Annals of Nuclear Medicine Vol. 20, No. 4, 325–328, 2006

A case of malignant pheochromocytoma with early intense uptake and immediate rapid washout of ^{99m}Tc-tetrofosmin characterizing the overexpression of anti-apoptotic Bcl-2

Shigetoshi WAKASUGI,* Toshiaki KINOUCHI,** Haruo TANIGUCHI,*** Kunihiko YOKOYAMA,**** Kazuki FUKUCHI,* Atushi NOGUCHI,* Masanori TAKESHITA* and Terumi HASHIZUME*

*Department of Nuclear Medicine, Osaka Medical Center for Cancer and Cardiovascular Diseases
**Department of Urology, Osaka Medical Center for Cancer and Cardiovascular Diseases
***Department of Pathology, Minoh Municipal Hospital
****Department of Biotracer Medicine, Kanazawa University Graduate School of Medical Sciences

Defective apoptotic program due to the overexpression of the anti-apoptotic Bcl-2 protein of the outer mitochondrial membrane may be a cause of the poor response of malignant pheochromocytoma to ^{131}I-MIBG therapy. We report a case of malignant pheochromocytoma which showed early intense uptake and immediate rapid washout of ^{99m}Tc-tetrofosmin characterizing the overexpression of anti-apoptotic Bcl-2 and which was refractory to ^{131}I-MIBG therapy.

Key words: malignant pheochromocytoma, overexpression of anti-apoptotic Bcl-2 protein, ^{99m}Tc-tetrofosmin, ^{131}I-MIBG therapy

INTRODUCTION

^{99m}Tc-labeled lipophilic cations such as ^{99m}Tc-methoxyisobutylisonitrile (MIBI) and ^{99m}Tc-tetrofosmin enter viable tumor cells via passive diffusion in response to plasma membrane and mitochondrial membrane potentials and accumulate primarily within mitochondria. Recent study indicates that breast carcinomas which fail to accumulate ^{99m}Tc-MIBI have an altered apoptotic program due to the overexpression of the anti-apoptotic protein Bcl-2.[1]

The overexpression of anti-apoptotic Bcl-2 correlates with resistance to chemotherapy and radiation therapy.[2,3] Non-invasive detection of the overexpression of anti-apoptotic Bcl-2 may provide rational criteria for therapeutic strategy.

We report a case of malignant pheochromocytoma which showed early intense uptake and immediate rapid washout of ^{99m}Tc-tetrofosmin characterizing the overexpression of anti-apoptotic Bcl-2 and which was refractory to ^{131}I-metaiodobenzylguanidine (MIBG) therapy.

Received November 16, 2005, revision accepted February 14, 2006.

For reprint contact: Shigetoshi Wakasugi, M.D., Division of Nuclear Medicine, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1–3–3 Nakamichi, Higashinari-ku, Osaka 537–8511, JAPAN.

E-mail: wakasugi-si@mc.pref.osaka.jp

CASE REPORT

A 72-year-old woman was diagnosed with a pheochromocytoma (8 cm in diameter) localized in the left adrenal by ultrasound, CT/MRI and ^{131}I-MIBG scintigraphy. After surgery, catecholamine metabolite levels and ^{131}I-MIBG scintigraphy normalized. The disease was in remission for approximately 7 years.

She then complained of back pain, palpitation and paroxysmal hypertension. Catecholamine metabolite levels were raised above normal levels. Bone scintigraphy showed increased activity in the spinous process of thoracic vertebrae (T5–7) and MRI demonstrated a hypervascular tumor (3 cm in diameter) in the region corresponding to the spinous process of T5–7 with slight compression of epidural sacs at the level of T6 (Fig. 1). ^{123}I-MIBG scintigraphy suggested metastatic lesions in thoracic vertebrae (T5–7) indicating maximal lesion, right ribs and right pelvis (Fig. 2). The diagnosis of metastatic malignant pheochromocytoma was proved by means of needle biopsy of the back tumor (Fig. 3). Four month later, she received the first dosage of 200 mCi (7400 MBq)

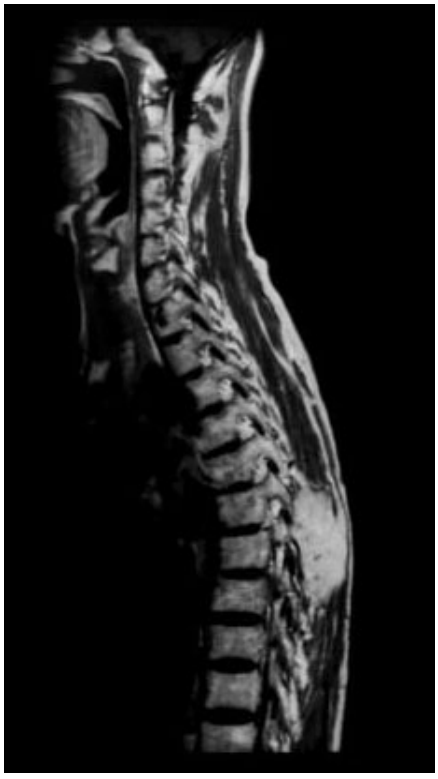


Fig. 1 Sagittal enhanced T1 weighted image shows hypervascular tumor (3 cm in diameter) in the region corresponding to the spinous process of T5–7 with slight compression of epidural sacs at level of T6.

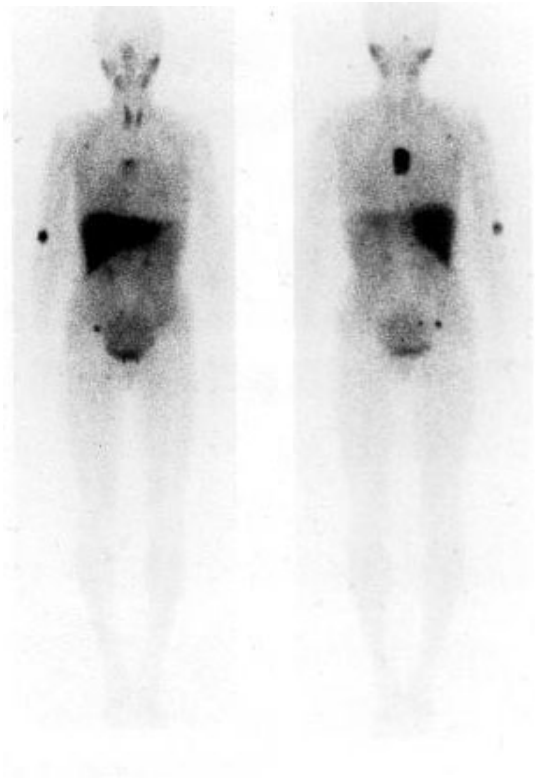


Fig. 2 ^{123}I-MIBG scintigraphy suggests metastatic lesions in thoracic vertebra (T5–7) indicating maximal lesion, right rib and right pelvis.

^{131}I-MIBG.

One-and-a-half years after the first ^{131}I-MIBG therapy, catecholamine metabolite levels remained elevated and she complained of increasing back pain, palpitation and paroxysmal hypertension. MRI showed increased size (6 cm in diameter) of the back tumor and stenosis of the spinal canal but no involvement of the spinal cord. ^{123}I-MIBG scintigraphy showed more extensive accumulation in the back tumor and appearance of multiple lesions in cranial bone, bilateral ribs and pelvis and a lesion in the left femur (Fig. 4).

Dynamic imaging with ^{99m}Tc-tetrofosmin demonstrated intense uptake in the back tumor in the early images after bolus injection and then immediate rapid washout from the tumor, with tumor accumulation disappearing within 7 min after injection (Fig. 5).

Time activity curve of tumor showed a relatively gentle down slope compared with very rapid clearance of blood pool activity of subclavicular vein, and continuous down slope different from the activity curve of the heart which showed slow down beginning at 1 min after injection and then plateau (Fig. 6). Tumor activity curve returned at the background level in the spine at 7 min after injection.

Immunohistochemical staining of biopsy specimens of the back tumor obtained prior to the first ^{131}I-MIBG therapy was performed. The results showed intense expression of apoptosis antagonist Bcl-2 (Fig. 7), no expression of apoptosis agonist Bax, no expression of DNA fragments in Tunel staining for evaluation of apoptosis, no expression of P-glycoprotein, proliferation rate of 10% in Mib-1 staining and spotty expression of P53.

Two years after the first ^{131}I-MIBG therapy, she received the second dosage of 200 mCi (7400 MBq) ^{131}I-MIBG. Three days later, she developed miserable spinal paraparesis and has been in hospital.

Fig. 3 Hematoxylin-eosin staining of biopsy specimens of the back tumor shows the characteristic small nests ("zellballen") pattern of proliferating tumor cells with granular cytoplasm and sporadic giant cells and hyperplasia of capillaries.

Fig. 4 ^{123}I-MIBG scintigraphy shows more extensive accumulation in the back tumor and appearance of multiple lesions in cranial bone, bilateral ribs and pelvis and a lesion in the left femur.

Fig. 5 Dynamic images (posterior view) with ^{99m}Tc-tetrofosmin demonstrate intense uptake in the back tumor in the early images after bolus injection and then immediate rapid washout from tumor, with tumor accumulation disappearing within 7 min after injection.

Fig. 3

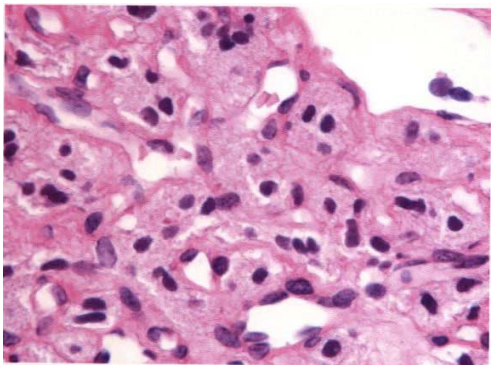
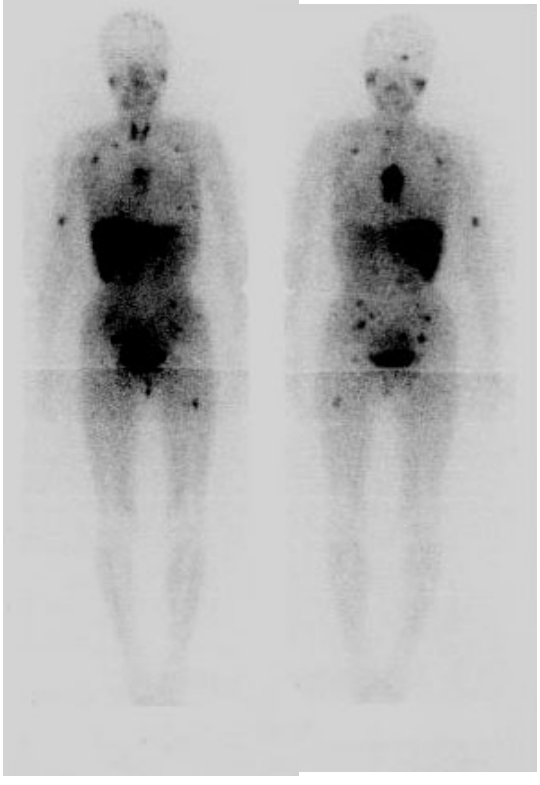


Fig. 4



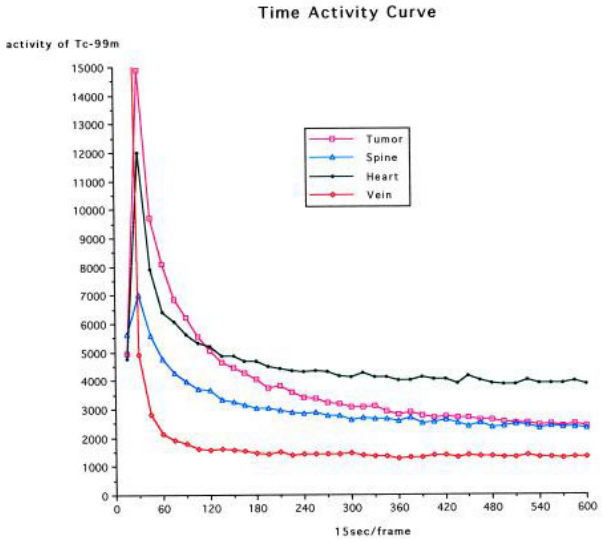


Fig. 6 Time activity curve of tumor shows a relatively gentle down slope compared with very rapid clearance of blood pool activity of subclavicular vein, and continuous down slope different from the activity curve of the heart which shows slow down beginning at 1 min after injection and then a plateau.

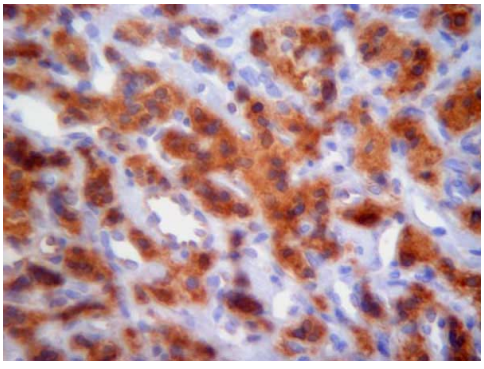
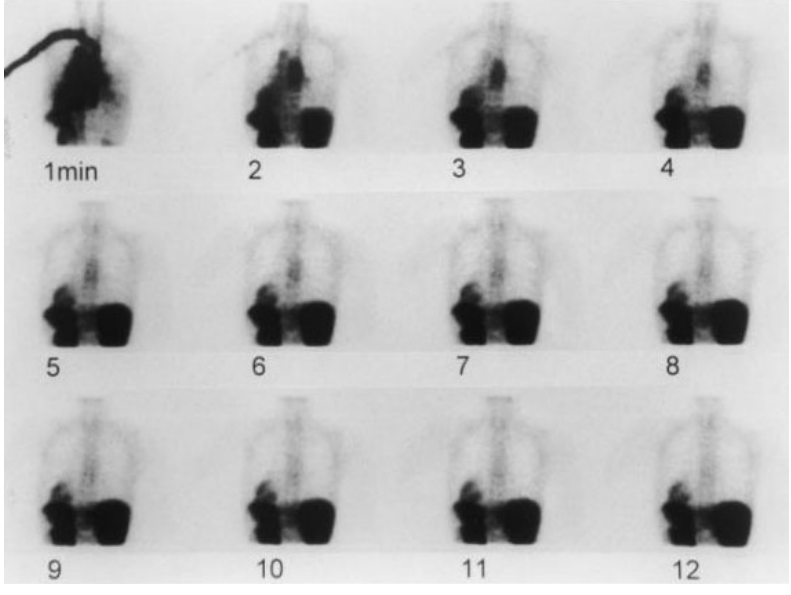


Fig. 7 Immunohistochemical staining of biopsy specimens of the back tumor obtained prior to the first ^{131}I-MIBG therapy shows intense expression of apoptosis antagonist Bcl-2.

Fig. 5



DISCUSSION

Overexpression of the anti-apoptotic Bcl-2 inhibits mitochondrial permeability as a shelter and prevents the trafficking of ^{99m}Tc-labeled lipophylic cations across mitochondrial membrane, and early accumulation within mitochondria is reduced despite the stabilization of mitochondrial membrane potentials.[1]

On the other hand, the early uptake of lipophilic cations at the early stage of cell apoptosis is also reduced by loss of influx in cells since electrical gradient failure occurs due to modification of the plasma membrane and mitochondrial membrane potentials.[4,5]

Also in the presence of multidrug resistance membrane protein (P-glycoprotein or MRP1), the early uptake of lipophilic cations may be reduced, although P-glycoprotein or MRP1-mediated washout of lipophilic cations is particularly evident at delayed time points after injection.[6,7]

Therefore, it seems difficult to detect the overexpression of the anti-apoptotic Bcl-2 from only static image at early time points.

From the results of dynamic imaging with ^{99m}Tc-tetrofosmin and immunohistochemical staining in the present case, it is suggested that passive influx of ^{99m}Tc-tetrofosmin in tumor cells is driven by an intact electrical gradient from outside the cell to mitochondria even in the overexpression of the anti-apoptotic Bcl-2, and early intense uptake of ^{99m}Tc-tetrofosmin in tumor indicates enhanced passive diffusion by large electrical gradient and increased blood flow in hypervascular tumor.

Immediately rapid washout of ^{99m}Tc-tetrofosmin from tumor demonstrates that the overexpression of the anti-apoptotic Bcl-2 prevents diffusion and retention of ^{99m}Tc-tetrofosmin in mitochondria and leads to rapid passive efflux from the cytosolic compartment of the cell.

Thus, early intense uptake and immediate rapid washout in dynamic imaging with ^{99m}Tc-tetrofosmin may be a characteristic pattern for the overexpression of anti-apoptotic Bcl-2 in tumor.

A comprehensive review of 116 reported patients with ^{131}I-MIBG treatment of malignant pheochromocytoma indicated that the tumor response rate was 30% and only five patients had a complete tumor response.[8]

Since malignant pheochromocytoma shows statistically significant higher frequency of Bcl-2 protein expression than benign pheochromocytoma,[9] defective apoptotic program due to the overexpression of the anti-apoptotic Bcl-2 may be the cause of the poor response of malignant pheochromocytoma to ^{131}I-MIBG therapy.

Our case report suggests that dynamic imaging of malignant pheochromocytoma with ^{99m}Tc-labeled lipophilic cations detects the overexpression of the anti-apoptotic Bcl-2 and predicts a poor response to ^{131}I-MIBG therapy.

REFERENCES

1. Del Vecchio S, Zanntti A, Aloj L, Caraco C, Ciamiello A, Salvatore M. Inhibition of early ^{99m}Tc-MIBI uptake by Bcl-2 anti-apoptotic protein overexpression in untreated breast carcinoma. *Eur J Nucl Med Mol Imaging* 2003; 30: 879–887.
2. Miyashita T, Reed JC. Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. *Blood* 1993; 81: 151–157.
3. Reed JC. Apoptosis-regulating proteins as a target for drug discovery. *Trends Mol Med* 2001; 7: 314–319.
4. Moretti JL, Hauet N, Caglar M, Rebillard O, Burak Z. To use MIBI or not to use MIBI? That is the question when assessing tumor cells. *Eur J Nucl Med Mol Imaging* 2005; 32: 836–842.
5. Vergote J, Di Benedetto M, Morretti JL, Azaloux H, Kouyoumdjian JC, Kraemer M, et al. Could ^{99m}Tc-MIBI be used to visualize the apoptotic MCF7 human breast cancer cells? *Cell Mol Biol (Noisy-le-grand)* 2001; 47: 467–471.
6. Kostakoglu L, Elahi N, Kiratli P, Ruacan S, Sayek I, Baltali E, et al. Clinical validation of the influence of P-glycoprotein on technetium-99m-sestamibi uptake in malignant tumors. *J Nucl Med* 1997; 38: 1003–1008.
7. Del Vecchio S, Ciarmiello A, Potena MI, Carriero MV, Mainolfi C, Botti G, et al. *In vivo* detection of multidrug-resistant (MDR1) phenotype by technetium-99m-sestamibi scan in untreated breast cancer patients. *Eur J Nucl Med* 1997; 24: 150–159.
8. Loh KC, Fitzgerald PA, Mattthay KK, Yeo PP, Price DC. The treatment of malignant pheochromocytoma with iodine-131 metaiodobenzylguanidine (^{131}I-MIBG): a comprehensive review of 116 reported patients. *J Endocrinol Invest* 1997; 20: 648–658.
9. Krijger RR, van der Harst E, van der Ham F, Stijnen T, Dinjens WK, Koper JW, et al. Prognostic value of p53, bcl-2, and c-erbB-2 protein expression in pheochromocytoma. *J Pathol* 1999; 188: 51–55.