^{99m}Tc-MIBI SPECT in small cell lung cancer patients before chemotherapy and after unresponsive chemotherapy

Yuka Yamamoto,* Yoshihiro Nishiyama,* Kotaro Fukunaga,* Katashi Satoh,*
Jiro Fujita** and Motoomi Ohkawa*

*Department of Radiology, Kagawa Medical University
**First Department of Internal Medicine, Kagawa Medical University

We evaluated the accumulation of ^{99m}Tc-MIBI in small cell lung cancer patients before chemo-therapy and after unresponsive chemotherapy. The pre-chemotherapeutic group included 22 newly diagnosed patients. These patients underwent a ^{99m}Tc-MIBI SPECT study before starting chemo-therapy. After chemotherapy, based on changes in tumor size, three different patterns of response (complete remission: CR, partial remission: PR and no change: NC) were defined. The post-chemotherapeutic group included 11 patients after chemotherapy who did not respond to chemo-therapy. These patients underwent a ^{99m}Tc-MIBI SPECT study after completion of chemotherapy. SPECT images were acquired 15 min (early) and 2 hr (delayed) after injection of ^{99m}Tc-MIBI. With a region of interest technique, the early ratio, delayed ratio and retention index were calculated. Early and delayed ratios in pre-chemotherapeutic patients were significantly higher than those in post-chemotherapeutic patients. There were no significant differences between the pre-chemo-therapeutic and post-chemotherapeutic patients in the retention index. In the pre-chemotherapeutic patients, early and delayed ratios for the CR and PR groups were significantly higher than those for the NC group. There were no significant differences in the retention index with respect to the tumor response. ^{99m}Tc-MIBI might be useful for evaluating the tumor chemosensitivity in patients with small cell lung cancer.

Key words: ^{99m}Tc-MIBI, small cell lung cancer, chemotherapy

INTRODUCTION

THE PRIMARY THERAPEUTIC METHOD for small cell lung cancer is chemotherapy, but the ability of tumors to develop resistance to chemotherapeutic agents is one of the greatest barriers to successful treatment of human cancers. Therefore, the development of effective thera-peutic regimens for these resistant neoplasms is an urgent problem in cancer chemotherapy.

Nuclear medicine imaging techniques may be appli-cable to the evaluation of therapeutic efficacy and the

prediction of therapeutic response in cancer. Non-cardiac uses of ^{99m}Tc-MIBI (hexakis 2-methoxy isobutyl-isonitrile), such as visualization of lung cancer, have also been investigated.[1-3] Multidrug resistance (MDR) in tu-mor cells has been correlated with overexpression of MDR1 gene coding for P-glycoprotein (Pgp), a plasma-membrane protein that functions as an energy-dependent transporter of cytotoxic lipophilic agents.[4] ^{99m}Tc-MIBI can be excluded from cytosol against its concentration gradient as a suitable transport substrate by Pgp.[5] In this study we evaluated the accumulation of ^{99m}Tc-MIBI in small cell lung cancer patients before chemotherapy and after unresponsive chemotherapy.

MATERIALS AND METHODS

Patients
Thirty-three patients (26 men and 7 women; age range

39–78 yrs) with pre-chemotherapeutic and post-chemo-therapeutic small cell lung cancer were investigated. Diagnosis was made by histopathologic analysis of sputum, computed tomography (CT)-guided needle biopsy or endoscopic samples.

The pre-chemotherapeutic group included 22 patients newly diagnosed before chemotherapy. These patients underwent a ^{99m}Tc-MIBI single photon emission computed tomography (SPECT) study before starting chemotherapy. After the SPECT study, these patients received multidrug chemotherapy regimens consisting of cyclophosphamide, doxorubicin, vincristine, etoposide, cisplatin, mitomycin-C and vindesine. Response to chemotherapy was evaluated by using dimensional criteria: the tumor size was measured on a CT scan performed before the start and within 2–4 weeks after the last chemotherapy. Based on the General Rule for Clinical and Pathological Record of Lung Cancer,[6] three different patterns of response were defined: complete remission (CR) (no evidence of disease), partial remission (PR) (50–99% reduction in tumor size) and no change (NC) (less than 50% decrease or increase in tumor size).

The post-chemotherapeutic group included 11 patients after completion of chemotherapy consisting of cyclophosphamide, doxorubicin, vincristine (CAV) and etoposide and cisplatin (VP) every 4 weeks for at least four times and who did not respond to chemotherapy (NC). These patients underwent a ^{99m}Tc-MIBI SPECT study after completion of chemotherapy.

The tumor size on CT ranged from 1.5 to 6.0 cm for pre-chemotherapeutic patients and 1.5 to 5.0 cm for post-chemotherapeutic patients. There were no significant differences among the two groups in tumor size. All patients provided written informed consent in agreement with the regulations of the university hospital ethical committee.

SPECT Imaging
SPECT imaging was performed with a large field-of-view gamma camera, with high resolution and a parallel-hole collimator (Picker Prism 2000; Picker International, Cleveland, OH). This camera was interfaced to a dedicated computer (ODYSSEY; Picker International). Doses of 600 MBq ^{99m}Tc-MIBI were injected intravenously. Early SPECT acquisition was performed 15 min after the injection of the radioisotope, whereas delayed SPECT images were acquired 2 hr after injection. For SPECT images of the chest, 72 projections were obtained with a 64×64 matrix for 45 sec/view in a step-and-shoot mode. With a dual head camera, the total actual acquisition time was 27 min. Image reconstruction was performed by filtered backprojection with a ramp filter. Transverse, coronal and sagittal sections were reconstructed. The system was 7 mm FWHM, and the slice thickness was 10 mm. Attenuation correction was not performed.
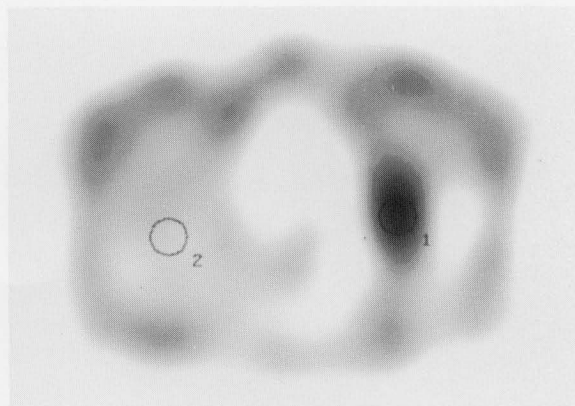

Fig. 1 ROI 1: the area with abnormal radioactivity, ROI 2: the contralateral normal lung.
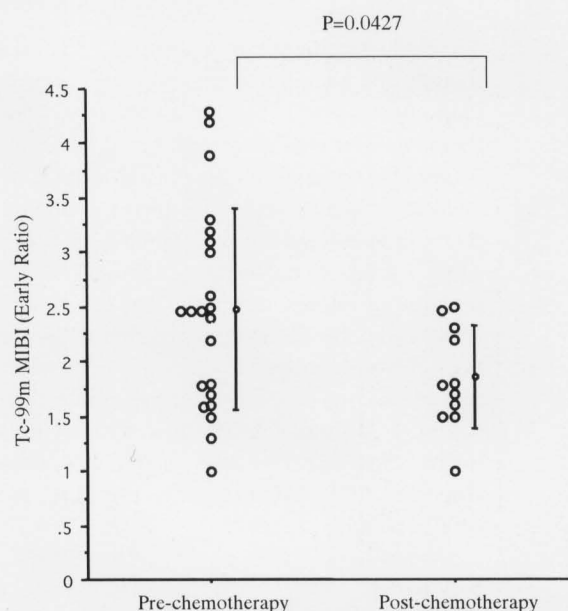

Fig. 2 Early ratio between pre-chemotherapeutic and post-chemotherapeutic patients. Points and bars are mean and standard deviations (S.D.).

Data Analysis
SPECT images were compared with chest CT and two nuclear medicine physicians (Y.Y., Y.N.) evaluated accumulation in lung tumors. For all patients studied, the transverse slices were evaluated first, followed by the coronal and sagittal views. Semiquantitative analysis of the abnormal uptake of ^{99m}Tc-MIBI was performed by drawing identical regions of interest (ROIs) over the tumor uptake and contralateral lung tissue areas on one transverse section that demonstrated the lesion most clearly on both early and delayed scans (Fig. 1). The mean ROI values (total counts/total pixels) were measured, and the tumor-to-normal (T/N) ratios were obtained. We called the T/N ratio of early image the early ratio and the T/N ratio of delayed image the delayed ratio. To semiquanti-
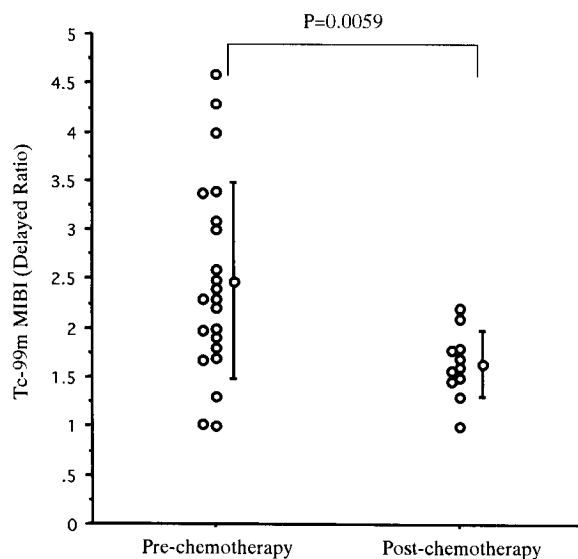
Fig. 3 Delayed ratio between pre-chemotherapeutic and post-chemotherapeutic patients. Points and bars are mean and standard deviations (S.D.).
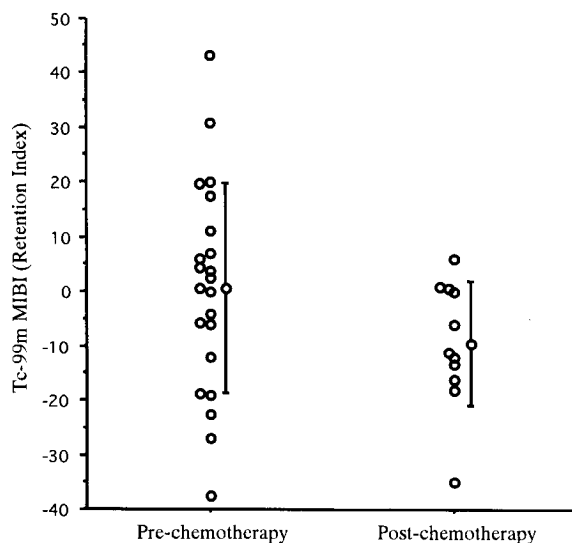

Fig. 4 Retention index between pre-chemotherapeutic and post-chemotherapeutic patients. Points and bars are mean and standard deviations (S.D.).

tatively evaluate the degree of retention in the lesion, the retention index was calculated with the following formula: (delayed ratio − early ratio) × 100/early ratio.[7] The T/N ratio and retention index were expressed as the mean ± S.D. Comparison of pre-chemotherapeutic and post-chemotherapeutic patients for each parameter on ^{99m}Tc-MIBI SPECT was performed with the Mann-Whitney U-test. Comparison between CR, PR and NC groups of each parameter on ^{99m}Tc-MIBI SPECT was performed with one-factor analysis of variance followed by the post-hoc test. Results were considered significant when $p < 0.05$.
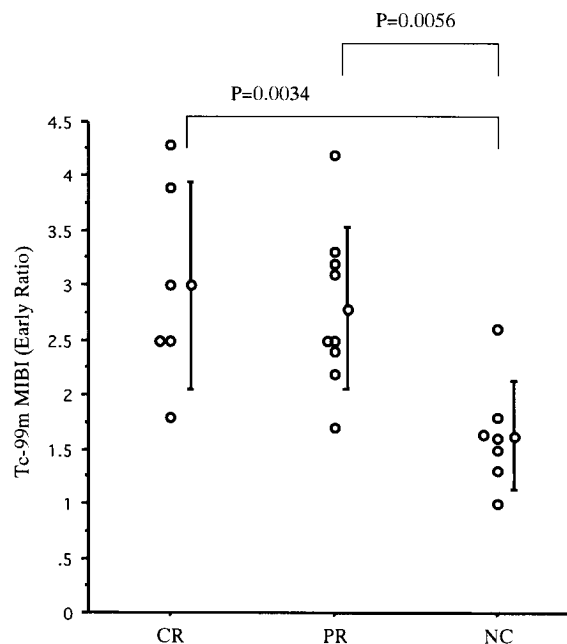

Fig. 5 Early ratio and responses to chemotherapy among pre-chemotherapeutic patients. Points and bars are mean and standard deviations (S.D.).

RESULTS

The early ratio was 2.48 ± 0.92 in pre-chemotherapeutic patients and 1.86 ± 0.47 in post-chemotherapeutic patients and this difference was significant ($p = 0.0427$) (Fig. 2). The delayed ratio was 2.48 ± 1.00 in pre-chemotherapeutic patients and 1.65 ± 0.34 in post-chemotherapeutic patients and this difference also was significant ($p = 0.0059$) (Fig. 3). The retention index was 0.66 ± 19.23 in pre-chemotherapeutic patients and −9.55 ± 11.46 in post-chemotherapeutic patients and there was no significant difference between the two groups (Fig. 4).

In pre-chemotherapeutic patients, after chemotherapy 6 patients experienced CR, 9 PR and 7 NC. The early ratio was 3.00 ± 0.94 in the CR group, 2.79 ± 0.74 in the PR group and 1.63 ± 0.50 in the NC group. Figure 5 shows that the early ratios for CR and PR groups were significantly higher ($p = 0.0034$ and $p = 0.0056$, respectively) than that of the NC group. The delayed ratio was 3.42 ± 1.04 in the CR group, 2.62 ± 0.53 in the PR group and 1.49 ± 0.38 in the NC group. Figure 6 shows that the delayed ratios for the CR and PR groups were also significantly higher ($p = 0.0001$ and $p = 0.0032$, respectively) than that of the NC group. The retention index was 14.66 ± 15.38 in the CR group, −3.94 ± 12.76 in the PR group and −5.42 ± 24.68 in the NC group, and there was no significant difference among the three groups (Fig. 7).

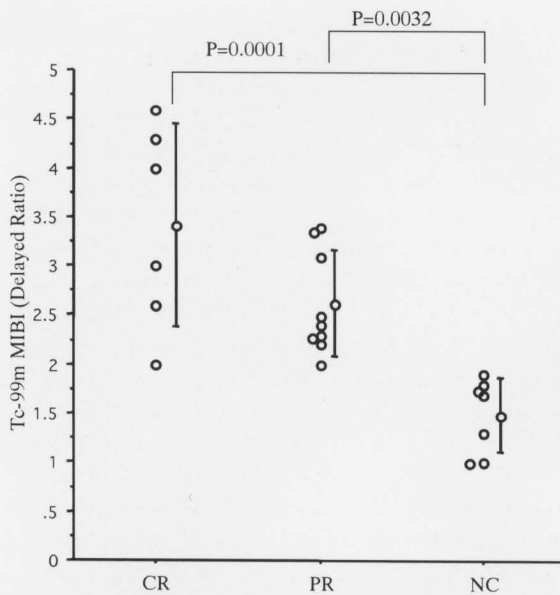Radiological findings in two typical cases are shown in Figures 8 and 9.

Fig. 6 Delayed ratio and responses to chemotherapy among pre-chemotherapeutic patients. Points and bars are mean and standard deviations (S.D.).
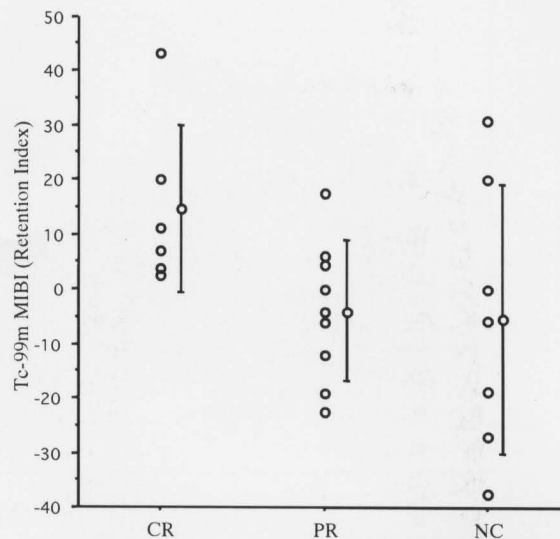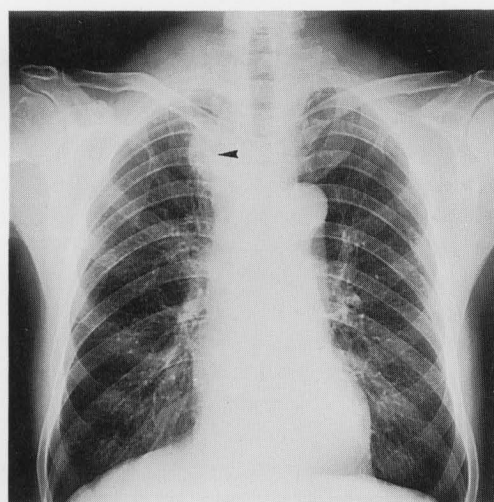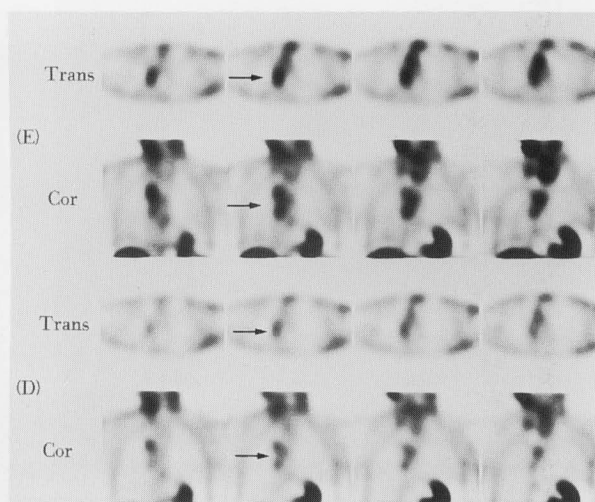
Fig. 7 Retention index and responses to chemotherapy among pre-chemotherapeutic patients. Points and bars are mean and standard deviations (S.D.).
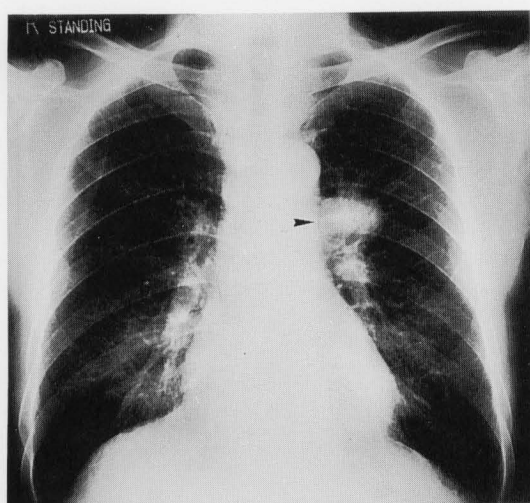

Fig. 8 Radiologic findings in a 71-yr-old male patient who was one of the 22 pre-chemotherapeutic patients. (a) Chest X-ray showing an abnormal mass shadow at the upper part of the right lung (arrowhead). (b) SPECT images of ^{99m}Tc-MIBI demonstrate an abnormal accumulation (early ratio 4.2, delayed ratio 3.4) corresponding to the lesion (arrows). After chemotherapy, the patient was classified as a partial remission. E = early image; D = delayed image; Trans = transverse; Cor = coronal.

DISCUSSION

Use of ^{201}Tl-chloride SPECT is attracting attention for the detection of lung cancer.[7] The mechanism of ^{201}Tl-chloride uptake in the cell has been reported to be related to the sodium-potassium adenosine triphosphate pump system.[8,9]

Since the late 1980s, there has been an increasing number of studies describing ^{99m}Tc-MIBI uptake in several tumors, including lung tumors.[1-3,10-14] The mechanism of ^{99m}Tc-MIBI uptake is different from that of ^{201}Tl-chloride. It has been shown that ^{99m}Tc-MIBI is attached to a low molecular weight protein in the lysosomes. The cationic charge and lipophilicity of ^{99m}Tc-MIBI, the mitochondrial and plasma membrane potentials of the tumor cell and the cellular mitochondrial content may play a significant role in the tumor uptake of this agent.[15] Since
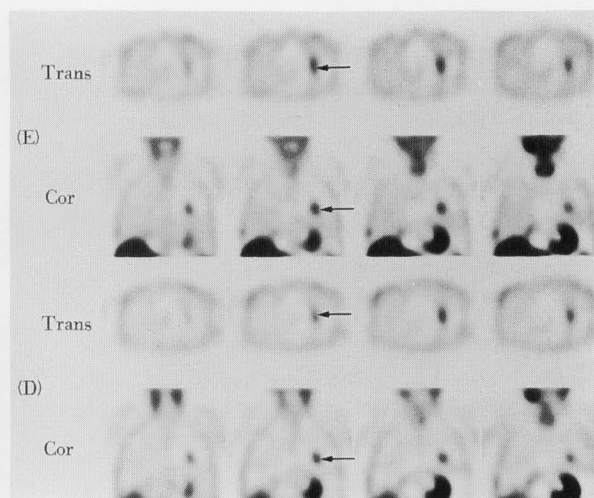
Fig. 9 Radiologic findings in a 74-yr-old male patient who was one of the 11 post-chemotherapeutic patients. He had been treated with a combination of 8 chemotherapeutic cycles using cisplatin, etoposide, cyclophosphamide, doxorubicin and vincristine but no response had been observed. (a) Chest X-ray showing an abnormal mass shadow at the upper part of the left lung (arrowhead). (b) SPECT images of ^{99m}Tc-MIBI demonstrate an abnormal accumulation (early ratio 2.5, delayed ratio 2.2) corresponding to the lesion (arrows). Compared to Figure 1, the uptake of ^{99m}Tc-MIBI in the lesion was faint. E = early image; D = delayed image; Trans = transverse; Cor = coronal.

malignant tumors maintain a more negative transmembrane potential due to their increased metabolic requirements, this has been postulated to cause increased accumulation of ^{99m}Tc-MIBI in malignant tumors. The uptake of ^{99m}Tc-MIBI may be also caused by indirect phenomena, such as increased tumor blood flow and capillary permeability.[2] A close relationship between ^{99m}Tc-MIBI uptake and tumor neoangiogenesis has been observed in other types of neoplasm.[16]

The mechanism of the chemotherapy effect is not clear. The results of chemotherapy may be conditioned by several factors; in particular, blood flow and vascularity of the tumor affect the bioavailability of the drug and its concentration in extracellular fluids in the neoplastic tissue.[17] On the other hand, oxidative metabolism, viability and MDR gene expression influence the sensitivity of neoplastic cells to the drugs.[18–21]

Recently many studies have found that ^{99m}Tc-MIBI is a suitable transport substrate of the Pgp efflux pump. The degree of overexpression of Pgp determines resistance to certain chemotherapeutic drugs such as anthracyclines, vinca alkaloids, epipodophyllotoxin, colchicine and actinomycin D.[5,22–26] This indicates that ^{99m}Tc-MIBI uptake by neoplastic cells is related to certain factors (histological: blood flow and vascularity; biological and biochemical: cellular metabolism and viability, and expression of MDR) that affect the pharmacological action of the drugs and the results of chemotherapy. Therefore, the degree of ^{99m}Tc-MIBI uptake by the tumor can be assumed to be the result of different balances among these factors and it may

be considered as a non-invasive *in vivo* tool to assess the chemosensitivity of the neoplastic mass. It has been suggested that determination of Pgp levels in patients at diagnosis or relapse may play a major role in the design of future treatment protocols,[27] but various investigations have revealed only low levels of MDR1 expression in patients with lung cancer, including both small and non-small cell lung cancers.[28,29] ^{99m}Tc-MIBI uptake in a tumor does not necessarily indicate that a cancer is sensitive to drugs associated with MDR, because there are many other mechanisms for resistance to multiple drugs, such as multidrug resistance associated protein, enhanced glutathione S-transferase, altered topoisomerase II, enhanced DNA repair and low levels of cytochrome p-450 reductase.[30,31] Patients with small cell lung cancer with higher uptakes of ^{99m}Tc-MIBI were more likely to respond to chemotherapy than those with lower uptakes.[32] One case report also showed that absence of ^{99m}Tc-MIBI uptake was associated with failure of chemotherapy.[33] These studies showed that the degree of ^{99m}Tc-MIBI uptake in small cell lung cancer correlated with response to chemotherapy. The information derived from ^{99m}Tc-MIBI imaging might be used in the prediction of the course of cancer by identifying patients who will not respond to conventional chemotherapy and could guide the design of the most effective therapy protocols.

Our results suggest that the assessment of ^{99m}Tc-MIBI uptake may discriminate between different degrees of response to chemotherapy in pre-chemotherapeutic patients. Reduced T/N ratios were found in post-chemo-

therapeutic patients compared with those in pre-chemo-therapeutic patients. A less favorable balance between the above factors—in particular, metabolic activity, vascularization and Pgp expression—may result in reduced ^{99m}Tc-MIBI uptake and chemoresistance in post-chemotherapeutic patients. ^{99m}Tc-MIBI scintigraphy may be utilized to monitor acquired drug resistance induced by chemotherapy, but there was a considerable overlap of ^{99m}Tc-MIBI uptake among subjects. We did not have sufficient numbers of patients with small cell lung cancer to arrive at a statistically meaningful conclusion. Further work in this area, including the factor of chemoresistance, needs to be conducted.

CONCLUSION

^{99m}Tc-MIBI uptake of small cell lung cancer without response to chemotherapy was lower than that of newly diagnosed small cell lung cancer. Before chemotherapy, small cell lung cancer with a higher ^{99m}Tc-MIBI uptake was more likely to respond to chemotherapy than that with a lower uptake. ^{99m}Tc-MIBI might be useful for evaluating the tumor chemosensitivity in patients with small cell lung cancer.

REFERENCES

1. Nishiyama Y, Kawasaki Y, Yamamoto Y, Fukunaga K, Satoh K, Takashima H, et al. ^{99m}Tc-MIBI and ^{201}Tl-chloride scintigraphy of primary lung cancer. *J Nucl Med* 1997; 38: 1358–1361.
2. Hassan IM, Sahweil A, Constantinides C, Mahnud A, Nair M, Omar YT, et al. Uptake and kinetics of ^{99m}Tc hexakis 2-methoxy isobutyl isonitrile in benign and malignant lesions in the lungs. *Clin Nucl Med* 1989; 14: 333–340.
3. Kao CH, Wang SJ, Lin WY, Hsu CY, Liao SQ, Yeh SH. Differentiation of single solid lesions in the lungs by means of single-photon emission tomography with technetium-99m methoxyisobutylisonitrile. *Eur J Nucl Med* 1993; 20: 249–254.
4. Gottesman NM. How cancer cells evade chemotherapy: Sixteenth Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res* 1993; 53: 747–754.
5. Piwnica-Worms D, Chiu ML, Budding M, Kronauge JF, Kramer RA, Croop JM. Functional imaging of multidrug-resistant P-glycoprotein with an organotechnetium complex. *Cancer Res* 1993; 53: 977–984.
6. The Japan Lung Cancer Society. General rule for clinical and pathological record of lung cancer, November 1995, forth edn.
7. Tonami N, Shuke N, Yokoyama K, Seki H, Takayama T, Kinuya S, et al. Thallium-201 single photon emission computed tomography in evaluation of suspected lung cancer. *J Nucl Med* 1989; 30: 997–1004.
8. Kishida T. Mechanism of thallium-201 accumulation to thyroid gland: clinical usefulness of dynamic study in thallium-201 chloride scintigraphy for differential diagnosis of thyroid nodules. *KAKU IGAKU (Jpn J Nucl Med)*

1987; 24: 991–1004.
9. Ito Y, Muranaka A, Harada T, Matsudo A, Yokobayashi T, Terashima H. Experimental study on tumor affinity of ^{201}Tl-chloride. *Eur J Nucl Med* 1978; 3: 81–86.
10. Aktolun C, Bayhan H, Kir M. Clinical experience with ^{99m}Tc-MIBI imaging in patients with malignant tumors: preliminary results and comparison with ^{201}Tl-chloride. *Clin Nucl Med* 1992; 17: 171–176.
11. Desai SP, Yuille DL. Visualization of a recurrent carcinoid tumor and an occult distant metastasis by technetium-99m-sestamibi. *J Nucl Med* 1993; 34: 1748–1751.
12. Strouse PJ, Wang DC. Incidental detection of bronchogenic carcinoma during ^{99m}Tc-SESTAMIBI cardiac imaging. *Clin Nucl Med* 1993; 18: 448–449.
13. Caner B, Kitapci M, Erbengi G, Gogus T, Bekdik C. Increased accumulation of ^{99m}Tc-MIBI in undifferentiated mesenchymal tumor and its metastatic lung lesions. *Clin Nucl Med* 1992; 17: 144–145.
14. Yamamoto Y, Nishiyama Y, Satoh K, Takashima H, Ohkawa M, Fujita J, et al. Comparative study of technetium-99m-sestamibi and thallium-201 SPECT in predicting chemotherapeutic response in small cell lung cancer. *J Nucl Med* 1998; 39: 1626–1629.
15. Chiu ML, Kronauge JF, Piwnica-Worms D. Effect of Mitochondrial and plasma membrane potential on accumulation of hexakis (2-methoxyisobutylisonitrile) technetium (I) in cultured mouse fibroblasts. *J Nucl Med* 1990; 31: 1646–1653.
16. Schillaci O, Scoparino F, Scarpini M, Mingazzini PL, Danieli R, Banci M, et al. Is ^{99m}Tc MIBI uptake in breast cancer related to neoangiogenesis? (abstract) *Eur J Nucl Med* 1994; 21: 747.
17. Ross EM. Pharmacodynamics: Mechanisms of drug action and the relationship between drug concentration and effect. In: Goodman Gilmann A, Rall TW, Nies AS, Taylor P, eds. *The Pharmacological Basis of Therapeutics*. New York; McGraw-Hill, 1992: 33–49.
18. Ihde DC. Chemotherapy of lung cancer. *N Engl J Med* 1992; 12: 1434–1441.
19. Gottesman MM, Pastan I. The multidrug transporter, a double edged sword. *J Biol Chem* 1988; 263: 12163–12166.
20. Endicott JA, Ling V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu Rev Biochem* 1989; 58: 134–171.
21. Croop JM, Gros P, Housman DE. Genetics of multidrug resistance. *J Clin Invest* 1988; 81: 1303–1309.
22. Ballinger JR, Hua HA, Berry BW, Firby P, Boxen I. ^{99m}Tc-sestamibi as an agent for imaging P-glycoprotein-mediated multi-drug resistance: *In vitro* and *in vivo* studies in a rat breast tumour cell line and its doxorubicin-resistant variant. *Nucl Med Commun* 1995; 16: 253–257.
23. Dimitrakopoulou-Strauss A, Strauss LG, Goldschmidt H, Lorenz WJ, Maier-Borst W, Van Kaick G. Evaluation of tumour metabolism and multidrug resistance in patients with treated malignant lymphomas. *Eur J Nucl Med* 1995; 22: 434–442.
24. Rao VV, Chiu ML, Kronauge JF, Piwnica-Worms D. Expression of recombinant human multidrug resistance P-glycoprotein in insect cells confers decreased accumulation of ^{99m}Tc-sestamibi. *J Nucl Med* 1994; 35: 510–515.
25. Komori T, Matsui R, Adachi I, Shimizu T, Sueyoshi K,

Narabayashi I. *In vitro* uptake and release of ^{201}Tl and ^{99m}Tc-MIBI in Hela cell. *KAKU IGAKU (Jpn J Nucl Med)* 1995; 32: 651–658.

26. Ford JM, Hait MN. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* 1990; 42: 155–199.

27. Chan HS, Haddad G, Thorner PS, DeBoer G, Lin YP, Ondrusek N, et al. P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. *N Engl J Med* 1991; 325: 1608–1614.

28. Chin K-V, Pastan I, Gottesman MM. Function and regulation of the human multidrug resistance gene. *Adv Cancer Res* 1993; 60: 157–180.

29. Goldstein LJ, Galski H, Fojo A, Willingham M, Lai SL, Gazdar A, et al. Expression of a multidrug resistance gene in human cancers. *J Natl Cancer Inst* 1989; 81: 116–124.

30. Eijdems EW, De Haas M, Coco-Martin JM, Ottenheim CP, Zaman GJ, Dauwerse HG, et al. Mechanisms of MRP overexpression in four human lung-cancer cell lines and analysis of MRP amplicon. *Int J Cancer* 1995; 60: 676–684.

31. Harris AL, Hochhauser D. Mechanisms of multidrug resistance in cancer treatment. *Acta Oncologica* 1992; 31: 205–213.

32. Bom HS, Kim YC, Song HC, Min JJ, Kim JY, Park KO. ^{99m}Tc-MIBI uptake in small cell lung cancer. *J Nucl Med* 1998; 39: 91–94.

33. Moretti JL, Caglar M, Boaziz C, Caillat-Vigneron N, Morere JF. Sequential functional imaging with ^{99m}Tc hexakis-2-methoxyisobutylisonitrile and ^{111}In-octreotide: can we predict the response to chemotherapy in small cell lung cancer? *Eur J Nucl Med* 1995; 22: 177–180.