therapy

We performed a retrospective review of 11 patients undergoing ^{131}I-MIBG therapy for malignant phaeochromocytoma for the first time from April 2006 to February 2010 in our institution. Patients with positive tracer uptake as assessed by ^{123}I-MIBG scan were selected for treatment. The dose of ^{131}I-MIBG was 100–200 mCi (3.7–7.4 GBq). We usually give two to three treatments of 100–200 mCi (3.7–7.4 GBq) with an at least 6-month interval. In this study, we analysed FDG PET and serum catecholamine assays which were performed 3 months before and after the first dose of ^{131}I-MIBG.

Patients with myelosuppression (haemoglobin <9.0 g/dl, white blood cell count <3,000 mm^{-3}, platelet count <100,000 mm^{-3}), patients who were pregnant or breast-feeding, and patients with an expected survival of less than 1 month or renal dysfunction with effective glomerular filtration rate <30 ml/min were excluded from this study.

To prevent solution (^{131}I-MIBG) leakage during administration, a central venous catheter was inserted through the right subclavian vein of all patients. ^{131}I-MIBG was infused intravenously for 30 min in a lead-shielded room with intravenous hydration.

Written informed consent was obtained from each patient before they were enrolled in the study, and the study protocol was approved by the Institutional Review Board. This study was conducted in accordance with the amended Helsinki Declaration. Our database and electronic charts were retrospectively reviewed to obtain follow-up data.

Imaging protocols

All patients underwent repeated FDG PET (eight scans) or PET/CT (three scans) 3 months before and after the first ^{131}I-MIBG therapy.

FDG was produced in our cyclotron facility. The patients were fasted for 6 h, and free of alcohol and caffeine for 12 h, before being given an intravenous injection of ^{18}F-FDG (5 MBq/kg). FDG PET/CT scans were performed with the Discovery STE (GE Healthcare, Waukesha, WI, USA) and Biograph 16 (Siemens Medical Solutions, Knoxville, TN, USA) scanners, with a 700-mm field of view (FOV) and slice thickness of 3.27 mm. The CT was acquired to correct PET transmission using the following parameters: 140 kV and 120–240 mAs to produce 128×128 matrix images. The patients were scanned in the arms-down position from head to thigh. Shallow breathing was advised to avoid motion artefacts and minimise misregistration of CT and PET images. Intravenous contrast material was not administered for CT scanning. After the CT scan, the PET

data were acquired, and acquisition time was 3 min per bed position. CT images were reconstructed by using the conventional filtered backprojection method. Axial full-width at half-maximum (FWHM) at 1 cm from the centre of the FOV was 6.3 mm. Intrinsic system sensitivity was 8.5 cps/kBq for three-dimensional acquisition.

Biochemical markers

Pre- and post-therapy assessment included the following markers of catecholamine metabolism: plasma norepinephrine, epinephrine and dopamine; and 24-h urine collection for fractionated catecholamines (norepinephrine, epinephrine and dopamine), metanephrine and normetanephrine. In this study, we adopted norepinephrine as a representative marker (upper reference limit <0.06 ng/ml in plasma).

Scan review and evaluation

FDG PET and PET/CT images were retrospectively reviewed by two experienced nuclear medicine physicians, who were blinded to all other imaging and clinical information, including biochemical markers. The SUV was used for image interpretation. FDG uptake of the observed lesions was evaluated using the maximum SUV (SUV_{max}), and tumour size was calculated on the basis of the sum of the longest diameter of the target lesions.

Statistical analysis

Responder (R) and non-responder (NR) patients were defined according to changes in symptoms, CT, MRI and ^{131}I-MIBG scan obtained after a period of at least 6 months of clinical follow-up. Symptoms included fatigue, flushing, sweat, diarrhoea, weight loss, etc. Symptomatic response was defined as either complete resolution or a subjective decrease in the intensity of symptoms, because the evaluation of these symptoms cannot help relying on a subjective evaluation excluding the one which can be measured by the numerical value. If a new lesion was detected by CT, MRI, PET or ^{131}I-MIBG scan after ^{131}I-MIBG therapy, the patient was classified into the NR group.

The average SUV_{max} of all lesions was defined as ASUV. The ratio of pre- and post-therapy ASUV was calculated (rASUV). If more than five lesions were identified by FDG PET, the average of the five highest SUV_{max} values was calculated (ASUV5). The ratio of pre- and post-therapy ASUV5 was also calculated (rASUV5). The lesion with the highest SUV_{max} was defined as MSUV. The ratio of pre- and post-therapy MSUV was calculated (rMSUV). In addition, the sum of the longest diameters of the lesions with the five highest SUV_{max} values (summed CT diameter) and the ratio of pre- and post-therapy summed CT diameter (rCT) were also calculated. For hormonal evaluation, the ratio of pre- and post-therapy serum catecholamine (rCA) was calculated.

The unpaired t test was used to evaluate the statistical significance of rASUV, rASUV5, rMSUV, rCT and rCA in the R and NR groups. Significance was set at $p<0.05$.

Results

The characteristics of the patients involved in the present study are summarised in Table 1. The patients were seven men and four women, aged 29–69 years [mean (SD) 49.4 (14.6) years]. Ten patients underwent surgery: three patients (27%) received radiotherapy, five patients (45%) received chemotherapy and two patients (18%) received both chemotherapy and radiotherapy before ^{131}I-MIBG administration. Of 11 patients, 8 (72%) had elevated serum catecholamine (norepinephrine) before ^{131}I-MIBG therapy, and 5 of 11 patients (45%) had elevated urinary catecholamine. The median dose of ^{131}I-MIBG was 7.4 GBq (3.7–7.4 GBq).

Table 1 Characteristics of patients

Patient	Age (years)/sex	Primary lesion	Previous treatment	Dose of ^{131}I-MIBG (mCi)
1	43/M	L adrenal	Op, chemo	100
2	34/F	L adrenal	Op	100
3	69/M	L neck	Op, chemo, RT	100
4	59/M	L adrenal	Op	150
5	51/M	L adrenal	Op, chemo, RT	150
6	29/F	Retroperitoneum	Op	200
7	39/F	L adrenal	Op, chemo	200
8	32/M	R adrenal	Op, RT	200
9	57/F	L adrenal	Op, chemo	200
10	63/M	R adrenal	Op	200
11	67/M	Bladder	None	200

M male, *F* female, *Op* operation, *chemo* chemotherapy, *RT* radiation therapy

FDG PET or PET/CT revealed 124 metastatic lesions in all patients (Table 2). Bone metastasis was observed in 8 of 11 patients (73%), followed by lung metastasis (64%). Liver, lymph node and retroperitoneal metastases were also observed (36%).

Table 3 summarises the data regarding ASUV, ASUV5, MSUV, summed CT diameter, serum CA, and each corresponding ratio between R and NR patients, the number of which was determined post-therapy (five and six patients, respectively). ASUV, ASUV5 and MSUV measurements were obtained for all patients, whereas the summed CT diameter and the serum CA were obtained for eight patients only. We also calculated the ratio of each index before and after therapy (Table 4). rASUV and rASUV5 were significantly different between R [rASUV 0.64 (0.18), rASUV5 0.68 (0.17)] and NR [rASUV 1.40 (0.54), rASUV5 1.37 (0.61)] patients (p=0.02 and p=0.04 for rASUV and rASUV5, respectively). Similarly, rCA was significantly different between R and NR patients (p=0.03). In contrast, rMSUV and rCT were not significantly different between R and NR patients (p=0.06 and p=0.07, respectively).

Figure 1 shows a representative case of the R patient. This patient (patient 1) underwent resection of the left adrenal mass and was treated with 100 mCi (3.7 GBq) ^{131}I-MIBG. The FDG PET/CT performed before therapy revealed intense FDG uptake of multiple metastatic lesions. Retroperitoneal metastasis and multiple lung metastases were noticed. Post-therapy FDG PET/CT revealed reduced FDG accumulation in these lesions. ASUV and ASUV5 were also decreased. Patient 7, a patient classified into the NR group, had multiple bone metastases (Fig. 2). ASUV

Table 2 Number of metastatic lesions detected by FDG PET

Patient	Metastasis				
	Bone	Lung	Liver	Retroperitoneum	Lymph node
1		++		+	
2	++	++		+	++
3	++	++	++		
4	++		++		
5	++	++			
6	++				
7		++			++
8		++		+	++
9	++		++		
10	++		++	+	
11	++	++			++

+ metastasis detected by FDG PET, ++ multiple metastases detected by FDG PET

Table 3 The disease response evaluated by different measures of pre- and post-therapy

Patients		ASUV			ASUV5			MSUV			CT diameter (mm)			Serum CA (ng/ml)		
		Pre	Post	rASUV	Pre	Post	rASUV5	Pre	Post	rMSUV	Pre	Post	rCT	Pre	Post	rCA
R	1	8.28	4.68	0.57	8.28	4.68	0.57	13.20	8.80	0.67	52.00	52.00	52.00	–	–	–
	2	5.90	2.77	0.47	11.32	7.48	0.66	16.50	12.10	0.73	101.00	91.00	0.90	8.61	4.19	0.49
	3	8.30	6.55	0.79	8.30	6.55	0.79	12.50	10.20	0.82	26.50	26.50	1.00	1.65	1.01	0.61
	4	6.01	3.01	0.50	6.78	3.32	0.49	8.60	3.00	0.35	39.00	35.00	0.90	7.71	6.08	0.79
	5	4.53	3.94	0.87	6.78	6.12	0.90	9.24	8.45	0.91	–	–	–	13.14	5.93	0.45
NR	6	2.50	4.09	1.64	2.50	4.09	1.64	3.19	3.43	1.08	189.00	189.00	1.00	0.94	2.65	2.82
	7	9.72	10.60	1.09	12.76	13.32	1.04	19.70	16.80	0.85	90.00	90.00	1.00	7.60	10.86	1.43
	8	4.53	4.76	1.05	5.74	4.27	0.74	8.69	6.36	0.73	115.00	134.00	1.17	0.70	0.65	0.93
	9	2.23	2.72	1.22	2.23	2.72	1.22	2.45	2.88	1.18	–	–	–	–	–	–
	10	3.54	8.54	2.41	3.75	9.26	2.47	4.56	8.47	1.86	–	–	–	–	–	–
	11	6.80	6.91	1.02	12.18	13.86	1.14	13.10	13.60	1.04	100.00	106.00	1.06	3.59	9.73	2.71

R responder, *NR* non-responder, *CA* norepinephrine was adopted for a catecholamine index, *CT diameter* sum of the longest diameter of five highest SUV_{max} lesions

Table 4 The ratio of pre- and post-therapy values according to the response to therapy

R responder, *NR* non-responder, *CA* norepinephrine was adopted for a catecholamine index

	rASUV	rASUV5	rMSUV	rCT	rCA
R	0.64±0.18	0.68±0.17	0.70±0.22	0.95±0.06	0.58±0.15
NR	1.40±0.54	1.37±0.61	1.12±0.34	1.06±0.08	1.97±0.94
p value	0.02	0.04	0.06	0.07	0.03

increased from 9.72 to 10.60; ASUV5 increased from 12.76 to 13.32. Serum norepinephrine levels also increased from 7.60 to 10.86 ng/ml. However, neither patient 1 nor patient 7 exhibited major changes in the size of the tumour lesions.

Discussion

^{131}I-MIBG therapy is not expected to result in remarkable tumour shrinkage or eradication in most of the patients. In malignant phaeochromocytoma, the 5-year survival rate is low. Thus, the goal of treatment is to delay disease progression and extend survival with quality of life. For the evaluation of therapeutic response, the combination of clinical, biochemical and radiological findings are estimated; however, no single parameter by which the therapeutic response is evaluable has been established. According to our study on ^{131}I-MIBG therapy evaluation, no significant difference was observed between R and NR patient groups with regard to changes in tumour size measured by CT. This is consistent with the fact that many cases do not show changes in tumour size before and after therapy. On the other hand, FDG uptake measured by SUV was significantly decreased in the R group as compared with the NR group.

At present, therapy evaluation by the Response Evaluation Criteria in Solid Tumors (RECIST) was mainly based on the measurement of length by CT. On the other hand, evaluation by the PET Response Criteria in Solid Tumors (PERCIST) has been used in recent years [15]. In the case of malignant phaeochromocytoma, anti-tumour effects are usually moderate compared with chemotherapy on common solid tumours, and tumour shrinkage is rare even in cases with improved symptoms. Therefore, it is unlikely that a significant difference was observed by CT imaging between the two groups in this study. A significant difference was observed only in the SUV, as assessed by FDG PET. Notably, evaluation is considered to be barely possible in the case of a subtle quantitative change in glucose metabolism.

The ratio of the highest SUV_{max} (rMSUV) was not significantly different between R and NR patient groups.

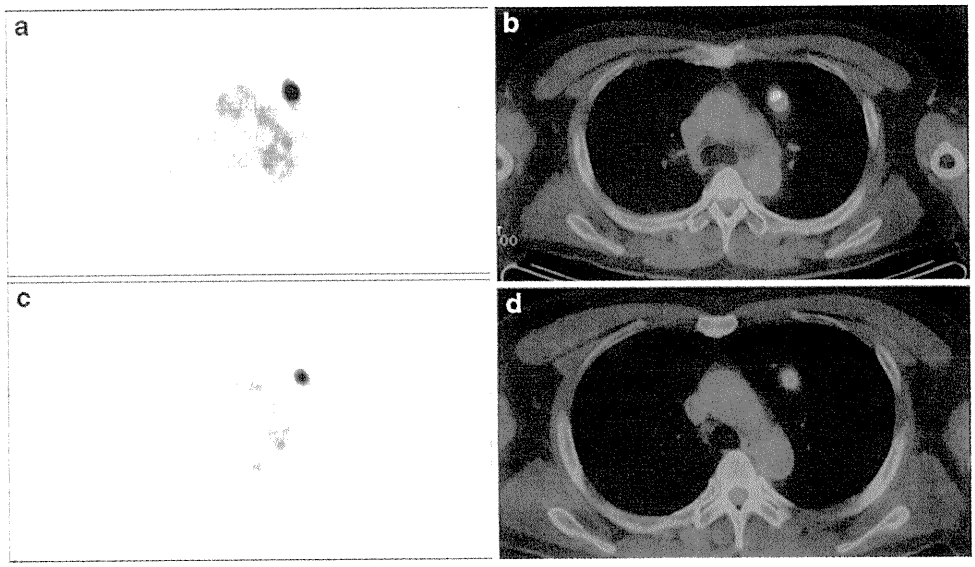

Fig. 1 A 43-year-old male patient with multiple lung metastases (patient 1). PET (**a**) and combined PET/CT (**b**) images obtained before ^{131}I-MIBG therapy showed FDG uptake in the lung metastasis. PET (**c**) and PET/CT (**d**) 3 months after ^{131}I-MIBG therapy showed FDG uptake decreased from 10.7 to 4.6

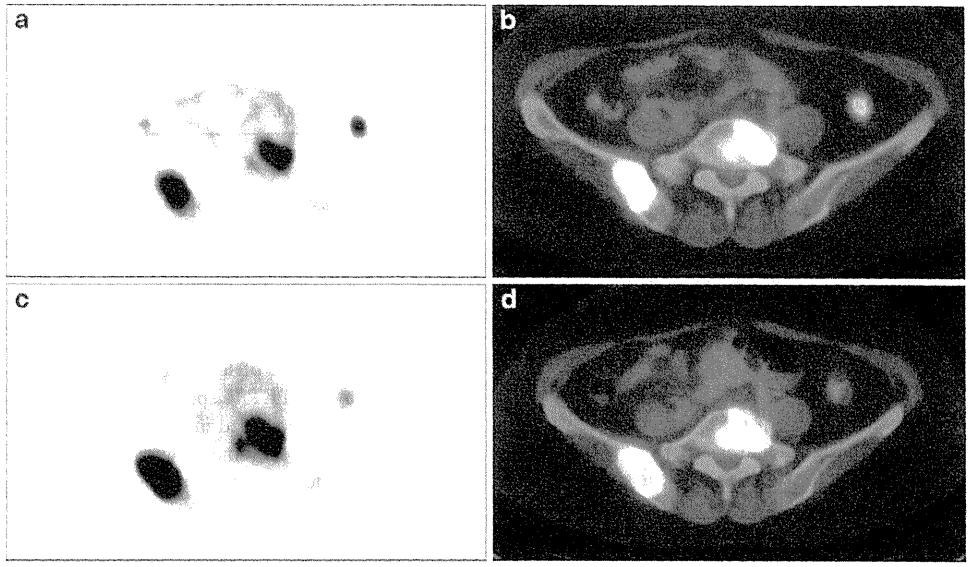

Fig. 2 A 57-year-old man (patient 7) underwent ^{131}I-MIBG therapy (200 mCi). PET (**a**) and PET/CT (**b**) images before ^{131}I-MIBG therapy showed FDG uptake in the right iliac bone and the L5 vertebra. Increased FDG uptake was also observed in the post-therapy study (from 12.8 to 17.3) (**c**, **d**)

On the other hand, a significant difference was observed for rASUV (calculated as the average of SUV changes for all lesions), or simplified rASUV5. On the basis of the above, we consider accurate evaluation to be impossible even using SUV without a comprehensive review of the accumulated changes in multiple lesions.

In our study, the most significantly different parameter between R and NR patients was rASUV. Unfortunately, in cases with a large number of metastases, determination of the SUV_{max} for all of the lesions by FDG PET is highly time-consuming. Therefore, ASUV5 (also statistically significant between R and NR patients) may be a suitable parameter for evaluation of the therapeutic effects. This method is less time-consuming than measurement of the SUV_{max} for all of the lesions.

In a study by Nwosu et al. [14], Kaplan-Meier analysis showed significantly increased survival (p=0.01) from the date of first ^{131}I-MIBG administration in patients with metastatic neuroendocrine tumours who reported symptomatic improvement. Patients with biochemical and radiological (MIBG scan, CT and MRI) responses did not show any statistically significant alteration in survival compared with NR patients. Ideally, assessment of the therapeutic effectiveness of ^{131}I-MIBG therapy should be based on Kaplan-Meier survival curves. However, these analyses are not available in malignant phaeochromocytoma because it is a rare disease, with only a few cases per year treated with ^{131}I-MIBG. In addition, evaluation of symptoms is a subjective parameter. Although the evaluation of symptoms is clinically useful, the assessment should be objective and reproducible. In our study, we used an objective method (based on SUV determination) for the quantitative evaluation of therapy response.

Our study suggests that FDG PET is useful for monitoring the efficiency of ^{131}I-MIBG therapy. However, we did not analyse MIBG scan as an evaluation procedure. Taggart et al. suggested that, for patients with MIBG-positive relapsed neuroblastoma, MIBG is more sensitive than FDG PET for disease detection and response evaluation after ^{131}I-MIBG therapy [16]. In that study, the therapeutic effect was assessed by comparing the number of lesions detected before and after ^{131}I-MIBG therapy by FDG PET coupled with MIBG scan. In malignant phaeochromocytoma, metastatic lesions rarely disappear after ^{131}I-MIBG therapy. The purpose of ^{131}I-MIBG therapy is usually to control disease progression. Therefore, it is inappropriate to use the change in the number of metastatic lesions detected by FDG PET as an evaluation method for malignant phaeochromocytoma. Therefore, our study is important because it provides a quantitative method for such evaluation.

Our study has some limitations. Firstly, the number of patients is limited. Secondly, the final assessment of therapeutic effectiveness is inadequate. Accurate definition of R and NR was important in this study. Ideally, patients should be classified into R and NR after a long-term

(several years) follow-up; however, we classified the patients after 1-year follow-up.

In conclusion, the results of the present study indicate that FDG PET is an alternative and useful method for evaluating the effect of ^{131}I-MIBG therapy as compared to CT in patients with malignant phaeochromocytoma. The change in SUV from pre- to post-therapy, as assessed by FDG PET, can be used as a quantitative index of assessment. In particular, the average SUV_{max} of the five highest lesions may be useful in the present clinical evaluation. Thus, FDG PET has the potential to predict or monitor the response to ^{131}I-MIBG therapy.

Conflicts of interest None.

References

1. Ilias I, Pacak K. A clinical overview of pheochromocytomas/paragangliomas and carcinoid tumors. Nucl Med Biol 2008;35: S27–34.
2. Lenders JW. Biochemical diagnosis of pheochromocytoma and paraganglioma. Ann Endocrinol (Paris) 2009;70:161–5.
3. Guller U, Turek J, Eubanks S, Delong ER, Oertli D, Feldman JM. Detecting pheochromocytoma: defining the most sensitive test. Ann Surg 2006;243:102–7.
4. Salmenkivi K, Heikkilä P, Haglund C, Arola J. Malignancy in pheochromocytomas. APMIS 2004;112:551–9.
5. Havekes B, Lai EW, Corssmit EP, Romijn JA, Timmers HJ, Pacak K. Detection and treatment of pheochromocytomas and paragangliomas: current standing of MIBG scintigraphy and future role of PET imaging. Q J Nucl Med Mol Imaging 2008;52:419–29.
6. John H, Ziegler WH, Hauri D, Jaeger P. Pheochromocytomas: can malignant potential be predicted? Urology 1999;53:679–83.
7. Eisenhofer G, Bornstein SR, Brouwers FM, Cheung NK, Dahia PL, de Krijger RR, et al. Malignant pheochromocytoma: current status and initiatives for future progress. Endocr Relat Cancer 2004;11:423–36.
8. Hoefnagel CA, Schornagel J, Valdés Olmos RA. [131I]metaiodobenzylguanidine therapy of malignant pheochromocytoma: interference of medication. J Nucl Biol Med 1991;35:308–12.
9. Sisson J, Shapiro B, Beierwaltes WH, Nakajo M, Glowniak J, Mangner T, et al. Treatment of malignant pheochromocytoma with a new radiopharmaceutical. Trans Assoc Am Physicians 1983;96:209–17.
10. Ackery DM, Troncone L. Session on the role of [131I]metaiodobenzylguanidine in the treatment of malignant phaeochromocytoma. Chairmen's report. J Nucl Biol Med 1991;35:318–20.
11. Safford SD, Coleman RE, Gockerman JP, Moore J, Feldman JM, Leight Jr GS, et al. Iodine-131 metaiodobenzylguanidine is an effective treatment for malignant pheochromocytoma and paraganglioma. Surgery 2003;134:956–62.
12. Gonias S, Goldsby R, Matthay KK, Hawkins R, Price D, Huberty J, et al. Phase II study of high-dose [131I]metaiodobenzylguanidine therapy for patients with metastatic pheochromocytoma and paraganglioma. J Clin Oncol 2009;27:4162–8.
13. Menzel C, Graichen S, Berner U, Risse JH, Diehl M, Döbert N, et al. Monitoring the efficacy of iodine-131-MIBG therapy using fluorine-18-FDG-PET. Acta Med Austriaca 2003;30:37–40.
14. Nwosu AC, Jones L, Vora J, Poston GJ, Vinjamuri S, Pritchard DM. Assessment of the efficacy and toxicity of (131)I-metaiodobenzylguanidine therapy for metastatic neuroendocrine tumours. Br J Cancer 2008;98:1053–8.
15. Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. J Nucl Med 2009;50:122S–50.
16. Taggart DR, Han MM, Quach A, Groshen S, Ye W, Villablanca JG, et al. Comparison of iodine-123 metaiodobenzylguanidine (MIBG) scan and [18F]fluorodeoxyglucose positron emission tomography to evaluate response after iodine-131 MIBG therapy for relapsed neuroblastoma. J Clin Oncol 2009;27:5343–9.

nature publishing group

Mineralocorticoid Receptor-Associated Hypertension and Its Organ Damage: Clinical Relevance for Resistant Hypertension

Hirotaka Shibata[1] and Hiroshi Itoh[1]

The role of aldosterone in the pathogenesis of hypertension and cardiovascular diseases has been clearly shown in congestive heart failure and endocrine hypertension due to primary aldosteronism. In resistant hypertension, defined as a failure of concomitant use of three or more different classes of antihypertensive agents to control blood pressure (BP), add-on therapy with mineralocorticoid receptor (MR) antagonists is frequently effective, which we designate as "MR-associated hypertension". The MR-associated hypertension is classified into two subtypes, that with elevated plasma aldosterone levels and that with normal plasma aldosterone levels. The former subtype includes primary aldosteronism (PA), aldosterone-associated hypertension which exhibited elevated aldosterone-to-renin ratio and plasma aldosterone levels, but no PA, aldosterone breakthrough phenomenon elicited when angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin II receptor blocker (ARB) is continued to be given, and obstructive sleep apnea. In contrast, the latter subtype includes obesity, diabetes mellitus, chronic kidney disease (CKD), and polycystic ovary syndrome (PCOS). The pathogenesis of MR-associated hypertension with normal plasma aldosterone levels is considered to be mediated by MR activation by pathways other than high aldosterone levels, such as increased MR levels, increased MR sensitivity, and MR overstimulation by other factors such as Rac1. For resistant hypertension with high plasma aldosterone levels, MR antagonist should be given as a first-line therapy, whereas for resistant hypertension with normal aldosterone levels, ARB or ACE-I should be given as a first-line therapy and MR antagonist would be given as an add-on agent.

Keywords: aldosterone; blood pressure; hypertension; mineralocorticoid receptor; resistant hypertension

American Journal of Hypertension, advance online publication 19 January 2012; doi:10.1038/ajh.2011.245

SIGNIFICANCE OF ALDOSTERONE AND MINERALOCORTICOID RECEPTOR IN HYPERTENSION AND CARDIOVASCULAR COMPLICATIONS

The role of aldosterone in the pathogenesis of hypertension and cardiovascular diseases has long been recognized through multiple mechanisms including inflammation, oxidative stress, fibrosis, and endothelial dysfunction. The beneficial effects of aldosterone blockade has been clearly demonstrated in patients with congestive heart failure by several randomized–controlled clinical trials, particularly Randomized Aldactone Evaluation Study (RALES),[1] Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS),[2] and Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF).[3] Based on the clinical studies, the addition of mineralocorticoid receptor (MR) antagonists to optimal medical therapy reduces morbidity and mortality among patients with mild to severe systolic heart failure.

The role of aldosterone in hypertension is best illustrated in primary aldosteronism (PA) which is characterized by aldosterone excess, suppressed plasma renin activity, and treatment-resistant hypertension. The widespread use of aldosterone-to-renin ratio as a screening test leads to higher prevalence of PA in hypertensive patients than previously predicted. The recent meta-analysis on the prevalence of PA showed that 4.3% of hypertensive patients in a primary care setting and 9.0% of referred patients have confirmed PA.[4] Patients with PA are at an increased cardiovascular risk by three to fivefold, as shown by higher rates of stroke, myocardial infarction, and arrhythmias compared to hypertensive individuals without PA.[5–9]

Resistant hypertension (RHTN) is defined as persistent hypertension with a failure of concomitant use of three or more different classes of antihypertensive agents to control blood pressure (BP) to <140/90 mm Hg, remains a common clinical problem.[10,11] The prevalence of PA is shown to be higher (14–21%) in patients with RHTN compared to the general hypertensive population. Besides PA, RHTN is frequently observed in several subsets of patients, including obstructive sleep apnea, obesity, diabetes mellitus, chronic kidney disease (CKD), and polycystic ovary syndrome (PCOS). Despite a variety of plasma aldosterone levels among these pathologic states, add-on therapy with MR antagonists is shown to be very effective in patients with RHTN, indicating MR overactivation independent of plasma aldosterone levels.[12]

[1]Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan. Correspondence: Hiroshi Itoh (hrith@sc.itc.keio.ac.jp)

Received 12 October 2011; first decision 23 October 2011; accepted 21 November 2011.

In the Framingham Offspring Study, higher baseline plasma aldosterone levels within the physiologic range were associated with an increased risk of BP elevation or development of hypertension after 4 years in 1,688 normotensive individuals.[13] The clinical observation suggests that MR overactivation plays a crucial role in not only cardiovascular complications but also a risk of developing hypertension.

ALDOSTERONE ACTION AND MR ACTIVITY

The MR is a member of the nuclear receptor superfamily and is essential for controlling sodium and potassium transport in epithelial cells, most notably in the kidney and colon.[14,15] Aldosterone induces activation of serum- and glucocorticoid-inducible kinase 1 and epithelial sodium channel which is followed by downstream actions that promote both ion transport and inflammation. In the kidney, the upregulation of intercellular adhesion molecule-1, monocyte chemotactic protein-1, interleukin-6, plasminogen activator inhibitor-1, and transforming growth factor-β contributes to vascular injury, tubulointerstitial inflammation and subsequent fibrosis, and glomerular injury. Aldosterone also plays an important role in nonepithelial cells, such as cardiac myocytes and vascular walls.

Nuclear receptor coregulators are composed of both coactivators and corepressors and are defined as nonreceptor proteins which interact with nuclear receptors to potentiate or attenuate transactivation. Among over 300 coregulators, the number of MR-interacting coregulators identified to date is very limited such as p160 family, RHA, ELL, Ubc9, and NF-YC.[16–22] Most of the canonical mechanisms of aldosterone are considered to be mediated by cytosolic/nuclear MR with multiple coregulators which are defined as genomic action. However, it is now generally accepted that aldosterone has acute effects, through rapid, so-called nongenomic signaling pathways.[23,24] Though aldosterone mediates rapid nongenomic effects via both MR-dependent and -independent pathways, the mechanism(s) of this MR-independent effect of aldosterone have remained a focus of controversy. Recent studies implicated that rapid vascular response to aldosterone is dependent on the availability of GPR30, which is originally an orphan G protein-coupled receptor. Functional significance of GPR30 and MR remains to be elucidated.[25–27]

There is one clinical observation that patients with autosomal dominant pseudohypoaldosteronism type 1, in whom plasma renin and aldosterone levels are elevated due to the MR gene mutations, present with no significant cardiovascular diseases or hypertension.[28] These findings suggest that the MR-independent aldosterone action alone may not lead to cardiovascular complications. Since the clinical significance of MR-independent aldosterone action has not been proved yet, the role of aldosterone in cardiovascular function may be mainly mediated by classical MR.

MR ACTIVITY AND HYPERTENSION

Previous reports have demonstrated the antihypertensive efficacy of high doses of spironolactone in subjects with PA and, to a lesser degree, subjects with RHTN.[10–12] Nishizaka *et al.*[29] showed that low-dose spironolactone provides significant additive BP reduction in African American and white subjects with RHTN with and without PA, indicating that MR is likely to be overactivated in patients with RHTN. Since the MR is activated by its agonistic ligands, elevation of plasma aldosterone levels obviously activate the MR activity in target tissues. In addition, the MR activity can be activated without elevation of plasma aldosterone levels in several diseased states through multiple mechanisms including increased MR expression levels, increased MR sensitivity, or MR overstimulation by other factors. In fact, RHTN frequently includes hypertension whose BP level is effectively controlled by MR antagonists. We therefore designate such MR antagonist–responsive RHTN in a broad sense as MR-associated hypertension. The MR-associated hypertension can be divided into two subtypes, that with high aldosterone and that with normal aldosterone levels (Figure 1, Tables 1 and 2). In the former subtype, plasma aldosterone levels are relatively higher (usually ≥150 pg/ml) in proportion to plasma renin activity. It is reasonable that RHTN with elevated plasma aldosterone levels can be effectively treated with MR antagonists. However, it should be noted that several subsets of patients with RHTN with normal plasma aldosterone levels can also be effectively controlled by add-on therapy with MR antagonist, which we define as MR-associated hypertension in a narrow sense (Table 2).

MR-ASSOCIATED HYPERTENSION WITH ELEVATED PLASMA ALDOSTERONE LEVEL

PA is a typical MR-associated hypertension with elevated plasma aldosterone level. The recommendation of PA clinical practice guideline consists of three consecutive steps: case

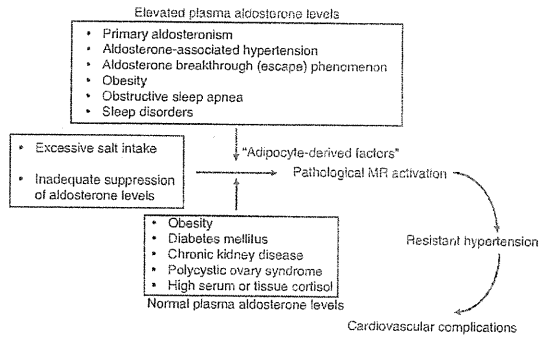

Figure 1 | Pathogenesis of MR-associated hypertension. MR, mineralocorticoid receptor.

Table 1 | MR-associated hypertension with elevated plasma aldosterone levels

Definition
MR antagonist–responsive hypertension
High plasma aldosterone levels (usually greater than 150 pg/ml) in proportion to plasma renin activity
Pathologic state
Primary aldosteronism
Aldosterone-associated hypertension
Aldosterone breakthrough (escape) phenomenon
Obesity
Obstructive sleep apnea
Sleep disorders (insomnia, sleep deprivation, shift working)

MR, mineralocorticoid receptor.

Table 2 | MR-associated hypertension with normal plasma aldosterone levels

Definition
MR antagonist–responsive hypertension
Normal plasma aldosterone levels
Pathologic state
Obesity
Diabetes mellitus
Chronic kidney disease
Polycystic ovary syndrome
High serum or tissue cortisol levels

MR, mineralocorticoid receptor.

detection, confirmatory, and lateralization tests.[30,31] The case detection of PA should be performed with combination of an increased plasma aldosterone concentration (pg/ml)/plasma renin activity (ng/ml/h) ratio of greater than 200 and a plasma aldosterone concentration of greater than 150 pg/ml.

Among several confirmatory tests, the Keio University Hospital (Tokyo, Japan) mostly relies on oral salt loading test. When 24-h urine aldosterone excretion is greater than 8 μg with a urinary Na excretion greater than 170 mmol, PA can be definitively diagnosed. Inadequately suppressed aldosterone levels under excessive salt intake have been shown to overstimulate the MR transactivation particularly in non-epithelial tissues such as myocardium and vasculature, thus resulting in the development and progression of several cardiovascular diseases.[5–9] Two most common subtypes of PA are aldosterone-producing adenomas (APAs) and idiopathic hyperaldosteronism (IHA) due to bilateral adrenal hyperplasia. The subtype diagnosis of PA is crucial because treatment option is dependent on its subtype; unilateral adrenalectomy for APAs and medical therapy including MR antagonist for IHA. The pathogenesis of APAs is due to autonomous overexpression of CYP11B2 in the tumors[32] or the *KCNJ5* potassium channel gene mutation, whereas that of IHA is totally unknown at present.[33]

Doi *et al.* have recently reported that mice lacking cryptochrome-1 (Cry1) and cryptochrome-2 (Cry2), which are clock genes that produce the circadian rhythm, show salt-sensitive hypertension with high plasma aldosterone and suppressed plasma renin activity levels.[34] Further research revealed that a newly identified steroidogenic enzyme, 3β-hydroxysteroid dehydrogenase type 6 (Hsd3b6) is exclusively overexpressed in the adrenal zona glomerulosa cells of *Cry1/Cry2* double-knockout mice, thus resulting in enhanced aldosterone secretion. They also showed that HSD3B1, a human ortholog of mouse Hsd3b6, is enriched in human adrenal zona glomerulosa cells, suggesting a role of this enzyme in aldosterone synthesis. It remains to be elucidated a possible role of HSD3B1 in the pathogenesis of IHA.

Several reports showed that a higher prevalence of metabolic syndrome in a large population of patients with PA compared with patients with essential hypertension.[35,36] Of interest, a recent retrospective study of 100 patients with PA showed that the prevalence of the metabolic syndrome and the body mass index (BMI) value were significantly higher in IHA compared with APA patients.[37] Similarly, in our institute, the BMI and homeostasis model assessment of insulin resistance were significantly correlated with urinary aldosterone excretion in patients with IHA (n = 54), but not with APA (n = 31) (Tables 3 and 4). It is tempting to speculate that the pathogenesis of aldosterone excess in IHA may be related with obesity and insulin resistance. Previous reports showed that secretory products from isolated human adipocytes strongly stimulated aldosterone secretion in human adrenocortical NCI-H295R cells, indicating that human adipocytes secrete aldosterone-releasing factors.[38,39] Several reports showed that plasma aldosterone levels are correlated with BMI and waist circumference in hypertensive subjects,[40,41] suggesting that the adipocyte-derived factors may play an important role in MR-associated hypertension with elevated plasma aldosterone levels particularly including IHA.

Second, high aldosterone level is also involved in RHTN other than PA. Sartoli *et al.*[42] demonstrated that high levels of plasma aldosterone in hypertensive patients increase the risk of poor BP control, despite the use of combination therapy with multiple antihypertensive agents, even when they do not meet the diagnostic criteria for PA. They designated such RHTN as "aldosterone-associated hypertension", which was defined as hypertension with an elevated aldosterone-to-renin ratio and plasma aldosterone levels, but no PA based on captopril suppression test. In this

Table 3 | Clinical features of two subtypes of primary aldosteronism

	APA	IHA	*P* value
Characteristics	Values (± s.d.)	Values (± s.d.)	
N	31	54	
Age, years	51 ± 11	51 ± 9	0.82
Sex (women)	12/31	30/54	0.14
BMI, kg/m^2	23.6 ± 4.5	24.8 ± 3.7	0.20
Waist circumstance, cm	83 ± 17	84 ± 9	0.84
Systolic BP, mm Hg	136 ± 20	140 ± 14	0.32
Diastolic BP, mm Hg	89 ± 16	86 ± 11	0.43
Diabetes mellitus	3/31	2/54	0.35
Dyslipidemia	1/31	9/45	0.085
Plasma aldosterone, pg/ml	404 ± 203	232 ± 96	<0.001**
Plasma-active renin, pg/ml	3.8 ± 2.6	6.5 ± 6.5	0.017*
Aldosterone:renin ratio	133.5 ± 80.9	55.1 ± 36.1	<0.001**
Urinary aldosterone, μg/day	23.9 ± 15.0	13.1 ± 7.5	0.01**
Serum potassium, mEq/l	3.7 ± 0.6	4.0 ± 0.4	0.009**

APA, aldosterone-producing adenoma; BMI, body mass index; BP, blood pressure; IHA, idiopathic hyperaldosteronism. *$P < 0.05$, **$P < 0.01$ between APA and IHA.

Table 4 | Correlation between urine aldosterone excretion and metabolic parameters

	APA		IHA	
	R	*P* value	R	*P* value
BMI	0.028	0.894	0.443	0.003**
HbA_{1c}	0.384	0.058	0.279	0.099
Glucose	−0.100	0.643	0.196	0.231
HOMA-R	−0.170	0.530	0.789	<0.001**
HDL-C	−0.109	0.582	−0.116	0.519
LDL-C	−0.027	0.089	0.086	0.613
TG	−0.100	0.605	0.175	0.279

APA, aldosterone-producing adenoma; BMI, body mass index; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-R, homeostasis model assessment of insulin resistance; IHA, idiopathic hyperaldosteronism; LDL-C, low-density lipoprotein cholesterol; R, correlation coefficient; TG, triglyceride.
**$P < 0.01$.

type of hypertension, adequate BP control was reached in a lower fraction of patients after a longer treatment period as compared with essential hypertensive patients.

Third, blocking the renin–angiotensin–aldosterone system with angiotensin-converting enzyme inhibitors (ACE-Is) or angiotensin II receptor blockers (ARBs) is often associated with an initial decrease in plasma aldosterone followed by a subsequent increase in aldosterone above pretreatment levels during prolonged treatment. This phenomenon is named as "aldosterone escape" or "aldosterone breakthrough" and it may contribute to drug resistance counteracting the antihypertensive actions of the drugs. Bomback and Klemmer[43] showed that an incidence of aldosterone breakthrough is 10–53% in patients with chronic heart or kidney disease on ACE-I or ARB therapy. However, the detailed molecular mechanisms for aldosterone breakthrough have not been elucidated. The add-on therapy of an MR antagonist in this context could result in an improved prognosis through a further and sustained BP reduction.

Obstructive sleep apnea (OSA) is characterized by recurrent episodes of partial or complete upper airway obstruction during sleep. OSA is particularly common in patients with RHTN.[44–48] Stimulation of sympathetic activity by OSA is likely representing the most important effect by which OSA increases BP through elevation of vascular resistance, greater cardiac output, and possibly stimulation of the renin–angiotensin–aldosterone system. Several adipocytokines, such as angiotensinogen, 12, 13-epoxy-9-keto-10(trans)-octadecenoic acid, and adipocyte-derived factors, would also stimulate aldosterone secretion.[44,46,48,49] Treatment of OSA with use of continuous positive airway pressure has generally shown a consistent but modest antihypertensive benefit due to suppression of the sympathetic activation. A positive correlation between OSA severity and aldosterone levels suggests two opposite possibilities[44,46,48]; either untreated OSA is stimulating aldosterone release or aldosterone excess is worsening OSA. Human studies, however, have not shown that treatment of OSA with continuous positive airway pressure to have a substantive effect on aldosterone levels. A preliminary study showed that treatment with a MR antagonist substantially reduces the severity of OSA,[45] suggesting that aldosterone-mediated chronic fluid retention as an important mediator of OSA severity in patients with RHTN.

Besides OSA, sleep disorders such as insomnia, sleep deprivation, and shift working have become common problems in many people. A sleep-inducing hormone, melatonin, rapidly suppresses the neural activity of suprachiasmatic nucleus through the melatonin type 1 receptor to induce sleep. Most sleep disorders are associated with disrupted circadian rhythm by which reduction of melatonin secretion leads to biological clock dysfunction. Based on a report that *Cry* knockout mice reveal a human-type IHA and salt-sensitive hypertension, a possible link among sleep disorders, melatonin, and elevated plasma aldosterone levels is suggested.[50]

MR-ASSOCIATED HYPERTENSION WITH NORMAL PLASMA ALDOSTERONE LEVEL

The prevalence of obesity is increasing and weight gain is associated with increases in BP. Mechanisms of obesity-related hypertension include insulin resistance, sodium retention, increased sympathetic nervous system activity, activation of the

renin–angiotensin–aldosterone, and altered vascular function. The clinical implications of the role of aldosterone in the metabolic syndrome and RHTN have been strongly suggested (Table 2, Figure 1).[51–55] It is therefore possible to hypothesize that the MR activity is overstimulated in the context of metabolic syndrome even in the absence of elevated plasma aldosterone levels.

Second, patients with diabetes often present with RHTN. ACE-Is or ARBs have become the cornerstone of the management of patients with hypertension plus diabetes and both drugs have been shown to have a beneficial effect on surrogate endpoints such as decreasing microalbuminuria, slowing progression from microalbuminuria to macroalbuminuria, and slowing the decline in renal function.[56,57] MR antagonists can be exceedingly helpful in selected diabetic patients. These drugs have been shown to reduce target organ disease and surrogate endpoints such as microproteinuria and left ventricular hypertrophy. The addition of spironolactone to a regimen that includes maximal ACE-I affords greater renoprotection than the addition of the ARB losartan in diabetic nephropathy, indicating that MR is overactivated in selected diabetic patients even in the absence of high plasma aldosterone levels.[57,58]

Plasma aldosterone levels have been correlated with alterations in kidney function in CKD, suggesting an association between kidney dysfunction and MR activation. ACE-Is or ARBs have become a first-line therapy for the management of patients with proteinuric CKD in a similar manner to diabetes. The use of MR antagonists as an add-on agent in CKD is recommended because of the possible direct MR activation as well as aldosterone breakthrough by ACE-Is or ARBs.[58–60] Beneficial effects of MR antagonists in CKD have been reported in diabetic nephropathy as well as nondiabetic proteinurias.[61–65] It is therefore suggested that direct activation of the MR that can occur in pathological states associated with CKD, even in the absence of increased circulating levels of aldosterone.

PCOS, which is characterized by chronic anovulation, hyperandrogenism, and insulin resistance, is considered metabolic disease associated with long-term health risks including cardiovascular disease and type 2 diabetes mellitus. Women with PCOS appear to have higher aldosterone levels than age- and BMI-matched controls.[66] A putative cause of the increased aldosterone levels, even if within normal limits, observed in PCOS could be the insulin resistance.

Under high serum or tissue cortisol levels such as Cushing's syndrome or inhibition of 11β-hydroxysteroid dehydrogenase type 2 by glycyrrhizin, clinical benefit of MR antagonists depends on displacement of cortisol from the cardiac as well as renal MR.[67,68]

PATHOGENESIS OF MR-ASSOCIATED HYPERTENSION

The MR action is principally activated by elevation of aldosterone levels and by alterations of MR status. The pathogenesis of MR-associated hypertension in a narrow sense is recognized to be overstimulation of MR independent of elevated plasma aldosterone levels (Figures 1 and 2). At present, putative molecular mechanisms of MR-associated hypertension are considered to include five mechanisms: increased MR gene transcription, increased MR sensitivity, MR stabilization, MR overstimulation by other factors, and activating mutation of the *MR* gene (Table 5).

Increased MR gene transcription

There are several pathological states in which MR gene transcription is increased. First, we have previously shown that MR expression and superoxide production was concomitantly increased in astrocytes of the striatum in the mouse 20-min middle cerebral artery occlusion model.[69] Treatment with spironolactone markedly stimulated the expression of neuroprotective or angiogenic factors, such as basic fibroblast growth factor and vascular endothelial growth factor, thus resulting in reduction of infarct size. These data indicate that increased MR transcripts play a pathological role in cerebral infarction *in vivo* and that MR antagonist therapy may thus provide a new therapeutic neuroprotective effects in the ischemic brain after stroke.

Second, rodent models of types 1 (streptozotocin-treated rat) and 2 (*db/db* mouse) diabetes mellitus developed albuminuria and histopathological evidence of renal injury as well as increased renal cortical levels of MR protein, MR messenger RNA (mRNA), transforming growth factor-β mRNA, and osteopontin mRNA. All of these changes were significantly reduced by treatment with eplerenone except for the elevated MR levels.[70] The beneficial effects of eplerenone were not

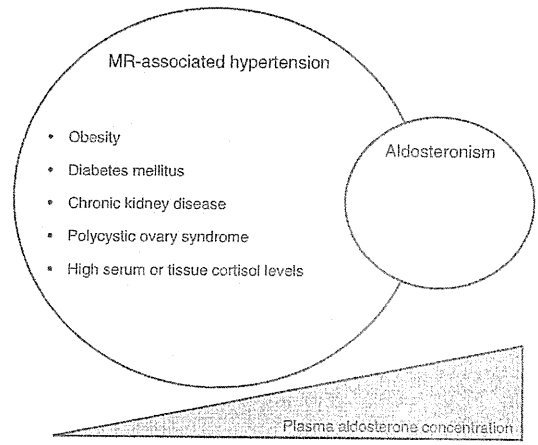

Figure 2 | MR-associated hypertension and putative diseases. MR, mineralocorticoid receptor.

attributable to changes in BP or glycemia. It is possible that increased intrarenal expression of MR may be one mechanism contributing to increased MR activity in diabetic kidneys.

Third, another study also showed increased MR mRNA levels in kidney biopsies from patients with chronic renal failure and heavy albuminuria, most of whom did not have diabetes.[71] Taken together with the above studies, increased expression of MR may be attributable to several pathological conditions, such as cerebral infarction, diabetes mellitus, and CKD.

Increased MR sensitivity

The MR activity is shown to be enhanced by post-translational modification, such as sumoylation, ubiquitylation, phosphorylation, and glycosylation.[15,18,72–74] First, treatment with H_2O_2 leads to global protein desumoylation including MR, thus resulting in enhancement of aldosterone-mediated MR transactivation by several fold *in vitro*.[18] Further analyses revealed that reduction of the MR sumoylation levels by overexpression of sumoylation-defective MR mutant, K89R/K399R/K494R/K953R significantly enhances MR transactivation by several fold *in vitro* (Figure 3). It is therefore speculated that oxidative stress enhances MR sensitivity through desumoylation of MR protein.

Second, cyclin-dependent kinase 5 (CDK5), a member of the CDK family of serine/threonine kinases, is essential for neuronal morphogenesis, function, and survival. Recent evidence suggests that aberrant CDK5 activation plays a role in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease. CDK5 phosphorylates MR, thus resulting in enhanced expression of brain-derived neurotrophic factor in rat cortical neuronal cells.[73] Therefore, increased MR sensitivity by CDK5-mediated phosphorylation of MR may reflect neuronal viability, synaptic plasticity, consolidation of memory, and emotional changes *in vivo*.

MR stabilization

There are several pathological states in which MR transcripts or proteins are stabilized. First, the MR expression is tightly regulated by osmotic stress through alteration of MR transcript stabilization.[75] Hypertonic conditions leads to a severe reduction in MR transcript and protein levels through induction of tonicity-induced expression of Tis11b, a mRNA-destabilizing protein, which may favor hypertonicity-dependent degradation of labile MR transcripts. Osmotic stress-regulated MR expression may explain an important molecular determinant for physiological abundant localization of MR at renal cortical collecting duct cells.

Second, the MR activation seems to involve the epidermal growth factor receptor (EGFR) for the development of fibrosis and vascular dysfunction.[76–79] The activated MR can rapidly transactivate the EGFR probably via the cytosolic tyrosine kinase, cSrc, resulting in extracellular signal-regulated kinase 1/2 (ERK1/2) activation. Furthermore, activated MR can enhance EGFR expression by a genomic mechanism. Increasing the number of EGFR makes a cell more sensitive for transactivating activity of other pathophysiological relevant stimuli, like angiotensin II, endothelin-1, or reactive oxygen species. Besides, our preliminary data showed that EGFR activation enhanced aldosterone-mediated MR transactivation via increased MR protein levels. The EGFR activation, coupling with activation of EGFR–tyrosine kinase and ERK, resulted in deubiquitylation and MR protein stabilization (Y. Mitsuishi,

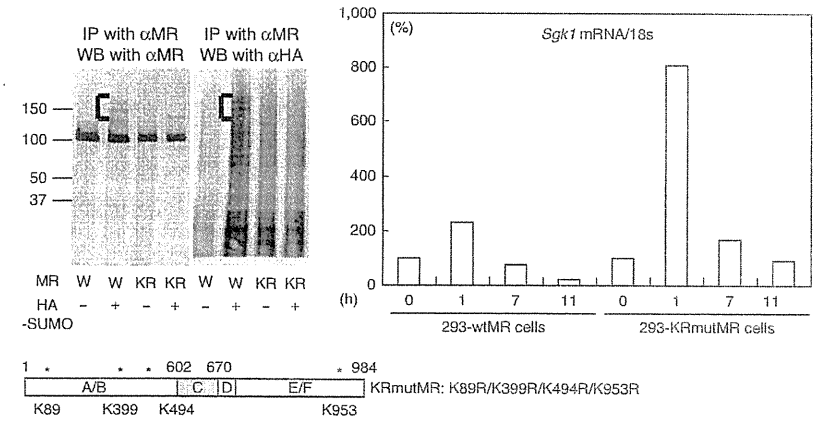

Figure 3 | Induction of endogenous Sgk1 mRNA levels was enhanced in stable transformants of sumoylation-inactivate MR mutant (KRmutMR). *Indicate four K residues (K89, K399, K494, and K953) which were mutated to R. αHA, anti-HA antibody; αMR, anti-MR antibody; HA, hemagglutinin; HA-SUMO, HA-tagged SUMO; IP, immunoprecipitation; mRNA, messenger RNA; MR, mineralocorticoid receptor; SUMO, small ubiquitin-related modifier; WB, western blot.

H. Shibata, I. Kurihara, H. Itoh, unpublished data). Activation of EGFR either activated by its peptide ligand or transactivated by factors such as reactive oxygen species, angiotensin II, or leptin may elicit vasconstriction and renal sodium retention.[80]

Third, it is believed that hyperglycemia is one of the most important metabolic factors in the development of both micro- and macrovascular complications in diabetic patients. One of the adverse effects of hyperglycemia is the chronic activation of protein kinase C (PKC).[81–84] Clinical trials using a PKCβ isoform inhibitor, ruboxistaurin, in diabetic patients showed 40% risk reduction in vision loss and decrease in urinary albumin excretion for diabetic nephropathy.[85,86] Our preliminary data showed that high glucose condition activate several PKC isoforms including PKCβ, thus resulting in enhancement of aldosterone-mediated MR transactivation via MR protein stabilization *in vitro* (T. Hayashi, H. Shibata, I. Kurihara, H. Itoh, unpublished data). These data suggest that some of the beneficial effects of PKCβ inhibitor are attributable to destabilization of MR proteins.

Fourth, another adverse effect of hyperglycemia is a O-linked N-acetylglucosamine (O-GlcNAc) glycosylation. The O-GlcNAc modification now appears to play a major role in glucotoxicity and participates in diabetic complications in various tissues.[72,87,88] Our preliminary data showed that high glucose condition enhances aldosterone-mediated MR transactivation by several fold via increased O-GlcNAc modification of MR and MR protein stabilization *in vitro* (R. Jo, H. Shibata, I. Kurihara, H. Itoh, unpublished data). The *in vitro* data may partially explain that add-on therapy with MR antagonist to maximum dose ACE-I further reduce albuminuria in diabetic nephropathy in a BP-independent manner.[57]

Table 5 | Putative molecular mechanisms of MR-associated hypertension

Molecular mechanisms	Pathological states
Increased MR gene transcription	
Unknown	Cerebral ischemia
Unknown	Diabetes mellitus
Unknown	Chronic kidney disease
Increased MR sensitivity	
Desumoylation	Oxidative stress
Phosphorylation by cyclin-dependent kinase 5	Neurodegenerative diseases
MR stabilization	
MR mRNA stabilization through reduction of Tis11b	Osmotic hypotonicity
Deubiquitylation	Hypercytokinemia (EGF, angiotensin II, leptin etc.)
Phosphorylation by PKCβ	High glucose
O-linked-β-N-acetylglucosamine modification	High glucose
MR overstimulation by other factors	
Rac1-mediated MR nuclear translocation	Excessive salt intake
Activating mutation of the MR gene (S810L)	Pregnancy-induced hypertension

EGF, epidermal growth factor; MR, mineralocorticoid receptor; mRNA, messenger RNA; PKCβ, protein kinase Cβ.

MR overstimulation by other factors

Excessive salt intake induced renal injury, such as heavy proteinuria, mesangial proliferation, and tubulointerstitial inflammation and fibrosis. Salt-induced renal injury was shown to be ameliorated by administration of MR antagonist, suggesting that the renal MR is pathologically activated even when plasma aldosterone level is suppressed. Recent reports showed a novel signaling crosstalk between MR and the small GTPase Rac1 that modulates MR function.[89] Salt excess increased expression levels of Rac1 in Dahl salt-sensitive, but not salt-insensitive rats.[90] Rac1 is shown to increase nuclear translocation of MR, thus resulting in enhanced MR activity. Elevation of Rac1 levels may therefore explain one mechanism of salt-sensitive hypertension. As shown before, alterations of abundance and/or MR affinity of coactivators and corepressors,[16–22] such as Ubc9, ELL, RHA, PGC-1α, PIAS1, and NF-YC play crucial roles in MR activation.

Activating mutation of the *MR* gene

The MR S810L mutation has been shown to be an activating mutation and it plays a crucial role in pregnancy-induced hypertension, since high progesterone levels during pregnancy act as an agonist for the mutant MR.[91]

CLINICAL MANAGEMENT OF MR-ASSOCIATED HYPERTENSION

Since MR-associated hypertension is a salt-sensitive RHTN, reduction of dietary salt intake is very effective to control BP. As a therapeutic approach for RHTN with high plasma aldosterone levels, MR antagonists, such as spironolactone and eplerenone,

Table 6 | Clinical management of MR-associated hypertension

Resistant hypertension with high plasma aldosterone levels
MR-antagonist (first-line)
Resistant hypertension with normal plasma aldosterone levels in the presence of obesity, diabetes mellitus, or CKD
ARB or ACE-I plus MR antagonist (add-on)

ARB, angiotensin II receptor blocker; ACE-I, angiotensin-converting enzyme inhibitor; CKD, chronic kidney disease; MR, mineralocorticoid receptor.

should be used as a first-line drug (Table 6). In contrast, ARBs or ACE-Is is frequently used as a first-line drug to control hypertension in patients with MR-associated hypertension, including obesity, diabetes mellitus, CKD, and POCS.[59,60] Based on the pathogenesis of MR-associated hypertension, low dose of MR antagonists should be given as an add-on agent for the treatment of RHTN with normal plasma aldosterone levels. However, given that patients with diabetes plus hypertension are prone to hyperkalemia due to hyporeninemic hypoaldosteronism, renal impairment, and/or ACE-I/ARB treatment, these drugs should be used cautiously and in low doses only. Serum potassium and creatinine levels will have to be monitored frequently.

Recently, dihydropyridine-type calcium channel blockers, such as nifedipine and amlodipine, are shown to have inhibitory effects on the MR transcriptional activity when they are given at higher doses.[92–94] Our preliminary data in patients with PA showed that switching from a nondihydropyridine-type calcium channel blocker, diltiazem to either amlodipine or nifedipine significantly reduced a transtubular potassium gradient, indicating that dihydopyridine-type calcium channel blockers have MR antagonistic activity in humans (I. Kurihara, H. Shibata, H. Itoh, unpublished data). Since clinical evidence of efficacy of MR antagonist in MR-associated hypertension is lacking, randomized and controlled clinical trial should be expected.

SUMMARY

In conclusion, we propose a clinical subtype of RHTN or organ damage which is effectively controlled by MR antagonists as "MR-associated hypertension" in a broad sense. The MR-associated hypertension is classified into two subtypes, one with elevated plasma aldosterone levels and the other with normal plasma aldosterone levels. This clinical entity includes a variety of pathological states, such as PA, obesity, OSA, diabetes mellitus, and CKD, in which plasma aldosterone levels are elevated in some but normal in others. The MR action is overactivated by elevated plasma aldosterone levels due to activation of sympathetic nerve activity, the renin–angiotensin–aldosterone system. and putative adipocyte-derived aldosterone-releasing factors, and also by several pathways other than elevated aldosterone levels, such as increased MR levels, increased MR sensitivity, and MR-interacting factors. Since the MR antagonist therapy as a first-line or add-on agent is effective, it is important to recognize this type of RHTN.

Acknowledgments: We thank Isao Kurihara, Sakiko Kobayashi, Kenichi Yokota, Noriko Suda, Ayano Murai-Takeda, Yuko Mitsuishi, Takeshi Hayashi, Yuichiro Motosugi, Rie Jo, Takako Ohyama, and Toshihiko Suzuki in Adrenal Endocrinology Research Group of Keio University School of Medicine, for their contributions.

Disclosure: The authors declared no conflict of interest.

1. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* 1999; 341:709–717.
2. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med* 2003; 348:1309–1321.
3. Zannad F, McMurray JJ, Krum H, van Veldhuisen DJ, Swedberg K, Shi H, Vincent J, Pocock SJ, Pitt B. Eplerenone in patients with systolic heart failure and mild symptoms. *N Engl J Med* 2011; 364:11–21.
4. Jansen PM, Boomsma F, van den Meiracker AH. Aldosterone-to-renin ratio as a screening test for primary aldosteronism–the Dutch ARRAT Study. *Neth J Med* 2008; 66:220–228.
5. Catena C, Colussi G, Nadalini E, Chiuch A, Baroselli S, Lapenna R, Sechi LA. Cardiovascular outcomes in patients with primary aldosteronism after treatment. *Arch Intern Med* 2008; 168:80–85.
6. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol* 2005; 45:1243–1248.
7. Rossi GP, Bernini G, Desideri G, Fabris B, Ferri C, Giacchetti G, Letizia C, Maccario M, Mannelli M, Matterello MJ, Montemurro D, Palumbo G, Rizzoni D, Rossi E, Pessina AC, Mantero F; PAPY Study Participants. Renal damage in primary aldosteronism: results of the PAPY Study. *Hypertension* 2006; 48:232–238.
8. Sechi LA, Colussi G, Di Fabio A, Catena C. Cardiovascular and renal damage in primary aldosteronism: outcomes after treatment. *Am J Hypertens* 2010; 23: 1253–1260.
9. Sechi LA, Novello M, Lapenna R, Baroselli S, Nadalini E, Colussi GL, Catena C. Long-term renal outcomes in patients with primary aldosteronism. *JAMA* 2006; 295:2638–2645.
10. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, White A, Cushman WC, White W, Sica D, Ferdinand K, Giles TD, Falkner B, Carey RM. Resistant hypertension: diagnosis, evaluation, and treatment. A scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Hypertension* 2008; 51:1403–1419.
11. Persell SD. Prevalence of resistant hypertension in the United States, 2003–2008. *Hypertension* 2011; 57:1076–1080.
12. Pimenta E, Calhoun DA. Resistant hypertension and aldosteronism. *Curr Hypertens Rep* 2007; 9:353–359.
13. Vasan RS, Evans JC, Larson MG, Wilson PW, Meigs JB, Rifai N, Benjamin EJ, Levy D. Serum aldosterone and the incidence of hypertension in nonhypertensive persons. *N Engl J Med* 2004; 351:33–41.
14. Pascual-Le Tallec L, Lombes M. The mineralocorticoid receptor: a journey exploring its diversity and specificity of action. *Mol Endocrinol* 2005; 19: 2211–2221.
15. Viengchareun S, Le Menuet D, Martinerie L, Munier M, Pascual-Le Tallec L, Lombès M. The mineralocorticoid receptor: insights into its molecular and (patho) physiological biology. *Nucl Recept Signal* 2007; 5:e012.
16. Murai-Takeda A, Shibata H, Kurihara I, Kobayashi S, Yokota K, Suda N, Mitsuishi Y, Jo R, Kitagawa H, Kato S, Saruta T, Itoh H. NF-YC functions as a corepressor of agonist-bound mineralocorticoid receptor. *J Biol Chem* 2010; 285:8084–8093.
17. Yang J, Young MJ. The mineralocorticoid receptor and its coregulators. *J Mol Endocrinol* 2009; 43:53–64.
18. Yokota K, Shibata H, Kurihara I, Kobayashi S, Suda N, Murai-Takeda A, Saito I, Kitagawa H, Kato S, Saruta T, Itoh H. Coactivation of the N-terminal transactivation of mineralocorticoid receptor by Ubc9. *J Biol Chem* 2007; 282:1998–2010.
19. Kobayashi S, Shibata H, Kurihara I, Yokota K, Suda N, Saito I, Saruta T. Ubc9 interacts with chicken ovalbumin upstream promoter-transcription factor I and represses receptor-dependent transcription. *J Mol Endocrinol* 2004; 32:69–86.
20. Kurihara I, Shibata H, Kobayashi S, Suda N, Ikeda Y, Yokota K, Murai A, Saito I, Rainey WE, Saruta T. Ubc9 and Protein Inhibitor of Activated STAT 1 Activate Chicken Ovalbumin Upstream Promoter-Transcription Factor-Mediated Human CYP11B2 Gene Transcription. *J Biol Chem* 2005; 280:6721–6730.
21. Shibata H, Kobayashi S, Kurihara I, Saito I, Saruta T. Nuclear receptors and co-regulators in adrenal tumors. *Horm Res* 2003; 59(Suppl 1):65–73.
22. Suda N, Shibata H, Kurihara I, Ikeda Y, Kobayashi S, Yokota K, Murai-Takeda A, Nakagawa K, Oya M, Murai M, Rainey WE, Saruta T, Itoh H. Coactivation of

SF-1-mediated transcription of steroidogenic enzymes by Ubc9 and PIAS1. *Endocrinology* 2011; 152:2266–2277.
23. Grossmann C, Gekle M. New aspects of rapid aldosterone signaling. *Mol Cell Endocrinol* 2009; 308:53–62.
24. Wendler A, Baldi E, Harvey BJ, Nadal A, Norman A, Wehling M. Position paper: Rapid responses to steroids: current status and future prospects. *Eur J Endocrinol* 2010; 162:825–830.
25. Funder JW. GPR30, mineralocorticoid receptors, and the rapid vascular effects of aldosterone. *Hypertension* 2011; 57:370–372.
26. Gros R, Ding Q, Sklar LA, Prossnitz EE, Arterburn JB, Chorazyczewski J, Feldman RD. GPR30 expression is required for the mineralocorticoid receptor-independent rapid vascular effects of aldosterone. *Hypertension* 2011; 57:442–451.
27. Krug AW, Pojoga LH, Williams GH, Adler GK. Cell membrane-associated mineralocorticoid receptors? New evidence. *Hypertension* 2011; 57:1019–1025.
28. Geller DS, Zhang J, Zennaro MC, Vallo-Boado A, Rodriguez-Soriano J, Furu L, Haws R, Metzger D, Botelho B, Karaviti L, Haqq AM, Corey H, Janssens S, Corvol P, Lifton RP. Autosomal dominant pseudohypoaldosteronism type 1: mechanisms, evidence for neonatal lethality, and phenotypic expression in adults. *J Am Soc Nephrol* 2006; 17:1429–1436.
29. Nishizaka MK, Zaman MA, Calhoun DA. Efficacy of low-dose spironolactone in subjects with resistant hypertension. *Am J Hypertens* 2003; 16:925–930.
30. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, Young WF Jr, Montori VM. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2008; 93:3266–3281.
31. Nishikawa T, Omura M, Satoh F, Shibata H, Takahashi K, Tamura N, Tanabe A; Task Force Committee on Primary Aldosteronism, The Japan Endocrine Society. Guidelines for the diagnosis and treatment of primary aldosteronism--the Japan Endocrine Society 2009. *Endocr J* 2011; 58:711–721.
32. Ogishima T, Shibata H, Shimada H, Mitani F, Suzuki H, Saruta T, Ishimura Y. Aldosterone synthase cytochrome P-450 expressed in the adrenals of patients with primary aldosteronism. *J Biol Chem* 1991; 266:10731–10734.
33. Takeda Y, Furukawa K, Inaba S, Miyamori I, Mabuchi H. Genetic analysis of aldosterone synthase in patients with idiopathic hyperaldosteronism. *J Clin Endocrinol Metab* 1999; 84:1633–1637.
34. Doi M, Takahashi Y, Komatsu R, Yamazaki F, Yamada H, Haraguchi S, Emoto N, Okuno Y, Tsujimoto G, Kanematsu A, Ogawa O, Todo T, Tsutsui K, van der Horst GT, Okamura H. Salt-sensitive hypertension in circadian clock-deficient Cry-null mice involves dysregulated adrenal Hsd3b6. *Nat Med* 2010; 16:67–74.
35. Fallo F, Veglio F, Bertello C, Sonino N, Della Mea P, Ermani M, Rabbia F, Federspil G, Mulatero P. Prevalence and characteristics of the metabolic syndrome in primary aldosteronism. *J Clin Endocrinol Metab* 2006; 91:454–459.
36. Iacobellis G, Petramala L, Cotesta D, Pergolini M, Zinnamosca L, Cianci R, De Toma G, Sciomer S, Letizia C. Adipokines and cardiometabolic profile in primary hyperaldosteronism. *J Clin Endocrinol Metab* 2010; 95:2391–2398.
37. Somlóová Z, Widimský J Jr, Rosa J, Wichterle D, Strauch B, Petrák O, Zelinka T, Vlková J, Masek M, Dvoráková J, Holaj R. The prevalence of metabolic syndrome and its components in two main types of primary aldosteronism. *J Hum Hypertens* 2010; 24:625–630.
38. Ehrhart-Bornstein M, Lamounier-Zepter V, Schraven A, Langenbach J, Willenberg HS, Barthel A, Hauner H, McCann SM, Scherbaum WA, Bornstein SR. Human adipocytes secrete mineralocorticoid-releasing factors. *Proc Natl Acad Sci USA* 2003; 100:14211–14216.
39. Krug AW, Vleugels K, Schinner S, Lamounier-Zepter V, Ziegler CG, Bornstein SR, Ehrhart-Bornstein M. Human adipocytes induce an ERK1/2 MAP kinases-mediated upregulation of steroidogenic acute regulatory protein (StAR) and an angiotensin II-sensitization in human adrenocortical cells. *Int J Obes (Lond)* 2007; 31:1605–1616.
40. Bochud M, Nussberger J, Bovet P, Maillard MR, Elston RC, Paccaud F, Shamlaye C, Burnier M. Plasma aldosterone is independently associated with the metabolic syndrome. *Hypertension* 2006; 48:239–245.
41. Kidambi S, Kotchen JM, Grim CE, Raff H, Mao J, Singh RJ, Kotchen TA. Association of adrenal steroids with hypertension and the metabolic syndrome in blacks. *Hypertension* 2007; 49:704–711.
42. Sartori M, Calò LA, Mascagna V, Realdi A, Macchini L, Ciccariello L, De Toni R, Cattelan F, Pessina AC, Semplicini A. Aldosterone and refractory hypertension: a prospective cohort study. *Am J Hypertens* 2006; 19:373–9; discussion 380.
43. Bomback AS, Klemmer PJ. The incidence and implications of aldosterone breakthrough. *Nat Clin Pract Nephrol* 2007; 3:486–492.
44. Dudenbostel T, Calhoun DA. Resistant hypertension, obstructive sleep apnoea and aldosterone. *J Hum Hypertens* 2011, in press.
45. Gaddam K, Pimenta E, Thomas SJ, Cofield SS, Oparil S, Harding SM, Calhoun DA. Spironolactone reduces severity of obstructive sleep apnoea in patients with resistant hypertension: a preliminary report. *J Hum Hypertens* 2010; 24:532–537.
46. Pedrosa RP, Drager LF, Gonzaga CC, Sousa MG, de Paula LK, Amaro AC, Amodeo C, Bortolotto LA, Krieger EM, Bradley TD, Lorenzi-Filho G. Obstructive sleep apnea: the most common secondary cause of hypertension associated with resistant hypertension. *Hypertension* 2011; 58:811–817.
47. Sim JJ, Yan EH, Liu IL, Rasgon SA, Kalantar-Zadeh K, Calhoun DA, Derose SF. Positive relationship of sleep apnea to hyperaldosteronism in an ethnically diverse population. *J Hypertens* 2011; 29:1553–1559.
48. Williams SK, Ravenell J, Jean-Louis G, Zizi F, Underberg JA, McFarlane SI, Ogedegbe G. Resistant hypertension and sleep apnea: pathophysiologic insights and strategic management. *Curr Diab Rep* 2011; 11:64–69.
49. Goodfriend TL, Ball DL, Egan BM, Campbell WB, Nithipatikom K. Epoxy-keto derivative of linoleic acid stimulates aldosterone secretion. *Hypertension* 2004; 43:358–363.
50. Kario K. Are melatonin and its receptor agonist specific antihypertensive modulators of resistant hypertension caused by disrupted circadian rhythm? *J Am Soc Hypertens* 2011; 5:354–358.
51. Briet M, Schiffrin EL. The role of aldosterone in the metabolic syndrome. *Curr Hypertens Rep* 2011; 13:163–172.
52. Kotchen TA. Obesity-related hypertension: epidemiology, pathophysiology, and clinical management. *Am J Hypertens* 2010; 23:1170–1178.
53. Krug AW, Ehrhart-Bornstein M. Aldosterone and metabolic syndrome: is increased aldosterone in metabolic syndrome patients an additional risk factor? *Hypertension* 2008; 51:1252–1258.
54. Sowers JR, Whaley-Connell A, Epstein M. Narrative review: the emerging clinical implications of the role of aldosterone in the metabolic syndrome and resistant hypertension. *Ann Intern Med* 2009; 150:776–783.
55. Tirosh A, Garg R, Adler GK. Mineralocorticoid receptor antagonists and the metabolic syndrome. *Curr Hypertens Rep* 2010; 12:252–257.
56. Grossman E, Messerli FH. Management of blood pressure in patients with diabetes. *Am J Hypertens* 2011; 24:863–875.
57. Mehdi UF, Adams-Huet B, Raskin P, Vega GL, Toto RD. Addition of angiotensin receptor blockade or mineralocorticoid antagonism to maximal angiotensin-converting enzyme inhibition in diabetic nephropathy. *J Am Soc Nephrol* 2009; 20:2641–2650.
58. Jansen PM, Danser AH, Imholz BP, van den Meiracker AH. Aldosterone-receptor antagonism in hypertension. *J Hypertens* 2009; 27:680–691.
59. Bomback AS, Kshirsagar AV, Amamoo MA, Klemmer PJ. Change in proteinuria after adding aldosterone blockers to ACE inhibitors or angiotensin receptor blockers in CKD: a systematic review. *Am J Kidney Dis* 2008; 51:199–211.
60. Bomback AS, Toto R. Dual blockade of the renin-angiotensin-aldosterone system: beyond the ACE inhibitor and angiotensin-II receptor blocker combination. *Am J Hypertens* 2009; 22:1032–1040.
61. Bertocchio JP, Warnock DG, Jaisser F. Mineralocorticoid receptor activation and blockade: an emerging paradigm in chronic kidney disease. *Kidney Int* 2011; 79:1051–1060.
62. Lu Y, Ku E, Campese VM. Aldosterone in the pathogenesis of chronic kidney disease and proteinuria. *Curr Hypertens Rep* 2010; 12:303–306.
63. Schrier RW, Masoumi A, Elhassan E. Aldosterone: role in edematous disorders, hypertension, chronic renal failure, and metabolic syndrome. *Clin J Am Soc Nephrol* 2010; 5:1132–1140.
64. Toto RD. Aldosterone blockade in chronic kidney disease: can it improve outcome? *Curr Opin Nephrol Hypertens* 2010; 19:444–449.
65. Volk MJ, Bomback AS, Klemmer PJ. Mineralocorticoid receptor blockade in chronic kidney disease. *Curr Hypertens Rep* 2011; 13:282–289.
66. Cascella T, Palomba S, Tauchmanovà L, Manguso F, Di Biase S, Labella D, Giallauria F, Vigorito C, Colao A, Lombardi G, Orio F. Serum aldosterone concentration and cardiovascular risk in women with polycystic ovarian syndrome. *J Clin Endocrinol Metab* 2006; 91:4395–4400.
67. Nagata K, Obata K, Xu J, Ichihara S, Noda A, Kimata H, Kato T, Izawa H, Murohara T, Yokota M. Mineralocorticoid receptor antagonism attenuates cardiac

hypertrophy and failure in low-aldosterone hypertensive rats. *Hypertension* 2006; 47:656–664.
68. Pitt B. Aldosterone blockade in patients with acute myocardial infarction. *Circulation* 2003; 107:2525–2527.
69. Oyamada N, Sone M, Miyashita K, Park K, Taura D, Inuzuka M, Sonoyama T, Tsujimoto H, Fukunaga Y, Tamura N, Itoh H, Nakao K. The role of mineralocorticoid receptor expression in brain remodeling after cerebral ischemia. *Endocrinology* 2008; 149:3764–3777.
70. Guo C, Martinez-Vasquez D, Mendez GP, Toniolo MF, Yao TM, Oestreicher EM, Kikuchi T, Lapointe N, Pojoga L, Williams GH, Ricchiuti V, Adler GK. Mineralocorticoid receptor antagonist reduces renal injury in rodent models of types 1 and 2 diabetes mellitus. *Endocrinology* 2006; 147:5363–5373.
71. Quinkler M, Zehnder D, Eardley KS, Lepenies J, Howie AJ, Hughes SV, Cockwell P, Hewison M, Stewart PM. Increased expression of mineralocorticoid effector mechanisms in kidney biopsies of patients with heavy proteinuria. *Circulation* 2005; 112:1435–1443.
72. Hart GW, Slawson C, Ramirez-Correa G, Lagerlof O. Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. *Annu Rev Biochem* 2011; 80:825–858.
73. Kino T, Jaffe H, Amin ND, Chakrabarti M, Zheng YL, Chrousos GP, Pant HC. Cyclin-dependent kinase 5 modulates the transcriptional activity of the mineralocorticoid receptor and regulates expression of brain-derived neurotrophic factor. *Mol Endocrinol* 2010; 24:941–952.
74. Yokota K, Shibata H, Kobayashi S, Suda N, Murai A, Kurihara I, Saito I, Saruta T. Proteasome-mediated mineralocorticoid receptor degradation attenuates transcriptional response to aldosterone. *Endocr Res* 2004; 30:611–616.
75. Viengchareun S, Kamenicky P, Teixeira M, Butlen D, Meduri G, Blanchard-Gutton N, Kurschat C, Lanel A, Martinerie L, Sztal-Mazer S, Blot-Chabaud M, Ferrary E, Cherradi N, Lombès M. Osmotic stress regulates mineralocorticoid receptor expression in a novel aldosterone-sensitive cortical collecting duct cell line. *Mol Endocrinol* 2009; 23:1948–1962.
76. Grossmann C, Gekle M. Non-classical actions of the mineralocorticoid receptor: misuse of EGF receptors? *Mol Cell Endocrinol* 2007; 277:6–12.
77. Grossmann C, Gekle M. Nongenotropic aldosterone effects and the EGFR: interaction and biological relevance. *Steroids* 2008; 73:973–978.
78. Grossmann C, Krug AW, Freudinger R, Mildenberger S, Voelker K, Gekle M. Aldosterone-induced EGFR expression: interaction between the human mineralocorticoid receptor and the human EGFR promoter. *Am J Physiol Endocrinol Metab* 2007; 292:E1790–E1800.
79. Krug AW, Allenhöfer L, Monticone R, Spinetti G, Gekle M, Wang M, Lakatta EG. Elevated mineralocorticoid receptor activity in aged rat vascular smooth muscle cells promotes a proinflammatory phenotype via extracellular signal-regulated kinase ½ mitogen-activated protein kinase and epidermal growth factor receptor-dependent pathways. *Hypertension* 2010; 55:1476–1483.
80. Beltowski J, Lowicka E. EGF receptor as a drug target in arterial hypertension. *Mini Rev Med Chem* 2009; 9:526–538.
81. Das Evcimen N, King GL. The role of protein kinase C activation and the vascular complications of diabetes. *Pharmacol Res* 2007; 55:498–510.
82. Geraldes P, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circ Res* 2010; 106:1319–1331.
83. Meier M, Menne J, Haller H. Targeting the protein kinase C family in the diabetic kidney: lessons from analysis of mutant mice. *Diabetologia* 2009; 52:765–775.
84. Meier M, Park JK, Overheu D, Kirsch T, Lindschau C, Gueler F, Leitges M, Menne J, Haller H. Deletion of protein kinase C-beta isoform *in vivo* reduces renal hypertrophy but not albuminuria in the streptozotocin-induced diabetic mouse model. *Diabetes* 2007; 56:346–354.
85. Aiello LP, Davis MD, Girach A, Kles KA, Milton RC, Sheetz MJ, Vignati L, Zhi XE. Effect of ruboxistaurin on visual loss in patients with diabetic retinopathy. *Ophthalmology* 2006; 113:2221–2230.
86. Tuttle KR, Bakris GL, Toto RD, McGill JB, Hu K, Anderson PW. The effect of ruboxistaurin on nephropathy in type 2 diabetes. *Diabetes Care* 2005; 28: 2686–2690.
87. Issad T, Masson E, Pagesy P. O-GlcNAc modification, insulin signaling and diabetic complications. *Diabetes Metab* 2010; 36:423–435.
88. Ozcan S, Andrali SS, Cantrell JE. Modulation of transcription factor function by O-GlcNAc modification. *Biochim Biophys Acta* 2010; 1799:353–364.
89. Shibata S, Nagase M, Yoshida S, Kawarazaki W, Kurihara H, Tanaka H, Miyoshi J, Takai Y, Fujita T. Modification of mineralocorticoid receptor function by Rac1 GTPase: implication in proteinuric kidney disease. *Nat Med* 2008; 14:1370–1376.
90. Shibata S, Mu S, Kawarazaki H, Muraoka K, Ishizawa K, Yoshida S, Kawarazaki W, Takeuchi M, Ayuzawa N, Miyoshi J, Takai Y, Ishikawa A, Shimosawa T, Ando K, Nagase M, Fujita T. Rac1 GTPase in rodent kidneys is essential for salt-sensitive hypertension via a mineralocorticoid receptor-dependent pathway. *J Clin Invest* 2011; 121:3233–3243.
91. Geller DS, Farhi A, Pinkerton N, Fradley M, Moritz M, Spitzer A, Meinke G, Tsai FT, Sigler PB, Lifton RP. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science* 2000; 289:119–123.
92. Dietz JD, Du S, Bolten CW, Payne MA, Xia C, Blinn JR, Funder JW, Hu X. A number of marketed dihydropyridine calcium channel blockers have mineralocorticoid receptor antagonist activity. *Hypertension* 2008; 51:742–748.
93. Fan YY, Kohno M, Nakano D, Hitomi H, Nagai Y, Fujisawa Y, Lu XM, Fu H, Du J, Ohmori K, Hosomi N, Kimura S, Kiyomoto H, Nishiyama A. Inhibitory effects of a dihydropyridine calcium channel blocker on renal injury in aldosterone-infused rats. *J Hypertens* 2009; 27:1855–1862.
94. Kosaka H, Hirayama K, Yoda N, Sasaki K, Kitayama T, Kusaka H, Matsubara M. The L-, N-, and T-type triple calcium channel blocker benidipine acts as an antagonist of mineralocorticoid receptor, a member of nuclear receptor family. *Eur J Pharmacol* 2010; 635:49–55.

INTERNATIONAL JOURNAL OF
UROLOGY

International Journal of Urology (2012) doi: 10.1111/j.1442-2042.2011.02950.x

Case Report

Pheochromocytoma crisis caused by *Campylobacter fetus*

Ichiro Abe,[1] Masatoshi Nomura,[1] Makiko Watanabe,[1] Shingo Shimada,[2] Michiko Kohno,[1] Yayoi Matsuda,[1] Masahiro Adachi,[1] Hisaya Kawate,[1] Keizo Ohnaka[1] and Ryoichi Takayanagi[1]

[1]Department of Medicine and Bioregulatory Science, Graduate School of Medical Science, Kyushu University, Fukuoka, and [2]Department of Internal Medicine, Kyushu Rosai Hospital, Labor Welfare Corporation, Kitakyushu, Japan

Abbreviations & Acronyms
CRP = C-reactive protein
DBP = diastolic blood pressure
SBP = systolic blood pressure

Correspondence: Masatoshi Nomura M.D., Ph.D., Department of Medicine and Bioregulatory Science, Graduate School of Medical Science, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan. Email: nomura@med.kyushu-u.ac.jp

Received 30 August 2011; accepted 11 December 2011.

Abstract: Pheochromocytoma crisis is a life-threatening endocrine emergency associated with symptoms of excess release of catecholamines. It might present spontaneously or be unmasked by triggers including trauma, surgery and certain medications that provoke catecholamine release by tumors. Here we report a case of pheochromocytoma crisis associated with abscess formation in the tumor and bacteremia of *Campylobacter fetus*, which was successfully treated with antibiotics and a surgical resection. This case appears to be the first reported case in the literature of abscess formation by *C. fetus* in pheochromocytoma, leading to catecholamine crisis.

Key words: abscess, antibiotics, *Campylobacter fetus*, catecholamine crisis, pheochromocytoma.

Introduction

Pheochromocytoma crisis is a rare, life-threatening endocrine emergency associated with symptoms of excess release of catecholamines by the tumor. It might present spontaneously or be unmasked by triggers, such as trauma, surgery, anesthesia, drug therapy and infection. Catecholamine crisis has several manifestation components including multiple organ failure, severe blood pressure variability, encephalopathy and high fever. A constellation of very dramatic manifestations occurs, which are progressive despite intensive medical management and lead to multiorgan failure. Consequently, a delay in making a definitive diagnosis and carrying out appropriate therapy results in further deterioration of the patient's condition. Therefore, it is important to recognize pheochromocytoma crisis as an emergency condition and to carry out thorough searches for coexisting diseases for proper diagnosis and treatment. It is uncommon for fever to be the presenting manifestation of pheochromocytoma. However, high fever is a common manifestation of pheochromocytoma crisis. The causes of fever might be multifactorial and often include an associated illness, most likely an infectious disease.

Campylobacter fetus is rarely isolated from humans, although it is ubiquitous in cattle, pigs and poultry. It is usually associated with opportunistic infections. Here, we report a case of pheochromocytoma crisis associated with abscess formation in the tumor and bacteremia of *C. fetus*, which was successfully treated with antibiotics and a surgical resection.

Case report

A 53-year-old woman was admitted with complaints of high fever, fatigue and stagger for 1 week. She had experienced a subarachnoid hemorrhage 2 years previously. Thereafter, she had been treated for hypertension. She was diagnosed with type 2 diabetes 6 months earlier. On physical examination, she presented with variable systolic blood pressure ranging from 120 to 220 mmHg. Her body temperature was 38.0°C, and her pulse rate was 110/min and regular. Her skin appeared normal, except for extreme perspiration. Laboratory tests showed