The value of Tc-99m tetrofosmin scintimammography in the assessment of P-glycoprotein in patients with breast cancer

Güler Silov[1] MD,
Zeynep Erdoğan[1] MD,
Ayşegül Özdal[1] MD,
Ahmet Tutuş[2] MD,
Yücel Tekin[3] MD,
Hatice Karaman[3] MD,
Özgül Turhal[1] MD

1. Department of Nuclear Medicine, Kayseri Training and Research Hospital, Kayseri, Turkey
2. Department of Nuclear Medicine, Erciyes University School of Medicine, Kayseri, Turkey
3. Department of Pathology, Kayseri Training and Research Hospital, Kayseri, Turkey

***

Keywords: Scintimammography
- ^{99m}Tc Tetrofosmin
- Multidrug resistance
- P- glycoprotein

Correspondence address:
Güler Silov MD, Kayseri Training and Research, Hospital Department of Nuclear Medicine, 38010 Kayseri, Turkey
E-mail: gulersilov@yahoo.com
Phone: 905446146154
Fax: 903523207313

Received:
23 Jule 2013
Accepted revised:
10 September 2013

Abstract

P-glycoprotein (Pgp) overexpression has been shown to be correlated with resistance to chemotherapy in patients with malignant breast tumors. *The aim* of our study was to investigate the role of technetium-99m-tetrofosmin (^{99m}Tc-TF) as a functional imaging agent reflecting Pgp expression in these tumors. *We prospectively studied 28 patients* (26 females, 2 males; mean age, 53.07±9.88 years; range, 38 to 70 years) with breast cancer to ascertain the relationship between the degree of accumulation (lesion/ nonlesion=L/NL) and percentage washout (WO%) rate of ^{99m}Tc-TF and expression of Pgp in tumor tissues. All patients received 555-740MBq of ^{99m}Tc-TF intravenously at the arm controlateral to the suffering breast. Planar images were obtained 10 and 120min post injection from prone lateral and anterior views with an acquisition time of 5min. Visual and semiquantitative measurements were performed. The L/NL ratios and WO% rates were calculated semiquantitatively. Immunohistochemical studies were performed on paraffin sections using a monoclonal antibody, JSB-1. The L/NL ratios and WO% rates were related with the level of Pgp determined immunohistochemically. *Our results showed* an inverse correlation between the L/NL ratios of ^{99m}Tc-TF and the density of Pgp expression in tumor tissues, whereas there was no appreciable correlation between the tumor WO% rates of ^{99m}Tc-TF and the level of Pgp expression (P=0.275). The values for the L/NL ratios were significantly lower for those tumors expressing Pgp at high levels than those with intermediate or no Pgp expression (P<0.002 and P<0.04). *In conclusion*, although our results warrant further studies, our data strongly suggest that ^{99m}Tc-TF imaging is useful to noninvasively determine the presence of multidrug resistance in patients with breast cancer.

Hell J Nucl Med 2013; 16(3): 218-222 *Epub ahead of print: 18 October 2013* *Published on line: 28 November 2013*

Introduction

The multidrug resistance (MDR) phenotype is frequently associated with the overexpression of transmembrane drug proteins such as the P-glycoprotein (Pgp) and/or multidrug resistance related protein-1 (MRP1). These proteins belong to the so-called ATP-binding cassette (ABC) superfamily and act as drug efflux pumps of a broad range of chemotherapeutic agents commonly used in the treatment of malignancies [1]. These proteins have been found to be overexpressed in both haematological and solid tumors and are considered as adverse prognostic factors. The ability to obtain in vivo and non-invasively information regarding the functional activity of MDR-related transporters, using probes that mimic the antineoplastic agents, provide a very useful tool in the clinical setting by determining the individual tumor susceptibility to chemotherapy [2]. This knowledge could serve as a critical tool for optimizing chemotherapeutic protocols on a patient-specific basis**.** Pgp is a primary transporter of anthracyclines, Vinca alkaloids, and epipodophylotoxins [3]. Reversal of MDR by nontoxic compounds (e.g., verapamil, cyclosporine A, PSC833, VX710, tariquidar and elacridar) that block the activity of all of ABC transporters have been an important target for cancer treatment [4]. Some of them have been applied in clinical practice, showing possible benefits in the treatment of cancer [5]. The emergence of non-invasive molecular imaging techniques using radiolabelled agents provides an interesting approach for functional assessment of the classical mechanism of MDR in cancer patients. Toward this objective, the clinically approved ^{99m}Tc-labelled cationic lipophilic complexes (sestamibi and tetrofosmin) have been characterized as transport substrates of Pgp and MRP1 and have been proposed as surrogate markers of chemotherapeutic agents for functional evaluation of MDR [6-8]. In this regard, we prospectively studied 28 patients with breast cancer to ascertain the relationship between the degree of accumulation (lesion/nonlesion=L/NL) and

percentage washout (WO%) rate of tetrofosmin (TF) and the expression of Pgp in tumor tissues.

Subjects and methods

Patients

Twenty-eight patients referred (26 females, 2 males; mean age, 53,07±9.88 years; range, 38 to 70 years) with primary breast cancer (tumor size ranged between 0.5cm and 9cm) were recruited for this study. Initial staging comprised complete and detailed physical examination, mammography, ultrasound. Informed consent to participate in the scintimammography study was obtained from each patient. The Ethical Committee of the Erciyes University Hospital Board approved this study.

Methods

^{99m}Tc-tetrofosmin scintimammography

All patients were injected with 555-740MBq of ^{99m}Tc-TF intravenously at the arm controlateral to the breast cancer sites. Image acquisitions were performed on a single-head gamma camera equipped with low energy general purpose (LEGP) collimator (Toshiba, Japan). Planar images were obtained 10 and 120min post injection from prone lateral and anterior views with acquisition time of 5min in a 256x256 matrix. Imaging was undertaken with the patient lying prone with her arms resting on her head. In order to prevent the "shine through" effect, the breast to be imaged was freely pendent, while the opposite breast compressed on the table. The camera was set for a 140-keV photo peak with 20% window. The interpretation of ^{99m}Tc-TF scintimammography was performed in consensus by 2 experienced nuclear medicine specialist physicians. Visually and semiquantitatively evaluations were performed. Visually the image findings were binary scored as positive or negative. In semiquantitative evaluation, L/NL ratios and WO% rates were calculated. WO% rates were calculated after correction of background activity of early and late regions of interest (ROI). Decay correction was applied for all cases from the late ROI. WO%=[(Te-Be)-(Tl-Bl)/(Te-Be)xT]x l00, Te=Lesion activity measured at l0min, Be= Background activity count at l0min, Tl=Lesion activity measured at l20min, Bl=Background activity measured at l20min, T=Time interval in hours between early and late imaging.

Immunohistochemical analysis

Expression of Pgp was assessed by immunohistochemistry (IHC), using the labelled streptoavidin-biotin (LSAB) method for surgical specimens from all patients [9]. The LSAB method is a standard IHC method and one of widely used techniques for immunhistochemical staining. Avidin, a large glycoprotein, can be labeled with peroxidase or fluorescein and has a very high affinity for biotin. Biotin, a low molecular weight vitamin, can be conjugated with a variety of biological molecules such as antibodies.The technique involves three layers. The first layer consists of unlabeled primary antibody. The second layer is biotinylated secondary antibody. The third layer is a complex of avidin-biotin peroxidase. The peroxidase is then developed by the DAB or other substrate to produce different colorimetric end products.

JSB-1 reacts with a conserved cytoplasmic epitope of the plasma membrane-associated 170kDa Pgp, member of superfamily of transmembrane transporters. JSB-1 detects Pgp overexpression in human tumor cells of all different histogenetic derivations. Hibridoma cell line obtained by fusion of lymph node cells from immunized mice (inbred Balb/Cstrain) with SP2/O mouse myeloma cells. Sections of 5μm thick were cut and mounted on slides couted with poly-L-lysine. Deparafinised slices was treated with hydrogen peroxide for 15min. Followed by washing in distilled water for 5min and in phosphate-buffered saline for 10min, sections in the citrate buffer solution of pH 6 were kept in the microwave at 50% power level for a period of 14min. After allowed to cool at room temperature for 30min, sections were washed in distilled water. Slices were treated with blocking solution. The sections were incubated with primer JSB-1 antibody at 1/20 concentration for 1h. After being washed in phosphate-buffered saline for 10min, the sections were treated with biotinylated seconder dilution buffer antibody for 10min. After being washed in phosphate-buffered saline for 10min, the sections were treated with streptoavidin peroxidase compound for another 10min. Followed by washing in phosphate-buffered saline for 10min, the sections were treated with freshly prepared diaminobenzidin (DAB) chromogen for 5min. Then sections were washed with distilled water for one minute and were counterstained with Meyer hematoxylin. Finally, sections were washed with distilled water and enclosed with glycerol. To avoid drying of the sections, all processing steps were made in the oven, which was set to room temperature with moist environment. Sections of normal kidney cortex were used as positive controls. Preparations were evxamined under a light microscope Olympus U-DO brand. Expression of Pgp was interpreted by two expert pathologists who were blinded to any clinical information. Tumors were graded according to the severity of staining. Immunostaining was examined in 10 high-power fields. The average percentage of the positive area was calculated for 10 fields for each of the tumors. Expression of Pgp was graded according to the percentage of stained tumor cells as (-=none), (0%-9%) (+=weak), (10%-69%) (++=moderate), (70%-100%) (+++=strong). Tumors were collected in three groups according to the distribution of Pgp. Accordingly, Group 1: Patients that were negative for Pgp, Group 2: Patients that were weak to moderate focal positive for Pgp, Group 3: Patients that were strong focal positive for Pgp.

Statistical analysis

Ratios of L/NL and levels of WO% data were expressed as mean±SD. One-way ANOVA test was used for the relationship between early L/NL ratios and Pgp groups. Pearson correlation test was used for the relationship between WO% levels and Pgp groups. A probability value less than 0.05 was considered as significant.

Results

Our results showed an inverse correlation between the L/NL ratios of ^{99m}Tc-TF and the density of Pgp expression in tumor tissues, whereas there was no appreciable correlation between tumor WO% rates of ^{99m}Tc-TF and the level of Pgp expression (P=0.275) (Table 1). Twenty-eight patients were examined with IHC staining. There was no staining in 8 patients (Fig. 1a), weak staining in 5 patients, moderate

staining in 7 patients and strong staining in 8 patients (Fig. 2a). Among the three groups L/NL ratios were significantly different (P=0.006). Mann-Whitney U test was used for binary analysis between groups. There were statistical significant differences between Group 1 (2.55±0.82) and Group 3 (1.80±0.41; P=0.04) and between Group 2 (2.76±0.63) and Group 3 (1.80±0.41; P=0.002). The values for the L/NL ratios were significantly lower for those tumors expressing Pgp at high levels than those with intermediate or no expression (P<0.002, P<0.04). There were no significant differences between group 1 (2.55±0.82) and 2 (2.76±0.63; P=0.355). There were no significant differences between WO% rates of tumor and the level of Pgp (P=0.275, r= -214).

Discussion

Determining the mechanisms of drug resistance is important for the development of effective therapeutic strategies. Two important mechanisms operating as drug efflux pumps are involved in drug resistance to chemotherapy in breast cancer, namely MRP and Pgp expressions [10-12]. Technetium-99m-TF is a lipophilic diphosphine compound generally accepted for imaging breast cancer [13].

Although its uptake mechanisms are not fully understood, it has been hypothesized that flow and metabolic status of cells are important, with intracellular uptake of the radiopharmaceutical dependent on mitocondria and the Na+/K+ pump. Several in vitro and in vivo studies found significant correlations between the uptake and/or the retention of this radiotracer and expression levels of Pgp [8, 14, 15].

The major finding of this study is that L/NL ratios were higher in patients without Pgp than in those with Pgp expression. Our findings were similar to and supported by previous studies [15, 16]. We found that the L/NL ratios calculated from ^{99m}Tc-TF scintigraphy were significantly lower in group 3 than in group 1. But in these studies [15, 16], researchers did not calculate tumor WO% rates. Our results indicated that ^{99m}Tc-TF scintigraphy using semiquantitative indices (L/NL ratios) could be used as a noninvasive in vivo test to detect Pgp expression (Fig. 1b, 2b).

Similar to our results, it was also shown that L/NL ratios were highest in patients with negative expression of Pgp, and lowest in patients with positive expression of Pgp using ^{99m}Tc sestamibi scintimammography and it was also found that ^{99m}Tc sestamibi scintimammography was useful for the determination of the presence of MDR due to Pgp expression in patients with breast cancer [17]. Several possible mechanisms of uptake and retention of cationic agents have been proposed in a variety of animal models. Net cell content of the cationic agent is a function of passive potential dependent influx and transporter mediated efflux. The decreased level of the substrates is the result of enhanced efflux due to overexpression of transporters. Unexpected interesting finding of their study [17] was that the WO% rates of ^{99m}Tc sestamibi did not show a statistical significance among the four groups. To the best our knowledge, WO% rates were calculated firstly in our study which used ^{99m}Tc-TF as radiotracer in breast MDR evaluation clinically, but an unexpected finding of the current study was also found that WO% rates of ^{99m}Tc-TF did not have statistical significance between the three groups (Groups 1-3). A linear relation between Pgp expression and WO% rates of ^{99m}Tc sestamibi in breast

Table 1. *Tumor L/NL ratios, WO% rates and Pgp levels of patients*

Patient No	L/NL	WO%	Pgp	Tumor Size (mm)
1	2,32	9	-	35x25x20
2	2,16	29	-	35x15x10
3	1,80	24	-	50x25x20
4	1,94	37	-	20x10x5
5	3,58	30	-	50x40x35
6	3,10	38	-	70x40x30
7	1,73	37	-	15x10x4
8	3,82	24	-	40x26x20
9	4,23	33	+	37x27x20
10	2,23	15	+	40x25x25
11	2,61	25	+	40x25x20
12	3,40	26	+	30x20x10
13	2,78	33	+	60x50x40
14	3,31	37	++	5 x45x25
15	1,97	36	++	43x40x40
16	2,46	33	++	25x0x20
17	2,84	32	++	50x25x20
18	2,71	22	++	90x50x30
19	2,08	29	++	30x30x25
20	2,51	34	++	12x8x3
21	1,56	21	+++	25x22x22
22	2,23	20	+++	32x32x27
23	1,86	31	+++	45x45x35
24	1,72	19	+++	50x40x35
25	1,80	11	+++	20x15x8
26	2,56	35	+++	60x45x40
27	1,40	14	+++	40x40x20
28	1,34	48	+++	30x2x18

AMC: Atypical medullary carcinoma, IDC: Invasive ductal carcinoma. The first 8 cases are in Group 1, the next 12 cases in Group 2 and the remaining 8 cases in Group 3. all patients were IDC, except No. 3 who was ACMC.

cancer has been reported previously [18]. Ongoing clinical studies addressed the question of whether a correlation exists between the efflux rate of ^{99m}Tc-TF and quantitative Pgp expression in breast cancer. Also the findings [19] of the biexponential efflux of ^{99m}Tc labelled monocationic agents could not explain our conflicting results, which showed the insignificant differences ^{99m}Tc-TF WO% rates among three of our groups. Because of in the second phase the clearance of ^{99m}Tc furifosmin and of ^{99m}Tc-TF were faster than that of ^{99m}Tc sestamibi in Pgp expressing tumors, these authors [19] suggest that ^{99m}Tc-TF was more desirable than ^{99m}Tc sestamibi in recognising Pgp expression. Nevertheless, our results did not confirm these suggestion and the optimum time for delayed imaging could be seen as a major problem in this regard. Furthermore, it is well known that artifacts caused by non-uniformity of the gamma camera and depth dependency of its spatial resolution as well as by scatter and radiation absorbtion may interfere with lesion and background semiquantification. Suboptimal functional capacity of the Pgp efflux pump was thought to be the possible mechanism to explain discordant findings. Discordant findings might also be explained by heterogeneous distribution of Pgp in tumor, in which a small biopsy specimen not always represents whole tumor Pgp expression.

Clinical studies investigating the expression of ABC transporters illustrate the conflicting results reported regarding the correlation of Pgp response to treatment [20, 21]. In general, studies using immunohistochemical methods to detect proteins conclude that high tumour levels of Pgp indicate shorter survival rates, and an increased risk of relapse [21]. These data suggest that assessing ABC transporter expression levels in breast tumours may help predict patients' response to chemotherapy.

In conclusion, although routine care of breast cancer patients does not include the determination of Pgp expression for treatment, our prelimary results show that ^{99m}Tc-TF scintimammography has the potential for accurate prediction of Pgp expressions in human breast cancers. However, additional further clinical studies with larger number of patients should be performed to determine the prognostic value of Pgp expression in breast cancer patients.

The authors declare that they have no conflicts of interest.

Bibliography

1. Borst P, Elferink RO. Mammalian ABC transporters in health and disease. *Annu Rev Biochem* 2002; 71: 537.
2. Gomes CMF, Abrunhosa AJ, Pauwels EK et al. P-glycoprotein versus MRP1 on transport kinetics of cationic lipophilic substrates: a comparative study using Tc-99m Sestamibi and Tc-99m Tetrofosmin. *Cancer Biother Radiopharm* 2009; 24: 215-27.
3. Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE et al. P-glycoprotein: From genomics to mechanism. *Oncogene* 2003; 22: 7468.
4. Aouali N, Eddabra L, Macadre J et al. Immunosuppressors and reversion of multidrug-resistance. *Crit Rev Oncol Hematol* 2005; 56-61.
5. Sandler A, Gordon M, De Alwis DP et al. A phase I trial of a potent P-glycoprotein inhibitor, zosuquidar trihydrochloride (LY335979), administered intravenously in combination with doxorubicin in patients with advanced malignancy. *Clin Cancer Res* 2004; 10: 3265.
6. Hendrikse NH, Franssen EJF, van der Graaf WTA et al. Visualization of multidrug resistance in vivo. *Eur J Nucl Med* 1999; 26: 283.
7. Gomes CMF. Cationic lipophilic radiotracers for functional imaging of multidrug resistance. *Current Radiopharmaceuticals* 2012; 5: 113-23.

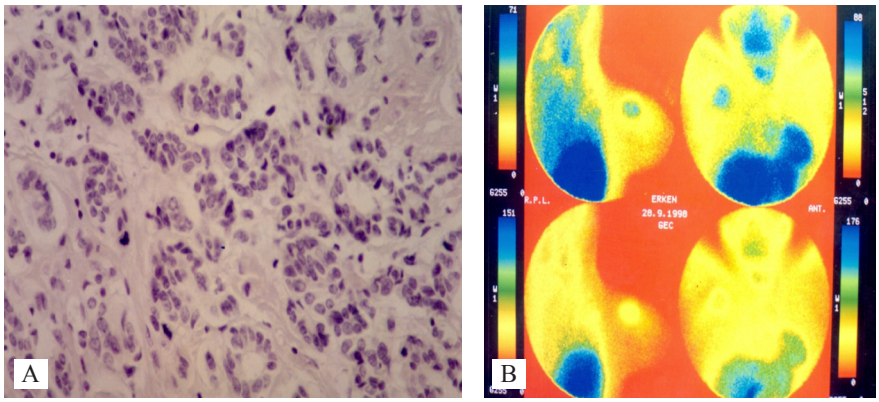

Figure 1. A. A 45 years old female patient with right invasive ductal carcinoma have no staining on IHC analysis. B. Scintimammography study of the same patient. There was clearly seen a mass lesion on the right breast (50x40x35mm macroscopic measurement).

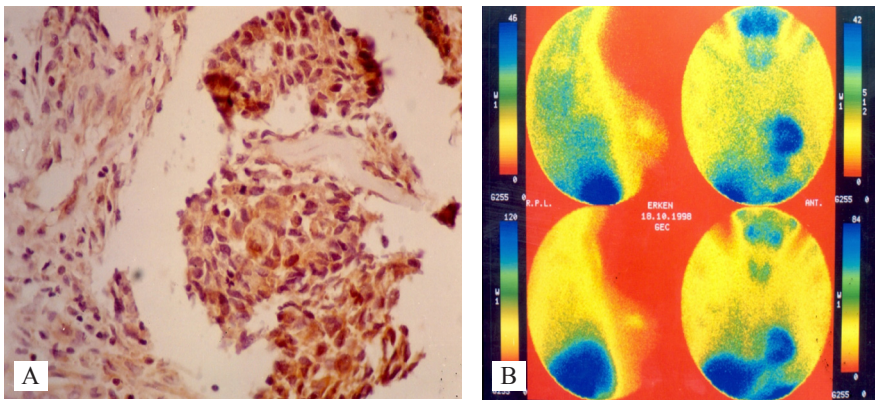

Figure 2. A. A 51 years old female patient with right invasive ductal carcinoma have +++ staining on IHC analysis. B. Scintimammography study of the same patient. There was slightly seen a mass lesion on the right breast (20x15x10mm macroscopic measurement).

8. Ballinger JR, Bannerman J, Boxen I et al. Technetium-99m-tetrofosmin as a substrate for P-glycoprotein: in vitro studies in multidrug-resistant breast tumor cells. *J Nucl Med* 1996; 37(9): 1578-82.
9. Scheper RJ, Bulte JWM, Brakkee JGP et al. Monoclonal Antibody JSB-1 Detects a Highly Conserved Epitope on the P-Glycoprotein associated with Multi-Drug-Resistance. *Int J Cancer* 1988; 42: 389-94.
10. Dexter DW, Reddy RK, Geles KG et al. Quantitative reverse transcriptase-polymerase chain reaction measured expression of MDR1 and MRP in primary breast carcinoma. *Clin Cancer Res* 1998; 4(6): 1533-42.
11. Filipits M, Suchomel RW, Dekan G et al. MRP and MDR1 gene expression in primary breast carcinomas. *Clin Cancer Res* 1996; 2(7): 1231-7.
12. Nooter K, Brutel de la Riviere G, Look MP et al. The prognostic significance of expression of the multidrug resistance-associated protein (MRP) in primary breast cancer. *Br J Cancer* 1997; 76(4): 486-93.
13. Mansi L, Rambaldi PF, Procaccini E. Scintimammography with Tc-99m tetrofosmin in the diagnosis of breast cancer and lymph node metastases. *Eur J Nucl Med* 1996; 23: 932-9.
14. Yapar Z, Kibar M, Yapar AF et al. The value of Tc-99m-tetrofosmin scintigraphy in the assessment of P-glycoprotein in patients with malignant bone and soft-tissue tumors. *Ann Nucl Med* 2003; 17(6): 443-9.
15. Tse-Jia Liu, Shih-Chuan Tsai, Yung-Jen Ho et al. Comparison of the Expression of P-Glycoprotein, Ki-67, and P-53 to Technetium-99m Tetrofosmin Mammoscintigraphic Findings. *Cancer Investigation* 2002; 20 (2): 199-205.
16. Lin CY, Kao CH. Usefulness of Technetium-99m-Tetrofosmin Mammoscintigraphy to Predict Expressions of P-Glycoprotein and Multidrug Resistance Related Protein: A Preliminary Report. *Ann Nucl Med Sci* 2002; 15: 59-64.
17. Kim IJ, Bae YT, Kim SJ et al. Determination and prediction of P-glycoprotein and multidrug-resistance-related protein expression in breast cancer with double-phase technetium-99m sestamibi scintimammography. Visual and quantitative analyses. *Oncology* 2006; 70(6): 403-10.
18. Ciarmiello A, Del Vecchio S, Silvestro P et al. Tumor clearence of technetium 99m-sestamibi as a predictor of response to neoadjuvant chemotherapy for locally advanced breast cancer. *J Clin Oncol* 1998; 16: 1677-83.
19. Liu Z, Stevenson GD, Barrett HH et al Imaging recognition of multidrug resistance in human breast tumors using ^{99m}Tc-labeled monocationic agents and a high-resolution stationary SPECT system. *Nucl Med Biol* 2004; 31: 53-65.
20. Moureau-Zabotto L, Ricci S, Lefranc JP et al. Prognostic impact of multidrug resistance gene expression on the management of breast cancer in the context of adjuvant therapy based on a series of 171 patients. *Brit J Cancer* 2006; 94(4): 473-80.
21. Surowiak P, Materna V, Matkowski R et al. Relationship between the expression of cyclooxygenase 2 and MDR1/P-glycoprotein in invasive breast cancers and their prognostic significance. *Breast Cancer Res* 2005; 7(5) 862-70.