FABAD J. Pharm. Sci., 24, 201-205, 1999

RESEARCH ARTICLES / BİLİMSEL ARAŞTIRMALAR

# Biodistribution of $^{99m}$Tc-Glutathione in mice with Osteosarcoma: Effect of Gamma Irradiation on Tumor Uptake

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***Biodistribution of $^{99m}$Tc-Glutathione in mice with Osteosarcoma: Effect of Gamma Irradiation on Tumor Uptake***

***Summary :*** *The aim of this study was to determine the efficacy of $^{99m}$Tc-glutathione (GSH) in scintigraphic demonstration of osteosarcoma tumor in mice and the effect of gamma irridiation of tumor on tumor uptake of $^{99m}$Tc-GSH. The biodistribution of $^{99m}$Tc-GSH was studied in 30 Balb C mice three weeks after implanting osteosarcoma tumor in their thighs. The mice were injected with 400 μCi of $^{99m}$Tc-GSH in 0.1 ml through the tail vein. They were divided into 2 equal groups. The tumors of mice in the second group were subjected to gamma irradiation for 10 min (20 Gy). The mice in both groups were sacrificed at 1, 3 and 6 h. Scintigrams were obtained at each time point. The organs, tumors, some muscle and some blood were removed, weighed and assayed for radioactivity. Tumor, liver and muscle sections were also obtained for gross autoradiographic studies. The tumors were well visualized on scintigrams. The tumor uptake values were 3.27±0.80, 1.53±0.69, and 1.51±0.55 for the control and 5.18±1.28, 0.399±0.120, and 1.67±1.05 %/g for the irradiated groups, respectively. Tumor/muscle concentration ratios were 34.03±12.2, 21.4±11.3 and 18.7±11.4 for the control and 18.8±7.2, 3.63±1.9, and 24.1±9.0 for irradiated groups, respectively. Gross autoradiography of tumor sections indicated focal sites of increased uptake within tumor tissue, indicating the presence of necrotic areas. In conclusion, $^{99m}$Tc-GSH accumulated in osteosarcoma tumor and resulted in high tumor to other tissue concentration ratios. The increase in uptake values after tumor irradiation might be a result of higher demand of tumor cells for GSH, due to formation of free radicals and oxidants as by-products of irradiation.*

***Key words:*** *$^{99m}$Tc-Glutathione, osteosarcoma, gamma irradiation, autoradiography*

*Received : 10.05.1999*
*Revised : 22.11.1999*
*Accepted : 22.11.1999*

***$^{99m}$Tc-glutatyonun Osteosarkomlu Farelerde Biyodağılımı : Gama Radyasyonunun Tümör Tutulumuna Etkisi***

***Özet :*** *Bu çalışmanın amacı $^{99m}$Tc-glutatyonun (GSH) osteosarkomlu farelerdeki biyodağılımının incelenmesi, tümör tutulumunun sintigrafik görüntüleme için yeterli olup olmadığının araştırılması ve gama radyasyonunun $^{99m}$Tc-GSH tümör tutulumuna olan etkisinin tayin edilmesidir. 30 Balb C türü farede osteosarkom tümörü bacağa implante edildi. 3 hafta sonra farelere kuyruk veninden 400 μCi/0.1 ml $^{99m}$Tc-GSH enjekte edildi. Fareler 2 eşit gruba ayrıldı. Bir gruptaki farelerde tümöre 20 Gy gama radyasyonu tatbik edildi. Fareler 1., 3., ve 6. saatte öldürülüp sintigrafik imajları alındı. Fareler kesildi, organları, tümörün tamamı, bir miktar karşı bacaktan adale çıkarıldı ve kan örneği alındı. Hepsi tartılıp radyoaktiviteleri ölçüldü. Otoradyografik çalışmalar için tümör, karaciğer ve adaleden kesitler alındı. Tümörler sintigrafide çok iyi görüntülendi. Tümör uptake değerleri (%/g doku olarak) kontrol grupta 3.27±0.80 (1 inci), 1.53±0.69 (3 üncü) ve 1.51±0.55 (6 ncı saatte), ışınlanan grupta ise 5.18±1.28 (1 inci), 0.399±0.120 (3 üncü) ve 1.67±1.05 (6 ncı saatte) idi. Tümör/adale oranları ise sırasıyla kontrolda 34.03± 12.2, 21.4±11.3 ve 18.7±11.4, ışınlanan grupta ise 18.8±7.2, 3.63±1.9 ve 24.1±9.0 idi. Tümör kesitlerinde nekrotik alanlarda $^{99m}$Tc-GSH tutulumu daha azdı. Netice olarak $^{99m}$Tc-GSH osteosarkomda yüksek oranda tutulum gösterdi ve tutulum diğer dokulardan daha yüksekti. Işınlamadan sonra gözlenen tümör tutulumundaki artış, tümör hücrelerinin ışınlamaya bağlı olarak açığa çıkan serbest radikaller ve oksidatif maddelerden kaynaklanan GSH'e olan ihtiyacının artmasıyla açıklanabilir.*

***Anahtar kelimeler:*** *$^{99m}$Tc-Glutatyon (GSH), osteosarkom, gama radyasyonu, otoradyografi*

## INTRODUCTION

Glutathione (GSH), a natural tripeptide, plays a critical role in detoxification reactions, protecting cells against damage from endogenous and exogenous free radicals, oxidants and other electrophilic substances[1-3]. There is increased demand for GSH in cell injury and cancer[4,5]. GSH has been successfully

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used in the treatment of hepatocellular carcinoma in rats[6]. $^{99m}$Tc labelled glutathione was introduced by our group[7] and successfully used for the demonstration of various tumors and metastases in man[8-12]. The localization mechanism has never been clarified but was attributed to the above mentioned considerations in addition to increased capillary permeability. The localization of $^{99m}$Tc-GSH in inflammatory tissues both in experimental animals[13] and man[14] was also demonstrated where the same mechanisms are in effect. Since free radicals and oxidants are formed as by-products of irradiation it might be possible to show the effect of irradiation of tumor as increased demand for GSH and consequently increased uptake of $^{99m}$Tc-GSH by tumor cells. Therefore, the aim of this study was to find the biodistribution of radiolabelled GSH in experimental animals bearing osteosarcoma tumors and to determine the effect of gamma irradiation of the tumor on tumor uptake of $^{99m}$Tc-GSH and consequently to clarify the localization mechanism(s) if possible.

## MATERIALS AND METHODS

### Radiolabelling of GSH with $^{99m}$Tc

GSH was labelled with $^{99m}$Tc according to a previous procedure[7] : 20 mg glutathione in reduced form (Sigma Chemical Co., USA) was dissolved in 2 ml distilled water. 0.2 mg in 0.2 ml of $SnCl_2.2H_2O$ was added. The pH was adjusted to 7 with 0.5 N NaOH. The mixture was passed through a 0.20 µm membrane filter (cellulose acetate, MFS) into a sterile vial. About 15-20 mCi of $^{99m}$Tc as pertechnetate, in 1-2 ml obtained from a generator (CIS Bio international, France) was added and left to react for 10 min at room temperature.

Chromatographic quality control of the prepared mixtures was carried out using mini-strips (1x10 cm) of ITLC-SG ready plates (Gelman Instruments, USA) with methyl ethyl ketone (MEK) or saline as solvents. The labelling efficiency was calculated by subtracting the percentage of migrated radioactivity in MEK from that in saline. Analytical grade reagents were used throughout.

### Animal experiments

Animal experiments were carried out within the relevant guidelines of the institution.

Biodistribution: Thirty Balb C mice were implanted with osteosarcoma tumor in their thighs. Three weeks were allowed for the growth of tumors (0.5-2 g). They were then injected with 400 µCi of $^{99m}$Tc-GSH in 0.1 ml through the tail vein. They were divided into 2 equal groups. The mice in the first group (control) were sacrificed at 1, 3, and 6 h post-injection (5 mice at each time point). The tumors of mice in the second group were subjected to gamma irradiation for 10 min (20 Gy) immediately following injection, and then sacrificed at the same time points. Planar scintigrams were obtained in mice of both groups and then the mice were dissected. The organs, tumors, some muscle and a blood sample were removed, weighed and assayed for radioactivity against a standard prepared from a 1/100 dilution of the injected solution. % uptake and % uptake/g tissue values were calculated. Tumor/muscle (T/M), tumor/blood (T/B), tumor/liver (T/L), tumor/intestine (T/I) and tumor/kidney (T/K) concentration ratios were also calculated.

$^{99m}$Tc Autoradiography: Tissue samples were obtained from tumor, muscle, and liver at the 3rd and 6th h. They were rapidly frozen and cut into 18 µm sections using a cryomicrotome (Microm HM 500 O). The sections were dried on a hot plate at 40°C for 1 hour. The dry tissue sections were exposed to a phosphorus screen for 5 days. After scanning with a Phosphorus Imager 445 Sl the data were analyzed with the program Image QuaNT (Molecular Dynamics). T/M and T/L ratios were calculated.

## RESULTS

The labelling efficiency of GSH with $^{99m}$Tc was >98%. The tumors were visualized on scintigrams in both groups. In addition, the kidneys and urinary bladder were also visualized, confirming the known biodistribution of $^{99m}$Tc-GSH (Figure 1). In bio-

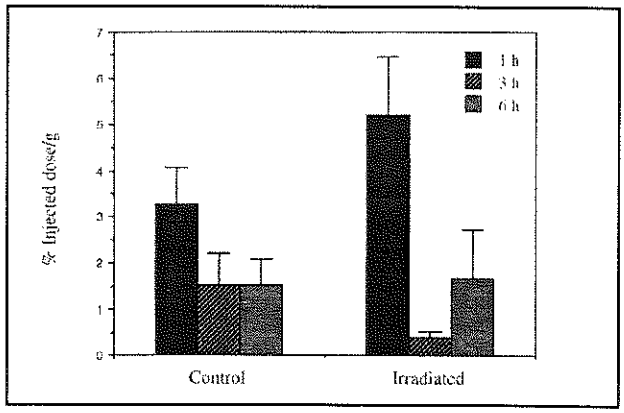


Figure 1. Scintigraphic image of a mouse showing accumulation of $^{99m}$Tc-GSH in the tumor (T), kidneys (K) and the urinary bladder (B) in the control group in the 1st h post-injection. The other structures are not visualized due to much lower radioactivity concentration.

distribution studies the kidneys, the excretory organs, had the highest uptake on %/g basis, followed by tumor and femur compared to other organs and tissues. There was a wash out of radioactivity from the tumor and the femur as time progressed, in parallel with blood clearance by renal excretion. The increased uptake initially observed might be partly due to blood radioactivity. There was an increased uptake/g tissue values in all organs, blood and tumor except kidneys in the irradiated group compared to the control group (Tables 1 and 2). The tumor uptake values in both groups are displayed in Figure 2. There was an increase in the

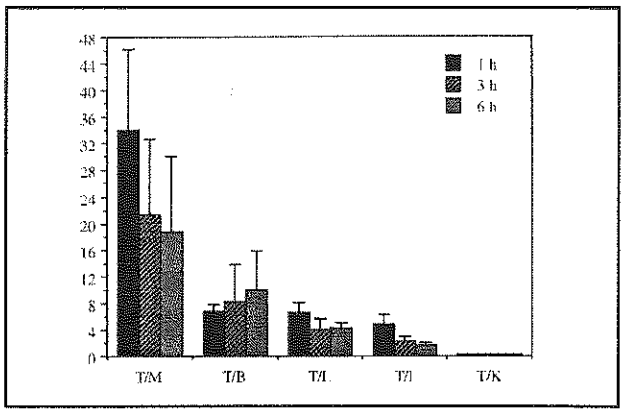


Figure 2. Tumor uptake of $^{99m}$Tc-GSH in control and irradiated groups of mice at times indicated.

1st h and a decrease in the 3rd h in irradiated tumor uptake values, which were significant compared to control values ($p<0.02$). The 6-h value was almost the same as the corresponding control value.

**Table 1.** Biodistribution of $^{99m}$Tc-GSH in mice with osteosarcoma tumor (control group).

| | % injected dose/g tissue* | | |
|---|---|---|---|
| Organ | 1h | 3h | 6h |
| Blood | 0.482±0.037 | 0.218±0.068 | 0.172±0.057 |
| Serum | 0.836±0.114 | 0.327±0.070 | 0.272±0.098 |
| Tumor | 3.27±0.80 | 1.53±0.69 | 1.51±0.55 |
| Heart | 0.199±0.029 | 0.127±0.034 | 0.077±0.018 |
| Lungs | 0.546±0.133 | 0.335±0.089 | 0.293±0.138 |
| Liver | 0.497±0.100 | 0.377±0.031 | 0.359±0.042 |
| Spleen | 0.191±0.036 | 0.127±0.018 | 0.135±0.022 |
| Stomach | 0.347±0.266 | 0.250±0.149 | 0.228±0.072 |
| Intestine | 0.763±0.313 | 0.724±0.057 | 0.906±0.102 |
| Muscle | 0.108±0.018 | 0.094±0.068 | 0.118±0.083 |
| Femur | 1.313±0.245 | 0.527±0.106 | 0.406±0.041 |
| Kidney | 12.6±1.6 | 9.44±2.50 | 10.1±1.4 |

* All values are mean±s.d.

**Table 2.** Biodistribution of $^{99m}$Tc-GSH in mice with osteosarcoma tumor (irradiated group).

| | % injected dose/g tissue* | | |
|---|---|---|---|
| Organ | 1h | 3h | 6h |
| Blood | 1.57±0.40 | 0.497±0.100 | 0.306±0.088 |
| Serum | 2.59±0.64 | 0.836±0.232 | 0.512±0.142 |
| Tumor | 5.18±1.28 | 0.399±0.120 | 1.67±1.05 |
| Heart | 0.488±0.153 | 0.188±0.035 | 0.127±0.008 |
| Lung | 0.372±0.180 | 0.463±0.129 | 0.319±0.012 |
| Liver | 0.946±0.166 | 0.595±0.111 | 0.435±0.030 |
| Spleen | 0.441±0.109 | 0.201±0.032 | 0.156±0.022 |
| Stomach | 0.726±0.129 | 1.04±0.10 | 0.347±0.222 |
| Intestine | 0.834±0.110 | 0.566±0.068 | 0.686±0.137 |
| Muscle | 0.271±0.069 | 0.115±0.30 | 0.069±0.027 |
| Femur | 3.58±1.90 | 1.21±0.14 | 0.492±0.177 |
| Kidney | 10.3±2.0 | 8.56±1.24 | 7.43±1.44 |

* All values are mean±s.d.

T/M, T/B, T/L, T/I, and T/K concentration ratios are shown in Figure 3 for the control (a) and for the irradiated (b) groups. Except for the kidneys, very

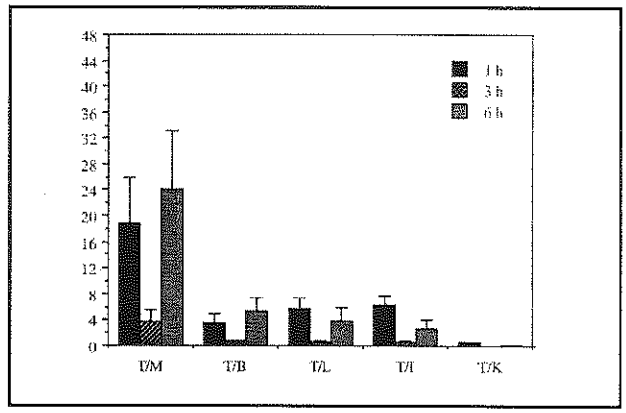


Figure 3. Concentration ratios of $^{99m}$Tc-GSH in contorl (a) and irradiated (b) groups of mice (T: Tumor, M: muscle, B: blood, L: liver, I: intestine and K: kidney).

high concentration ratios are obtained in this experimental tumor model in the control group. The result of irradiating the tumor was decreased T/M rations in the 1st and 3rd h, which returned to the control value at 6th h post-injection. The decrease in the 3rd h was striking in T/B, T/L and T/I ratios as well.

T/M and T/L ratios obtained from autoradiographic studies are shown in Table 3. Gross autoradiography of tumor sections indicated focal sites of increased uptake within tumor tissue compared

to liver and muscle microsections, where the distribution of radioactivity was uniform.

**Table 3.** Tumor/muscle (T/M) and tumor/liver (T/L) concentration ratios obtained from images of $^{99m}$Tc-GSH autoradiography.

| Time | T/M | T/L |
|---|---|---|
| 3h | 8.38±2.20 | 1.55±0.73 |
| 6h | 6.94±1.87 | 0.74±0.34 |

## DISCUSSION

The accumalation of $^{99m}$Tc-GSH in this experimental tumor model was sufficient for scintigraphic visualization (3.27±0.80% injected dose/g tumor in the 1st h) and was higher compared to other organs and tissues except for the kidneys and the urinary bladder. The biodistribution of $^{99m}$Tc-GSH and its excretion via the kidneys confirmed previous experimental[7,13] and clinical[8-12] studies. The urinary bladder can be emptied in man. This offers a distinc advantage for the demonstration of metastases in the abdominal and thoracic regions. In addition to high tumor uptake, it has the advantage of visualizing primary bone tumors such as osteosarcoma against a low blood background without interference from normal bone radioactivity observed with non specific agents such as $^{99m}$Tc-MDP, which localizes, predominantly in bone. The visualization of cardiac blood-pool, the major blood vessels and the nasal region might be due to the plasma protein-bound fraction of $^{99m}$Tc-GSH which was found to be 30%[13]. The wash-out of radioactivity from the tumor and other tissues as time progressed might be a result of clearance of $^{99m}$Tc-GSH blood pool activity not internalized by the cells.

T/M and T/L ratios obtained from autoradiographic studies (Table 3) are lower than the values obtained from biodistribution studies. This might be attributed to the thin section of sampling in autoradiography and non-homogeneous distribution of radioactivity in tumor sections, which was evident in images of the obtained sections. Necrotic areas in tumors did not concentrate $^{99m}$Tc-GSH and appeared as cold areas on images. This problem may be solved by using small tumors (<0.5 g), because the tumor size was large (0.5-2 g) in the present study, by shortening the waiting period for the growth of tumor.

Increased blood flow and capillary permeability is the main underlying localization mechanism in all agents targeting tumor. Tissues demanding high nutrition may display high levels of GSH or GSH-related enzyme activity. GSH levels may parallel blood flow in certain tissues with reduced levels in hypoxic tissues[16]. However, this non-specific mechanism, which was also observed in localization of radiopharmaceuticals in inflammation[13,14], cannot alone account for the very high levels of tumor uptake of $^{99m}$Tc-GSH. The $^{99m}$Tc-GSH uptake of turpentine-induced abscesses and abscess-to-other tissue concentration ratios obtained in mice were considerably lower compared to the results of present investigation[13]. Similarly, in scintigraphic studies in man, the arthritic-to-normal knee uptake ratios[14] were lower compared to the ratios obtained in tumoral and metastatic lesions[8,11,12]. This may indicate that $^{99m}$Tc-GSH is a tumor-avid radiopharmaceutical.

Another mechanism might be easy and rapid diffusion of $^{99m}$Tc-GSH into tumoral tissues, achieving high concentration ratios due to its small molecular size compared to proteins or protein-associated agents such as $^{67}$Ga[17,18]. However, back diffusion is also possible due to the small size of the molecule. The blood clearance and wash-out of $^{99m}$Tc-GSH from tumoral and other tissues was not as fast as the wash-out of other highly water-soluble $^{99m}$Tc complexes such as citrate and DTPA[17,18]. Protein binding at the site of localization might account for the prolonged retention of $^{99m}$Tc-GSH at the target tissue. The protein-bound fraction may also diffuse out of the leaky capillaries, although more slowly than the free $^{99m}$Tc-GSH, and become entrapped at the target site. Back diffusion is retarded because of the large size of the molecule. This may also contribute to the uptake, though to a lesser degree.

GSH takes part in DNA repair, enzyme activity and the protection of cells. It also plays an important role in melanin synthesis. High levels of GSH in human melanoma metastases have been noted in human aspirates[19,20]. Successful scintigraphic visualization of human melanoma metastases with $^{99m}$Tc-GSH can be attributed to increased demand for GSH. In reduced form GSH is used in detoxification processes. It functions through the sulphide group by reducing the oxidants and free radicals[1]. There is obviously more demand for GSH in cell injury and cancer[4,21]

When the tumor was irradiated we observed a significant increase in tumor uptake of $^{99m}$Tc-GSH in the 1 st h post-injection compared to the control group followed by a decrease in the 3rd h and reaching the control value again by the 6th h. This might be due to increased demand of tumor cells for GSH after irradiation, causing the formation of free radicals and oxidants. Here, the question arises as to the biological effectiveness of $^{99m}$Tc-GSH compared to free GSH as a reducing agent. GSH is a good chelating agent, having amine and thiol groups for complexation with metallic ions. It is possible that endogenous GSH forms complexes with metallic ions already present in plasma such as zinc, which is known to make a complex with 2 GSH molecules such as $^{99m}$Tc[15]. In this respect endogenous GSH may not be too different from i.v. administered $^{99m}$Tc-GSH. This point should be further investigated.

In conclusion, $^{99m}$Tc-GSH has the advantages of high target-to-non target ratios and low blood background. Like other peptides, it is accumulated and excreted by the kidneys which are visualized on scintigrams. There is minimal liver uptake and no abdominal, thoracic or bone activity. It is prepared by a simple and rapid procedure with a high labelling efficiency. Limited clinical studies reported with this agent so far indicate its usefulness in tumor diagnosis and follow-up. It is a potential radiopharmaceutical and needs to be further evaluated.

## Acknowledgement

The author is indebted to the Alexander von Humboldt Stiftung for a research fellowship.

## REFERENCES

1. Reed DJ. Regulation of reductive processes by glutathione. *Biochem. Pharmacol.*, 35: 7-13, 1986.
2. Reed DJ. Glutathione: Toxicological implications. *Ann. Rev. Pharmacol. Toxicol.*, 30: 603-631, 1990.
3. Kaplowitz N, Aw TY, Ookhtens M. The regulation of hepatic glutathione. *Ann. Rev. Pharmacol. Toxicol.*, 25: 715-44, 1985.
4. Reed DJ, Fariss MW. Glutathione depletion and susceptibility. *Pharmacol. Rev.*, 36: 25-33, 1984.
5. Meister A, Anderson ME. Glutathione. *Ann. Rev. Biochem.*, 52: 711-60. 1983.
6. Novi AM. Regression of aflatoxin B1-increased hepatocellular carcinomas by reduced glutathione. *Science*, 212: 541-542, 1981.
7. Ercan MT, Bekdik CF, Şarizi T. $^{99m}$Tc-glutathione: labelling and distribution in rats. *Int. J. Appl. Rad. Isotopes.*, 29: 697-698, 1978.
8. Ercan MT, Aras T, Aktaş A et al. Accumulation of $^{99m}$TC-glutathione in head and neck tumors. *Nucl-Med.*, 33, 224-228, 1994.
9. Duman Y, Burak Z, Ercan MT et al. Clinical evaluation of metastases of malignant melanoma imaging with Tc-99(m)-glutathione and Tc-99(m)-melanoma antibody: A comparative study. *Nucl. Med. Commun.*, 16: 927-935, 1995.
10. Tutuş A, Erkılıç K, Ercan MT et al. Scintigraphic visualization of ocular melanoma with $^{99m}$Tc-glutathione. *Ann. Nucl. Med.*, 11, 143-146, 1997.
11. Burak Z, Duman Y, Sabah D et al. Clinical value of $^{99m}$Tc-glutathione (GSH) in diagnosis and follow-up of mesenchymal tumors. *Eur. J. Nucl. Med.*, 21: S116, 1994.
12. Duman Y, Kumanlıoğlu K, Özcan Z et al. A comparative study with $^{99m}$Tc-glutathione (GSH) and Tl-201 in the evaluation of osteosarcoma. *Eur. J. Nucl. Med.*, 22: 822, 1995.
13. Ercan MT, Ünlenen E, Aktaş A. Technetium-99m glutathione for imaging inflammatory lesions. *Nucl. Med. Commun.*, 15: 533-9, 1994.
14. Ercan MT- Ünlü M, İnanır S et al. $^{99m}$Tc-glutathione for imaging arthritic joints in man. *T.J.N.M.*, 4: 6-10, 1995.
15. Ercan MT. $^{65}$Zn-glutathione: Biodistribution and effect of carrier zinc on pancreas uptake. *Int. J. Appl. Radiat. Isot.*, 32: 417-421, 1981.
16. Babich JW. $^{99m}$Tc HMPAO retention and the role of glutathione: The debate continues. *J. Nucl. Med.*, 9: 1681-1683, 1991.
17. Ercan MT, Ünlenen E. Accumulation of some small molecular weight complexes of $^{99m}$Tc in experimental abscesses. *Nucl. Med. Biol.*, 21: 143-149, 1994.
18. Ercan MT, Aras A, Ünlenen E et al. $^{99m}$Tc-citrate versus $^{67}$Ga-citrate for the scintigraphic visualization of inflammatory lesions. *Nucl. Med. Biol.*, 20: 881-887, 1993.
19. Coates A, Tripp E. Glutathione content of human malignant melanoma cell lines: Correlation of flow cytometric and biochemical assays and application to human tumor aspirates. *Melanoma Res.*, 1: 327-332, 1992.
20. Mannarvik B, Castro VM, Danielson VH et al. Expression of class Pi glutathione transferase in human malignant melanoma cells. *Carcinogenesis*, 8: 1929-1932, 1987.
21. Estrela JM, Hernandez R, Terradez P et al. Regulation of glutathione metabolism in Ehrlich ascites tumour cells. *Biochem. J.*, 286 (Part 1): 257-262, 1993.