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The Effects of ^{99m}Technetium-methylendiphosphonate and ^{99m}Technetium-methoxyisobutylisonitrile on Erythrocyte Antioxidant Enzyme Activities

[^{99m}Teknesyum-metilendifosfonat ve ^{99m}Teknesyum-metoksiizobutilizonitril'in Eritrosit Antioksidan Enzim Aktiviteleri Üzerine Etkileri]

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ABSTRACT

Purpose: To investigate the effects of ^{99m} Technetium-methylendiphosphonate and ^{99m}Technetium-methoxyisobutylisonitrile, which are used for scanning of patients with bone metastasis and coronary artery disease, respectively, on antioxidant enzyme activities in erythrocytes.

Methods: Blood samples were collected from patients with coronary artery disease (n=20) at four different times that are just before (I) and 1h (II), 3h (III), 24h (IV) after administration of ^{99m}Technetium- methoxyisobutylisonitrile and from patients with breast cancer (n=20) at two different times that are just before (I) and 1h (II) after administration of ^{99m}Technetium-methylendiphosphonate in dose used clinically, 20 mCi/patient, before scintigraphy. Selenium dependent glutathione peroxidase, copper/zinc superoxide dismutase and catalase activities were determined in all samples to evaluate ^{99m}Technetium-methylendiphosphonate / ^{99m}Technetium-methoxyisobutylisonitrile induced alteration of antioxidant capacity in erythrocytes.

Results: Based on our findings, both ^{99m}Technetium labeled agents led to decrease copper/zinc superoxide dismutase and catalase activities within 1h after injection when compared to the uninjected condition. Contrary to the decrease of selenium dependent glutathione peroxidase activity in patients administered with ^{99m}Technetium-methoxyisobutylisonitrile, this enzyme activity increased in the ^{99m}Technetium-methylendiphosphonate group.

Conclusion: It is marked that the altered activities in all three antioxidant enzymes returned to the baseline 24h after injection of ^{99m}Technetium-methoxyisobutylisonitrile in clinical dose that suggests reversible enzyme inhibition.

Key words: gamma radiation, ^{99m}Technetium, scintigraphy, oxidative stress, antioxidant enzyme, erythrocyte

ÖZET

Amaç: Kemik metastazı gelişmiş olan hastaların ve koroner arter hastalarının taranması amacıyla kullanılan sırasıyla ^{99m}Teknesyum-metilendifosfonat ve ^{99m}Teknesyum-metoksiizobutilizonitril'in, eritrosit antioksidan enzim aktivitesi üzerindeki etkilerinin incelenmesi amaçlandı.

Yöntem: Sintigrafi öncesinde; koroner arter hastalarından (n=20), klinik kullanım dozu olan 20mCi ^{99m}Teknesyum-metoksiizobutilizonitril verilmeden hemen önce (I) ve verildikten 1 saat (II), 3 saat (III), 24 saat (IV) sonra olmak üzere dört farklı zamanda ve meme kanseri hastalarından (n=20) ise 20mCi ^{99m}Teknesyum- metilendifosfonat verilmeden hemen önce (I) ve verildikten 1 saat (II) sonra olmak üzere iki farklı zamanda kan örnekleri alındı. ^{99m}Teknesyum-metilendifosfonat veya ^{99m}Teknesyum-metoksiizobutilizonitril ile uyarım sonucunda eritrosit antioksidan kapasitesindeki değişikliğin değerlendirilmesi için, selenyum bağımlı glutatyon peroksidaz, bakır/çinko süperoksit dismutaz ve katalaz aktiviteleri tüm örneklerde ölçüldü.

Bulgular: Bulgularımıza göre; her iki ^{99m}Teknesyum işaretli ajan, enjeksiyon öncesindeki şartlar ile kıyaslandığında enjeksiyondan 1 saat sonra bakır/çinko süperoksit dismutaz ve katalaz aktivitelerinde bir azalmaya neden oldu. ^{99m}Teknesyum-metoksiizobutilizonitril enjekte edilen hastalarda, selenyum bağımlı glutatyon peroksidaz aktivitesinde bir azalma gözlenmesine karşılık, bu enzim aktivitesi ^{99m}Teknesyum-metilendifosfonat grubunda yükseldi.

Sonuçlar: Bütün bu sonuçlar; tüm üç antioksidan enzimin değişen aktivitelerinin, ^{99m}Teknesyum-metoksiizobutilizonitril enjeksiyonundan 24 saat sonra bazal düzeye geri döndüğünü açıkça göstermektedir. Bu ise, geri dönüşümlü bir enzim inhibisyonu ile ilişkili olduğunu kuvvetle muhtemel kılmaktadır.

Anahtar Kelimeler: Gama radyasyonu, ^{99m}Teknesyum, Sintigrafi, Oksidatif stres, Antioksidan enzim, Eritrosit

Introduction

^{99m}Technetium is widely used in medicine, comprising 85% of all nuclear medicine procedures and has ideal characteristics for nuclear scan. The main advantage of this radioisotope is that it has a half-life of six hours which is long enough to examine metabolic processes yet short enough to minimize the radiation dose to the patient (1). ^{99m}Technetium labeled agents have been used to image the skeleton, brain, thyroid, lungs, liver, spleen, kidneys, bone marrow, salivary glands, heart, blood pool, infections and numerous specialized medical studies (1).

^{99m}Technetium-methylendiphosphonate (^{99m}Tc-MDP) and ^{99m}Technetium-methoxyisobutylisonitrile (^{99m}Tc-MIBI also called ^{99m}Tc-sestamibi) are two of the most common agents examined in the present study. ^{99m}Tc-MDP is a gamma ray emitting radioactive substance, which is administered intravenously to the patients for bone scintigraphy (2). On the other hand, ^{99m}Tc-MIBI is a lipophilic cationic complex that has been approved as a tracer to image the myocardial perfusion (3, 4). It distributes in the myocardium proportionally to the myocardial blood flow although ^{99m}Tc-MIBI does not undergo significant redistribution. Single photon emission computed tomography (SPECT) imaging of the heart is performed using a gamma camera to detect the gamma rays emitted by the ^{99m}Tc-MIBI as it decays.

In contrast to importance of technetium as radiopharmaceuticals in diagnosis, the agents labeled with technetium disrupt the cells due to gamma irradiation. The doses and duration of absorbed irradiation and the susceptibility of the tissue against irradiation are the factors that cause variations on living cells (5). Ionizing radiation is known to generate reactive oxygen species (ROS) in irradiated tissues (6). These free radicals react with cellular macromolecules, such as DNA, RNA, proteins, membranes, etc., and cause cell dysfunction and mortality. These reactions take place in tumor cells as well as normal cells when exposed to radiation (7,8). However, there are special defense systems in aerobic cells to regulate the influx of ROS. One of them is nonenzymatic compounds such as low-molecular-weight antioxidant molecules, reduced glutathione (GSH), and the other is enzymatic mechanisms such as copper/zinc superoxide dismutase (Copper/zinc superoxide), catalase (CAT) and selenium-dependent glutathione peroxidase (Se-GSH-Px). Copper/zinc superoxide is the first line of defense against oxygen-derived free radicals and catalyses the dismutation of superoxide anion radical ($O_2^{\cdot -}$) into hydrogen peroxide (H_2O_2). H_2O_2 can be transformed into H_2O and O_2 by CAT. Se-GSH-Px reduces lipid or nonlipid hydroperoxides as well as H_2O_2 while oxidizing GSH. Oxidized glutathione (GSSG) is reduced back to GSH by glutathione reductase in the presence of NADPH (9,10).

^{99m}Tc-MDP and ^{99m}Tc-MIBI were injected intravenously to patients with bone metastasis and coronary artery disease, respectively, for scintigraphy. To our knowledge, the present study is the first investigation in the literature to evaluate the antioxidant conditions against oxidative stress occurred by technetium labeled agents, ^{99m}Tc-MDP and ^{99m}Tc-MIBI. Thus, aim of this study was to determine the alterations in the activities of erythrocyte antioxidant enzymes (Cu/Zn-SOD, CAT, GSH-Px) caused by ^{99m}Tc-MDP and ^{99m}Tc-MIBI in the clinically used dose in patients with breast cancer and with coronary artery disease.

Materials And Methods

Study population

Patients (n=40), who underwent scintigraphy in Nuclear Medicine Clinic at the Akdeniz University Hospital within one year were chosen as the study group. They were divided into two subgroups based on the type of disease and technetium labeled agent. 20 patients with known breast cancer and 20 patients with known coronary artery disease were examined using a ^{99m}Tc-MDP and ^{99m}Tc-MIBI protocol, respectively. All the patients referred to our institution for routine myocardial imaging were eligible. The patients were aged between 40 and 70 years (62 ± 11.3 years). Written informed consents were taken from all subjects. The blood samples were collected from coronary artery patients just before and 1h, 3h, 24h after injection of ^{99m}Tc-MIBI, and from breast cancer patients just before and 1h after ^{99m}Tc-MDP administration of clinical doses of 20 mCi. According to the procedure of ^{99m}Tc-MIBI sintigraphy, coronary artery patients were taken under examination again after 24 h. However, breast cancer patients were not examined 3 h and 24 h after administration of ^{99m}Tc-MDP due to problems in recruiting these patients.

Blood samples and erythrocyte separation

Blood samples were obtained by venipuncture, collected in vacutainers containing sodium heparin (15 IU/ml). Plasma and buffy coat were removed followed by centrifugation at 3000 x g for 3 minutes. Erythrocytes were washed three times with 0.9% NaCl solution and hemolysed by adding cold distilled water. The enzyme activities of Cu/Zn-SOD, CAT and Se-GSH-Px were assessed in these hemolysates.

Biochemical analysis in erythrocytes

Hemoglobin concentrations of the hemolysates were determined by using the cyanomethemoglobin method (11). Cu/Zn-SOD, CAT and Se-GSH-Px activities were measured by a spectrophotometer (Shimadzu UV-1601-Japan).

Cu/Zn-SOD activity

Misra and Fridovich (12) method was used to determine the activity of Cu/Zn-SOD (EC 1.15.1.1), which is based

on the inhibition of adrenaline auto-oxidation reaction in an alkaline medium by the enzyme. Cu/Zn-SOD activities were expressed as units/g of hemoglobin (one unit, U = the amount of the sample which inhibits the transformation of adrenaline into adrenochrome by 50%).

Se-GSH-Px activity

The quantification of Se-GSH-Px (EC 1.11.1.9) activity was performed by the method of Paglia and Valentine (13), which is based on the oxidation of glutathione by cumene hydroperoxide through Se-GSH-Px in the presence of glutathione reductase and NADPH. Oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^{+}$. The decrease in NADPH was recorded at 340 nm and the molar absorptivity of NADPH, 6.22×10^{-3} L mol^{-1} cm^{-1} was used to calculate the enzyme activity. Se-GSH-Px activity was expressed as units/g of hemoglobin (one unit, U = 1 μmol of NADPH transformed / min).

CAT activity

CAT (EC 1.11.1.6) activity was determined according to the method of Aebi (14). The principle of the assay is based on the determination of the rate constant (s−1, k) of H_2O_2 decomposition by CAT. The rate constant was calculated by the following formula: k(2.3/8t)(a/b) log(A1/A2). In this formula, A_1 and A_2 are the absorbance values of H_2O_2 at t_1 (0^{th} s) and t_2 (15^{th} s), *a* is the dilution factor, *b* is the hemoglobin content of erythrocytes. CAT activities were expressed as k/g of hemoglobin (k: rate constant of the first order reaction).

Statistical analysis

Data were analyzed using the statistical package SPSS for Windows version 14.0 (SPSS Inc., Chigago, IL.). Results were expressed as mean ± standard error (SE). Statistical comparisons between groups were performed by paired samples test and p values <0.05 were accepted as statistically significant.

Results

The specific activities of erythrocyte Cu/Zn-SOD, CAT, Se-GSH-Px in patients treated with ^{99m}Tc-MIBI for scanning of coronary artery disease by scintigraphy were summarized in Table 1. Additionally, same enzyme activities in patients with breast cancer treated with ^{99m}Tc-MDP for bone scintigraphy were represented in Table 2. Based on our data, all three antioxidant enzyme activities were decreased after ^{99m}Tc-MIBI treatment within 1h and 3h. Although the decrease in enzyme activity was significant for CAT ($p<0.01$) and Se-GSH-PX ($p<0.01$), it was not significant for Cu/Zn-SOD. However, it is marked that the loss of antioxidant activities was returned to baseline within 24h after ^{99m}Tc-MIBI treatment. Parallel to the data of ^{99m}Tc-MIBI group, ^{99m}Tc-MDP leads to a decrease in Cu/Zn-SOD and CAT activities, but an increase in Se-GSH-Px. CAT activities reduced significantly after Tc-99m-MDP administration ($p<0.01$) similar to the ^{99m}Tc-MIBI group. However, the decline of Cu/Zn-SOD activities was not significant neither in ^{99m}Tc-MDP nor ^{99m}Tc-MIBI treatment groups. In contrast to the data of ^{99m}Tc-MIBI group, enhanced Se-GSH-Px activities were obtained in the patient group administered with Tc-99m-MDP ($p<0.01$).

Discussion

^{99m}Tc-MDP and ^{99m}Tc-MIBI-induced alteration of antioxidant capacity in erythrocytes was evaluated in patients with breast cancer and coronary artery disease, who were scanned by scintigraphy. Phosphate analogues such as MDP can be labeled with ^{99m}Tc and used for bone imaging because of their good localization in the skeleton and rapid clearance from soft tissues (15). ^{99m}Tc-MDP is concentrated predominantly in the mineral phase of the bone, which consists of crystalline hydroxyapatite and amorphous calcium phosphate. Using an in vitro assay, the competitive adsorption of ^{99m}Tc-MDP to pure inorganic hydroxyapatite has been 40 times that to pure organic bone matrix (16). ^{99m}Tc-MIBI accumulation in the cell also is related to cell membrane potential, and passage through this membrane is thought to involve passive diffusion (17,18). The uptake of MIBI tracers is known to be partly related to the Na^{+}/H^{+} antiporter system within the cell membrane (17). It is thought that either ^{99m}Tc-MIBI behaves like Na^{+} or its uptake depends on the intracellular concentration of Na^{+} (19). ^{99m}Tc-MIBl is localized mostly inside mitochondria because of the negative mitochondrial membrane potential (20). Both ^{99m}Tc-MDP and ^{99m}Tc-MIBI are accumulated in the cell and this state is a common special feature for these agents.

Based on our findings, it can clearly be said that these agents cause antioxidant enzyme inhibition and do not cause any oxidative stress within the erythrocytes. When we investigated the effects of ^{99m}Tc-MIBI on antioxidant enzyme activities, a decline in Cu/Zn-SOD, CAT and Se-GSH-Px activity was obtained within 1h and 3h after intravenous administration, which was only significantly for CAT ($p<0.01$) and Se-GSH-Px ($p<0.01$) activities. This reduction may result from the inhibition of enzymes by the interaction between ^{99m}Tc-MIBI and their active sites. Annad et al. (21) reported that gamma radiation causes hemoglobin oxidation due to damage to the porphyrin ring. Therefore the decreased CAT activity may be related to the changes in the porphyrin ring of enzyme structure due to ^{99m}Tc-MIBI. However, it is marked that the decreased activity of the three enzymes 1h and 3h after ^{99m}Tc-MIBI treatment were significantly returned to baseline levels in 24h, when compared to the non-treated conditions ($p>0.05$). These findings suggest an inhibitory effect of ^{99m}Tc-MIBI on Cu/Zn-SOD, CAT and Se-GSH-PX activities which was reversible in erythrocytes.

In addition to ^{99m}Tc-MIBI, it was shown that ^{99m}Tc-MDP led to changes in Cu/Zn-SOD, CAT and Se-GSH-Px activities in erythrocytes of breast cancer patients 1h after treatment. These changes were determined as a decrease in Cu/Zn-SOD, CAT ($p<0.01$) and as an increase in Se-GSH-Px ($p<0.01$). The decrease in Cu/Zn-SOD was not significant, similar to the findings in ^{99m}Tc-MIBI group. However, the stimulation in Se-GSH-Px activity was found to be significant ($p<0.01$). The reason cannot be an increase in its own substrate, $O_2^{\cdot-}$, since no increase in Cu/Zn-SOD activity is responsible of generating $O_2^{\cdot-}$. So it strongly seems that there is a specific activation of Se-GSH-Px by treatment of ^{99m}Tc-MDP, contrary to the decreases in Cu/Zn-SOD and CAT activities. However, this activation does not result from the gene induction since the erythrocytes do not contain a nucleus. ^{99m}Tc-MDP may directly combine with the active site of enzyme and play an activator role in Se-GSH-Px activity.

To our knowledge, there are no reports concerning the effects of ^{99m}Tc-MDP or ^{99m}Tc-MIBI on antioxidant enzyme activities in erythrocytes. Thus, we unfortunately did not have a chance to compare our findings with any previous data. However, we discussed our findings based on such investigations involving ionizing radiation and antioxidant activities. The results of these studies concerning the decrease of the three antioxidant enzymes support our findings (5,22,23,24,25,26). Cicek et al. (5) reported that erythrocyte Cu/Zn-SOD, CAT and Se-GSH-Px activities in patients having thyroid scintigraphy reduced 1h after injection of ^{99m}Tc-pertechnetate, but was only significantly in CAT. Mansour (7), Groen et al. (23), Kaya et al (24), Nikishkin et al. (25) and Sabitha & Shyamaledevi (26) confirmed the decreases in antioxidant enzymes after irradiation in erythrocytes. However, we observed that the loss of enzyme activity induced by ^{99m}Tc-MDP / ^{99m}Tc-MIBI resulted from antioxidant enzyme deficiencies because the altered enzyme activities were increased to baseline levels in sample with 24h group after injection. That's why, that seems strongly related to reversible enzyme inhibition.

The results obtained in this investigation strongly supports other findings that ionizing radiation induces the decrease of antioxidant enzyme activities, Cu/Zn-SOD, CAT, Se-GSH-Px, in erythrocytes. However, this is the first report to investigate the effects of two technetium labeled agents widely used in clinical screening for patients with bone metastasis and coronary artery disease, ^{99m}Tc-MDP and ^{99m}Tc-MIBI, respectively. And it has been firstly reported that effects of ^{99m}Tc-MIBI on antioxidant enzymes were reversible because altered activities were returned to baseline levels after 24h of ^{99m}Tc-MIBI injection.

Table 1. Effects of 99mTc-MIBI administration on Cu/Zn-SOD, CAT, Se-GSH-Px activities in erythrocytes of patients with coronary artery disease.

	Cu/Zn-SOD	CAT	Se-GSH-Px
	(U/g Hb)	(k/g Hb)	(U/g Hb)
Before 99mTc-MIBI administration	742 ± 66.2	408 ± 25	1.73 ± 0.12
1 h after administration	667 ± 31.7	318 ± 18.8[a]	1.45 ± 0.15[a]
3 h after administration	652 ± 29	344 ± 21.7[a,b]	1.46 ± 0.12[a]
24 h after administration	781 ± 34.2	442 ± 17.1	1.76 ± 0.09

[a] $p<0.01$ compared to before 99mTc-MIBI administration group.
[b] $p<0.01$ compared to 1 hour after 99mTc-MIBI administration group.

Table 2. Effects of 99mTc-MDP administration on Cu/Zn-SOD, CAT, Se-GSH-Px activities in erythrocytes of patients with breast cancer.

	Cu/Zn-SOD	CAT	Se-GSH-Px
	(U/g Hb)	(k/g Hb)	(U/g Hb)
Before 99mTc-MIBI administration	1259 ± 58.4	137 ± 20.5	1.29 ± 0.14
1 h after administration	1207 ± 55.8	110 ± 16[a]	1.68 ± 0.18[a]

[a] $p<0.01$ compared to before 99mTc-MDP administration group.

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