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Tc-99m glutathione scintigraphy in the detection of primary tumor and staging of patients with lung cancer: a preliminary report

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SUMMARY

The aim of this preliminary study was to investigate the potential role of Tc-99m GSH scintigraphy in the diagnosis and staging of patients with lung cancer. Twenty five patients with newly biopsy-proven primary lung cancer were included in this study. Whole body imaging was performed at the 1st and 3rd hours after the injection of 15 mCi (555 MBq) of Tc-99m GSH. Thorax SPECT imaging was also performed at the 1st hour in all patients. SPECT and whole body imaging showed increased tracer uptake in primary lesion sites in 23 and 22 of 25 patients with lung cancer, respectively. The tumor/background activity ratios in early and late images were 1.29 to 2.35 (mean 1.74) and 1.41 to 4.03 (mean 2.01), respectively. Mediastinum could not be clearly evaluated due to high blood pool activity of Tc-99m GSH. According to the results of this study, detection rate of distant metastases with Tc-99m GSH is not adequate. As a nonspecific tumor imaging agent, Tc-99m GSH scintigraphy has not enough specificity for using in a routine fashion in lung cancer. However, it could be a promising radiopharmaceutical due to having potential to visualize in vivo GSH metabolism which is important in chemo- or radio-therapy resistance.

Key words: *Lung cancer, SPECT, staging, Tc-99m glutathione scintigraphy*

ÖZET

Akciğer kanserli hastalarda primer tümör tanısı ve evrelemede Tc-99m glutatyon sintigrafisinin rolü: ön sonuçlar

Bu öncü çalışmanın amacı akciğer kanserli hastalarda tanı ve evrelemede Tc-99m GSH sintigrafisinin potansiyel rolünün araştırılmasıdır. Bu çalışmaya yeni tanı konulmuş ve biyopsi ile tanısı doğrulanmış 25 akciğer kanserli hasta dahil edildi. On beş mCi (555 MBq) Tc-99m GSH enjeksiyonu sonrası 1. ve 3. saatte tüm vücut taraması ve 1. saatte toraks SPECT görüntülemesi yapılmıştır. SPECT ve tüm vücut taraması sırası ile 25 hastanın 23 ve 22'sinde primer tümöre ait artmış aktivite tutulumu saptanmıştır. Tümör/geri plan aktivitesi erken ve geç görüntülerde sırası ile 1.29-2.35 (ortalama 1.74) ve 1.41-4.03 (ortalama 2.01) olarak bulunmuştur. Tc-99m GSH'a ait yüksek kan havuzu aktivitesi nedeni ile mediyastinal lenf nodları net olarak değerlendirilememiştir. Çalışma sonuçlarımıza göre Tc-99m GSH sintigrafisinin uzak metastaz tespitindeki rolü yeterli duyarlılıkta bulunmamıştır. Tc-99m GSH sintigrafisi, non-spesifik bir tümör görüntüleme ajanı olarak akciğer kanserlerinde rutinde kullanım için yeterli özgüllüğe sahip değildir. Bununla birlikte, kemo- veya radyo-terapi direncinin değerlendirilmesinde öneme sahip olabilecek GSH metabolizmasını in vivo gösterebilme potansiyeli nedeniyle umut verici bir ajan olduğu düşünülmektedir.

Anahtar kelimeler: *Akciğer kanseri, SPECT, evreleme, Tc-99m glutatyon sintigrafisi*

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Introduction

Glutathione (GSH, γ--glutamylcysteinylglycine) is a tripeptide which has an important metabolic role in detoxification reactions, protecting cells against damage from endogenous and exogenous free radicals, oxidants and other electrophilic substances such as chemotherapeutics and also from radiation (1-4). Some studies have shown that there is an increased demand for GSH in cell injury and cancer cells (5-7).

GSH can be easily labeled with Tc-99m pertechnetate and labeling efficiency is very high (8,9). Tc-99m GSH scintigraphy has potential to image the in vivo GSH metabolism of tumor cells. Up to date, its labeled form has been studied as a potential radiopharmaceutical for several tumors including head and neck tumors (8) and malignant melanoma (10).

Increased GSH levels and GSH peroxidase activity have been reported in small (SCLC) and non-small cell lung carcinomas (NSCLC) (11-18), and there has been heightened cellular extraction of GSH from circulation (19,20). Moreover, tumor-bearing lung is capable of extracting higher amounts of GSH from the pulmonary circulation compared to normal lung (21). The aim of this preliminary study was to investigate the potential role of Tc-99m GSH scintigraphy, as a functional imaging method, in the evaluation of patients with newly diagnosed lung cancer.

Material and Methods

Patients: Twenty five patients (23 male, 2 female, aged 37-69, mean 60.5 years) with newly biopsy-proven primary lung cancer were included in this study (Table I). All patients were evaluated by means of computerized tomography (CT), ultrasonography (US) and bone scintigraphy for the detection of primary tumor extention, before the onset of specific treatment. Patients with metastatic lung disease or lung cancer with other known lung pathologies were excluded from the study. Approval of the local ethics

Table I. Whole body scintigraphic and pathological results of the study population

No	Age	Diagnosis	Early tumor/ background	Late tumor/ background
1	37	Adenocarcinoma	1.62	1.66
2	56	Adenocarcinoma	2.20	2.41
3	54	Adenocarcinoma	2.30	4.03
4	56	Adenocarcinoma (Poorly differentiated)	1.29	1.41
5	66	Adenocarcinoma (Poorly differentiated)	1.60	1.73
6	58	Adenocarcinoma	1.65	1.68
7	48	Adenosquamous carcinoma	-	-
8	63	Small cell carcinoma	1.62	1.73
9	67	Small cell carcinoma	1.60	1.73
10	66	Small cell carcinoma	-	-
11	46	Large cell anaplastic carcinoma	1.79	1.88
12	60	Large cell anaplastic carcinoma	1.40	1.44
13	68	Squamous cell carcinoma	1.48	1.74
14	64	Squamous cell carcinoma	2.35	3.64
15	54	Squamous cell carcinoma	1.50	1.80
16	69	Squamous cell carcinoma	-	-
17	54	Squamous cell carcinoma	1.73	1.89
18	69	Squamous cell carcinoma	2.13	2.24
19	69	Squamous cell carcinoma	1.77	1.94
20	67	Squamous cell carcinoma	1.86	2.01
21	57	Squamous cell carcinoma	1.56	1.94
22	62	Squamous cell carcinoma	1.45	1.73
23	55	Squamous cell carcinoma	1.67	1.75
24	69	Squamous cell carcinoma	1.94	2.22
25	61	Squamous cell carcinoma	1.80	1.82

committee was obtained and the study was fully explained to each patient by obtaining their informed consent prior to the study.

Tc-99m GSH scintigraphy: The reduced form of GSH (Sigma Chem. Co., U.S.A) was labeled with Tc-99m pertechnetate and labeling efficiency was controlled as described previously (8,9). Briefly, 20 mg GSH was dissolved in 2 ml water and after pH neutralization, 45-50 mCi (1665-1850 MBq) Tc-99m pertechnetate was added to the solution to label GSH. A sample of Tc-99m GSH was spotted on one end of ITLC-SG strip (1x10cm) (Gelman Instrument Co., USA) and the strip was dipped into a vial containing methyl ethyl ketone (MEK) or saline as solvents. Free pertechnetate migrated with MEK and free pertechnetate and Tc-99m GSH migrated with saline in ITLC-SG strips. The chromatography strip was cut into two pieces and they were counted in a dose calibrator (Veenstra inst VDC-202, NL). The labeling efficiency of Tc-99m GSH was determined (%migrated in saline-% migrated in MEK). Radiochemical purity of Tc-99m GSH assessed by ITLC-SG was around ≥95%.

Whole body (WB) imaging was performed at the 1st and 3rd hours after the injection of 555 MBq (15 mCi) of Tc-99m GSH by using large field of view gamma camera equipped with a low energy, all purpose collimator (Millennium, GE Medical Systems, Milwaukee, WI). Single photon emission computed tomography (SPECT) imaging of the thorax was also performed to evaluate the lymph node involvement in the mediastinum at first hour. The SPECT acquisition protocol was 64 projection images over a range of 360°, sampling times of 28 seconds and 64x64 matrices. Image reconstruction was done using filtered back projection with a ramp filter.

Data analysis: Two experienced physicians blinded to patient's clinical findings evaluated Tc-99m GSH WB and SPECT images. Only intense abnormal accumulation clearly separated from normal structures was considered as positive finding for malignant tumor focus. For semiquantitative analysis, a tumor-to-background (T/Bg) ratio was calculated by drawing equal-sized regions of interests (ROI) around the tumor and an area of normal lung tissue in the contra lateral site on early and late WB scans.

Statistical analysis: Wilcoxon's Matched-Pairs Signed-Ranks test and Kruskal Wallis test were used to evaluate any difference between early and late T/Bg ratios and any difference in T/Bg ratios of the subgroups, respectively. Statistical analysis was performed by using the SPSS 10.0 Statistical Package Program for Windows (SPSS Inc., Chicago, Illinois, USA). Differences were considered significant at $p<0.05$.

Results

Increased tracer uptake in primary lesion sites in 23 and 22 of 25 patients in the study group were demonstrated by SPECT and WB Tc-99m GSH imaging, respectively (Table I). The T/Bg ratios in early and late images ranged from 1.29 to 2.35 (mean 1.74) and 1.41 to 4.03 (mean 2.01), respectively. A representative case with lung adenocarcinoma is shown in Figure 1. Although enlarged lymph nodes in the mediastinum were detected in 13 of 25 patients (52%) by CT imaging, we could not observe any uptake in the mediastinum either on WB or SPECT images. Twelve of 25 patients in the study group had a total of 17 distant metastatic sites (9 bone, 4 liver and 4 CNS metastases), which were detected with conventional imaging methods. Six of these metastatic foci (2 bone, 2 liver and 2 central nervous system) were accurately identified by Tc-99m GSH scintigrapy. On the other hand, one metastatic focus in paraaortic lymph node and another one in the left adrenal gland could not be detected by Tc-99m GSH scintigrapy in two patients.

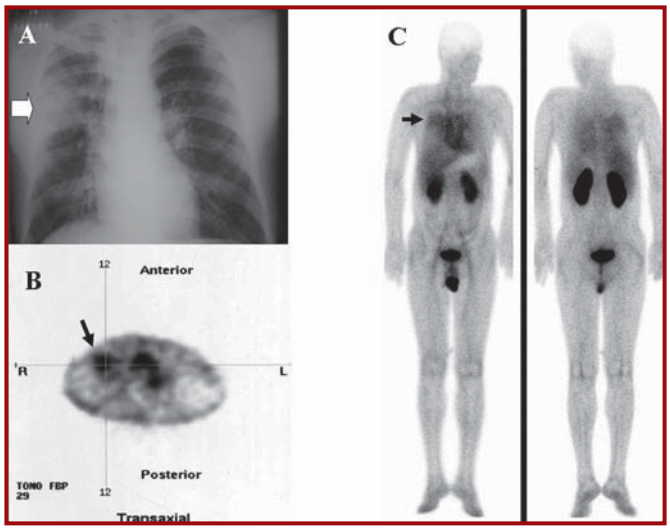

Figure 1. Chest X-ray graph (A) of a patient with adenocarcinoma reveals the tumor mass in the middle lobe of the right lung. Transaxial SPECT image (B) and anterior-posterior whole-body image (C) also demonstrate the increased Tc-99m GSH uptake in the tumor (arrows)

Statistical results are summarized in the Table II. There is no significant difference between the sensitivity for tumor detection among the subgroups in both early and late imaging ($p>0.05$). On the other hand, T/Bg ratios derived from late images showed A significant increase than the early ones ($p<0.001$).

Table II. Descriptive statistics of the study

Diagnosis	*n*	*Early tumor/ background*	*Late tumor/ background*	*p***
Squamous cell carcinoma	13	1.770±0.272	2.061±0.528	
Adenocarcinoma	6	1.776±0.390	2.153±0.978	
Small cell carcinoma	3	1.610±0.014	1.730±0.000	
Large cell carcinoma	2	1.595±0.275	1.660±0.311	
Adenosquamous carcinoma	1	-	-	
Malignant	25	1.730±0.0617	2.010±0.135	<0.001
p*		0.778	0.219	

*Kruskal Wallis test, ** Wilcoxon's Matched-Pairs Signed-Ranks test

Discussion

According to our results, the efficiency of Tc-99m GSH to show the primary lung cancer is satisfactory. Average T/Bg ratios derived from both early and late images are very close to calculations derived measurements from tumor specimens under in vitro conditions (21). Tc-99m GSH has been used to image the inflammatory lesions previously (9). Moreover, it has been shown moderate Tc-99m GSH uptake in primary lung cancer, as well. So, it is not expected to be helpful in differential diagnosis of solitary pulmonary nodule.

Tc-99m GSH imaging did not show any pathological uptake in the mediastinum in 13 cases that have enlarged lymph nodes on their CT scans. Actually, elevated blood pool activity probably caused by protein binding restricts to assess the lesions located in the mediastinum, even with SPECT imaging. Moreover, the detection rate of Tc-99m GSH scintigraphy in distant metastases demonstrated by conventional methods in these patients was also low. Although the decreased uptake mechanism in distant metastases is not clear, altered GSH metabolism in these metastatic foci may be one of the reasonable explanations. According to the overall results of this small patient group, Tc-99m GSH scintigraphy seems not to be helpful in the staging of lung cancer.

Uptake of the Tc-99m GSH in primary lesions increases in time. A decrease in background activity due to renal clearance of the radiopharmaceutical or an increase in tumoral uptake can probably be underlying reasons. Therefore, late imaging as in bone scan can increase the detection rate of primary lesion and quality of the scan.

It can be concluded that Tc-99m GSH is not helpful in the clinical management of patients with lung cancer, but one point should be considered that Tc-99m GSH scintigraphy can reflect the in vivo GSH metabolism of a malignant pulmonary lesion. Since, we can assume that GSH labeled with Tc-99m behaves like GSH in the circulation, it may demonstrate the GSH metabolism of a malignant lesion. Many reports suggest the elevated cellular GSH as the source of resistance to chemotherapeutic agents or radiation therapy in patients with cancer (22-26). On the contrary, depletion of cellular GSH level may cause to increase cytotoxic effects of anticancer drugs (27-30). Therefore, functional imaging with Tc-99m GSH may provide important physiological information about chemotherapy or radiotherapy resistance by monitoring the GSH levels in vivo.

Although Tc-99m GSH seems to be a nonspecific tumor imaging agent like Ga-67 citrate, Tl-201 chloride and Tc-99m MIBI, and it is not helpful in staging of lung cancer, it is a promising radiopharmaceutical due to having potential to visualize in vivo GSH metabolism, which is important in chemo- or radio-therapy resistance. Further studies are needed to determine the value of Tc-99m GSH scintigraphy in the evaluation of both resistance in radiation and chemotherapy in lung cancer.

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References

1. Reed DJ. Regulation of reductive processes by glutathione. Biochem Pharmacol 1986; 35: 7-13.
2. Andersen HR, Nielsen JB, Nielsen F, Grandjean P. Antioxidative enzyme activities in human erythrocytes. Clin Chem 1997; 43: 562-568.
3. Reed DJ. Glutathione: toxicological implications. Annu Rev Pharmacol Toxicol 1990; 30: 603-631.
4. Harlan JM, Levine JD, Callahan KS, Schwartz BR, Harker LA. Glutathione redox cycle protects cultured endothelial cells against lysis by extracellularly generated hydrogen peroxide. J Clin Invest 1984; 73: 706-713.
5. Meister A, Anderson ME. Glutathione. Annu Rev Biochem 1983; 52: 711-760.
6. Reed DJ, Fariss MW. Glutathione depletion and susceptibility. Pharmacol Rev 1984; 36 (Suppl 2): 25S-33S.
7. Rahman I. Regulation of nuclear factor-kappa B, activator protein-1, and glutathione levels by tumor necrosis factor-alpha and dexamethasone in alveolar epithelial cells. Biochem Pharmacol 2000; 60: 1041-1049.
8. Ercan MT, Aras T, Aktas A, Kaya S, Bekdik CF. Accumulation of 99mTc-glutathione in head and neck tumors. Nuklearmedizin 1994; 33: 224-228.
9. Ercan MT, Unlenen E, Aktas A. 99Tcm-glutathione for imaging inflammatory lesions. Nucl Med Commun 1994; 15: 533-539.
10. Duman Y, Burak Z, Ercan MT, et al. Clinical evaluation of metastases of malignant melanoma imaging with 99Tcm-glutathione and 99Tcm-anti melanoma antibody: a comparative study. Nucl Med Commun 1995; 16: 927-935.
11. Mock D, Whitestone B, Freeman J. Gamma-glutamyl transpeptidase activity in human oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol 1987; 64: 197-201.
12. Dawson JR, Vahakangas K, Jernstrom B, Moldeus P. Glutathione conjugation by isolated lung cells and the isolated, perfused lung. Effect of extracellular glutathione. Eur J Biochem 1984; 138: 439-443.
13. O'Brien ML, Tew KD. Glutathione and related enzymes in multidrug resistance. Eur J Cancer 1996; 32: 967-978.
14. Hanigan MH, Ricketts WA. Extracellular glutathione is a source of cysteine for cells that express gamma-glutamyl transpeptidase. Biochemistry 1993; 32: 6302-6306.
15. Hanigan MH, Frierson HF Jr, Brown JE, Lovell MA, Taylor PT. Human ovarian tumors express gamma-glutamyl transpeptidase. Cancer Res 1994; 54: 286-290.
16. Cook JA, Pass HI, Iype SN, et al. Cellular glutathione and thiol measurements from surgically resected human lung tumor and normal lung tissue. Cancer Res 1991; 51: 4287-4294.
17. Oberli-Schrammli AE, Joncourt F, Stadler M, et al. Parallel assessment of glutathione-based detoxifying enzymes, O6-alkylguanine-DNA alkyltransferase and P-glycoprotein as indicators of drug resistance in tumor and normal lung of patients with lung cancer. Int J Cancer 1994; 59: 629-636.
18. Dempo K, Elliott KA, Desmond W, Fishman WH. Demonstration of gamma-glutamyl transferase, alkaline phosphatase, CEA and HCG in human lung cancer. Oncodev Biol Med 1981; 2: 21-37.
19. Griffith OW, Bridges RJ, Meister A. Transport of gamma-glutamyl amino acids: role of glutathione and gamma-glutamyl transpeptidase. Proc Natl Acad Sci 1979; 76: 6319-6322.
20. Anderson ME, Meister A. Transport and direct utilization of gamma-glutamylcyst(e)ine for glutathione synthesis. Proc Natl Acad Sci 1983; 80: 707-711.
21. Blair SL, Heerdt P, Sachar S, et al. Glutathione metabolism in patients with non-small cell lung cancers. Cancer Res 1997; 57: 152-155.
22. Biaglow JE, Varnes ME, Clark EP, Epp ER. The role of thiols in cellular response to radiation and drugs. Radiat Res 1983; 95: 437-455.
23. Hamilton TC, Winker MA, Louie KG, et al. Augmentation of adriamycin, melphalan, and cisplatin cytotoxicity in drug-resistant and sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion. Biochem Pharmacol 1985; 34: 2583-2586.
24. Lee FYF, Flannery DJ, Siemann DW. Prediction of tumor sensitivity to 4-hydroperoxycyclophosphamide by a glutathione-targeted assay. Br J Cancer 1991; 63: 217-222.
25. Richardson ME, Siemann DW. Thiol manipulation as a means of overcoming drug resistance in a novel cyclophosphamide-induced resistant cell line. Int J Radiat Oncol Biol Phys 1992; 22: 781-784.
26. Suzukake K, Petro BJ, Vistica DT. Reduction in glutathione content of L-PAM resistant L1210 cells confers drug sensitivity. Biochem Pharmacol 1982; 31: 121-124.
27. Lee FYF, Siemann DW, Sutherland RM. Changes in cellular glutathione content during adriamycin treatment in human ovarian cancer, a possible indicator of chemosensitivity. Br J Cancer 1989; 60: 291-298.
28. Mitchell JB, Cook JA, DeGraff W, Glatstein E, Russo A. Glutathione modulation in cancer treatment: Will it work? Int J Radiat Oncol Biol Phys 1989; 16: 1289-1295.
29. Ku RH, Billings RE. The role of mitochondrial glutathione and cellular protein sulfhydryls in formaldehyde toxicity in glutathione-depleted rat hepatocytes. Arch Biochem Biophys 1986; 247: 183-189.
30. Lewis AD, Hayes JD, Wolf CR. Glutathione and glutathione-dependent enzymes in ovarian adenocarcinoma cell lines derived from a patient before and after the onset of drug resistance: intrinsic differences and cell cycle effects. Carcinogenesis 1988; 9: 1283-1287.