Effects of 99mTc sestamibi on antioxidant defense system and lipid peroxidation in the heart of Sprague Dawley

Gokhan Cesur¹, Duygu Kumbul Doguc², Mustafa Yildiz³, Serdal Ogut⁴, Mumin Polat⁴ and Kurtulus Ongel⁵

Abstract
Nuclear medicine has been using radiopharmaceuticals for the diagnostic and therapeutic purposes of many diseases. Technetium-99m methoxyisobutylisonitrile (99mTc sestamibi) is a lipophilic complex that has a positive-loaded isonitril group. Aim of the study is to investigate whether 99mTc sestamibi, which is one of the mostly used radiopharmaceuticals in nuclear medicine field, causes oxidative damage or not in rats’ heart after an injection. A total of 16 male Sprague Dawley rats were randomly divided into two groups: group I: 99mTc sestamibi group, 99mTc sestamibi administered intravenously with the dose of 25MBq; group II: control group, one dose of isotonic sodium chloride was administered intravenous with the same volume as 99mTc sestamibi group. Malondialdehyde (MDA) and total oxidant status (TOS) were used as markers of oxidative stress-induced heart impairment. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and total antioxidant status (TAS) activities were studied to evaluate the changes in the antioxidant status. In the 99mTc sestamibi group (group I), animals treated with 99mTc sestamibi produced a significant decrease in the activities of antioxidant enzymes (SOD and CAT), while MDA level increased when compared with control group (group II) in myocardial tissue (p < 0.05). On the other hand, the GSH-Px activities were significantly increased in the 99mTc sestamibi-treated rats compared with the untreated rats (p < 0.05). There was no significant difference in the TAS and TOS levels of plasma.

Keywords
99mTc sestamibi, antioxidant defense system, lipid peroxidation, heart, Sprague Dawley

Introduction
Nuclear medicine has been using radiopharmaceuticals for the diagnostic and therapeutic purposes of many diseases. The technetium radiopharmaceuticals have been used for imaging many organs like liver, heart, thyroid, kidney and bones. They were developed by the help of some physiological properties such as adsorption, distribution, metabolism and excretion of various technetium-99m (99mTc) complexes.

99mTc methoxyisobutylisonitrile (99mTc sestamibi) is a lipophilic complex that has a positive loaded isonitril group. 99mTc sestamibi is prepared from a kit and requires boiling to affect labeling. Myocardial holding is related to the blood flow.

¹Department of Physiology, Faculty of Medicine, Adnan Menderes University, Aydin, Turkey
²Department of Biochemistry, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey
³Department of Nuclear Medicine, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey
⁴Blood Bank, Suleyman Demirel University Hospital, Isparta, Turkey
⁵Department of Family Medicine, Izmir Tepecik Research and Implementation Hospital, Izmir, Turkey

Corresponding author:
Gokhan Cesur, Department of Physiology, School of Medicine, Adnan Menderes University, Aydin 09100, Turkey
Email: gokhancesur@hotmail.com
Myocardial cleaning is very slow and almost there is no redistribution after 3–4 h from the injection. It accumulates in the mitochondria of the cell (Lomonte et al., 2006). 99mTc sestamibi diffuses passively out of the blood and apparently localizes in the mitochondria on the basis of their negative electrical potentials (Beller et al., 1993).

99mTc sestamibi is mostly used for the clinical imaging of the myocardium (Hatada et al., 2004). The agent has some advantages; there is a limited washout from the myocardium over a 4-h period. Moreover, it is rapidly cleared from the blood. Initially, Tc-99m sestamibi was thought to bind with high affinity to a cytosolic protein moiety. However, a source of error was proposed in the subcellular fractionation technique, and a direct analysis of heart cell aggregates has indicated specific mitochondrial retention (Crane et al., 1993).

Oxidative stress is thought to play an important role in the tissue injury by many mechanisms like ischemia, reperfusion, inflammation, aging and various diseases. It is a reaction process of biomolecules with O₂ or reactive oxygen species (ROS) (Sasaki et al., 1996). Under normal conditions, there is a balance between ROS generated and the antioxidants present. ROS generated are detoxified by the antioxidants present in the body (Sun, 1990). ROS including superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (OH⁻) and peroxynitrite (ONOO⁻) acts as subcellular messengers in such complex processes, such as mitogenic signal transduction, gene expression and regulation of cell proliferation, when they are generated excessively or when enzymatic and nonenzymatic defense systems are impaired (Halliwell and Gutteridge, 1999). The targets for these highly reactive free radicals include proteins, lipids, carbohydrates and nucleic acids. Antioxidants can offer electrous that stabilize radical molecules or regulate reactions that convert ROS into less destructive forms. The cell contains oxygen radical scavenger systems. The oxygen radical scavenger mechanisms consist of scavenger enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) (Halliwell and Gutteridge, 1990).

Aim of this study is to investigate whether 99mTc sestamibi, which is one of the mostly used radiopharmaceuticals in nuclear medicine field, causes oxidative damage or not in rats’ heart after an injection.

Material and methods

Animal model

The study was approved by the Institutional Review for Animal Research Board of Suleyman Demirel University and conducted in accordance with the institutional guidelines. The Medical Faculty Experimentation Ethics Committee approved the experimental procedures of the study (ethical committee approval number: 05.06.2010/02). The experiment was designed to obtain reliable data with minimum number of animals. A total of 16 male Sprague Dawley rats of approximately 5 months old, weighing 150–200 g were used in the experiments. The animals had tap water and normal rat chow available ad libitum. Rats were randomly divided into two groups; group I: Tc-99m sestamibi group (n = 8), 99mTc sestamibi administered intravenously with the dose of 25 MBq; group II: control (sham) group (n = 8), one dose of isotonic sodium chloride was administered intravenous with the same volume as 99mTc sestamibi group.

Preparation of 99mTc-MIBI

99mTc, as sodium pertechnetate, was milked from a 99Mo/99mTc generator (Monrol AS, Istanbul, Turkey) just before radiolabeling procedure. MIBI (Cardio-Spect, Medi-Radiopharma Ltd, Budapest, Hungary) was purchased as freeze-dried commercial kit containing methoxyisobutylisonitrile, 0.12 mg. 99mTc sestamibi was prepared by adding 25 MBq of 99mTc pertechnetate in 3 mL saline to the kit and boiled for 10 min. Quality control procedures were performed with this mixture according to the manufacturer’s instructions after cooling it to room temperature. Labeling efficiency was greater than 95%.

Experimental protocol

Rats were anesthetized with a cocktail of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally. Under anesthesia, each rat received a bolus intravenous injection of 99mTc sestamibi (25 MBq) as a reference agent through the tail vein (Tsopelas et al., 2006). Rats were killed by dissection 60 min after the injection of radiopharmaceutical. Blood and heart samples were collected through a cardiac puncture.
Biochemical determinations

At the end of the study, heart tissue of the decapite rats were collected in vitreous tubes fully filled with phosphate tampon at pH 7.4. Each rat tissue was weighed and diluted 10 times with phosphate tamponate at pH 7.4. Homogenization was performed by Janke U Kunkel Ultraturrax T 25™ (Germany) tissue dividing machine and UW-2070 Bandeun Electronic™ (Germany) sonicator. Tissue specimen was centrifuged for 15 min at 5000 r/min. with Eppendorf 5415-R (Germany) centrifugal and specimen was taken into the eppendorf tubes after supernatants were taken away. Protein indication was carried out in homogenized specimen supernatants with Lowry method (Van kampfen and Zijlstra, 1965). Later, tissue homogenates were reserved and stored at −80°C. Before each parameter, specimens were taken out and untied one by one.

Determination of lipid peroxidation

To measure malondialdehyde (MDA), one of the lipid peroxidation products, Draper and Hadley (1990) double heating method was used. The principle of the method depends on the measurements of the absorbance of MDA-thiobarbituric acid complex at 532 nm. Results were given as nanomoles per gram.

Determination of SOD activity

SOD activity was measured with spectrophotometric method by the help of Randox commercial kit. Hypoxanthine/xanthine is catalyzed to superoxide radical by xanthine oxidase. Formed superoxide radical react with INT (2-(4-iodophenyl)-3-(4-nitrophenol)-5 phenyl tetrazolium chloride to form a red coloured formazon compound. SOD activity is measured according to this reaction’s inhibition degree. SOD activity is defined as U/gr. protein. (Woolliams et al., 1983).

Determination of GSH-Px activity

GSH-Px activity was measured with spectrophotometric method by the help of Randox commercial kit. GSH-Px catalyzes glutathione by oxidation in the presence of abundant hydroperoxide. In the presence of glutathione reductase and NADPH, oxide glutathione turns to reducte glutathione and NADPH turns to NADP⁺. Decrease in the absorbance of NADPH at 340 nm can be measured spectrophotometrically. Determination of GSH-Px activity was explained in different studies (Paglia and Valentine, 1967; Woolliams et al., 1983).

Determination of CAT activity

CAT activity was detected according to Aebi method (Aebi, 1984). CAT catalyzes the deterioration of H₂O₂ to water and molecular oxygen. In the study, CAT activity was detected spectrophotometrically at 240 nm, according to the decrease in H₂O₂ concentration during a unique time. CAT activities were detected by UV spectrophotometric method, which depends on the breaking into pieces theory by CAT. CAT activity expressed as kilounits per gram protein.

Measurement of the total oxidant and antioxidant status

Total antioxidant status (TAS) and total oxidant status (TOS) levels were measured spectrophotometrically using a commercial kit (Rel Assay Diagnostics, Gaziantep, Turkey) by the Erel methods (Erel, 2004, 2005). These methods are automatic and colorimetric. The TAS measurement method is based on the bleaching of the characteristic color of a more stable 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation by antioxidants. The TOS method is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in an acidic medium and the measurement of the ferric ion by xylanol orange. The TAS and TOS results were expressed in millimoles of trolox equivalent per liter and micromoles of H₂O₂ per liter, respectively, and the precision error of this assay is lower than 3%.

Data analysis

All data were statistically analyzed with commercially available statistics software package (SPSS for Windows v. 16.0, Chicago, IL, USA). Data are expressed as minimum, maximum, median (range) and SD. To compare values for oxidative stress tests, Mann-Whitney U test was used. p < 0.01 was considered statistically significant. Moreover, Somers’D test was used to detect the direction of the meaningful relations.

Results

The results presented in Tables 1 and 2 indicate the evidence of the degree of MDA, TAS, TOS and antioxidant enzymatic changes in cardiac tissue and plasma in experimental groups. The mean MDA, CAT, SOD and GSH-Px values in both the groups are shown in Table 1. In the 99mTc sestamibi group, animals treated with 99mTc sestamibi produced a
significant decrease in the activities of antioxidant enzymes (SOD and CAT) in myocardial tissue, while MDA level increased when compared with control group.

The level of MDA in the heart was increased in 99mTc sestamibi-treated rats compared with the rats of control group \((p < 0.01)\). CAT levels were decreased in the 99mTc sestamibi-treated group compared with the control group \((p < 0.01)\). In the 99mTc sestamibi-treated rats, the SOD activities were significantly lower than in the control group \((p < 0.01)\). On the other hand, the GSH-Px activities were significantly \((p < 0.01)\) increased in the 99mTc sestamibi-treated rats when compared with the untreated rats.

The mean values of TAS, TOS and oxidative stress index (OSI) in plasma in both the groups are shown in Table 2. There were no significant difference in the levels of plasma TAS and TOS between the 99mTc sestamibi-treated rats and untreated rats. But, OSI was increased in 99mTc sestamibi-treated rats when compared with control rats \((p < 0.01)\).

### Table 1. MDA levels and the activities of antioxidant enzymes in heart tissue in control and experimental groups (values are given as mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Control group ((n = 8))</th>
<th>Experimental group ((n = 8))</th>
<th>(p) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g)</td>
<td>2.42 ± 1.013</td>
<td>5.85 ± 1.38</td>
<td>0.003</td>
</tr>
<tr>
<td>CAT (kU/g)</td>
<td>19.64 ± 5.32</td>
<td>7.87 ± 3.57</td>
<td>0.001</td>
</tr>
<tr>
<td>SOD (U/g)</td>
<td>9.05 ± 91.18</td>
<td>6.54 ± 115.38</td>
<td>0.004</td>
</tr>
<tr>
<td>GSH-Px (U/g)</td>
<td>1.36 ± 426.69</td>
<td>7.14 ± 208.59</td>
<td>0.011</td>
</tr>
</tbody>
</table>

MDA: malondialdehyde; CAT: catalase; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase.

### Table 2. TOS, TAS and OSI in plasma in control and experimental groups (values are given as mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Control group ((n = 8))</th>
<th>Experimental group ((n = 8))</th>
<th>(p) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (mmol trolox equivalent/L)</td>
<td>1.49 ± 0.06</td>
<td>1.44 ± 0.07</td>
<td>0.141</td>
</tr>
<tr>
<td>TOS (μmol H(_2)O(_2)/L)</td>
<td>1.43 ± 0.05</td>
<td>1.47 ± 0.06</td>
<td>0.247</td>
</tr>
<tr>
<td>OSI (TOS/TAS × 10)</td>
<td>0.096 ± 0.04</td>
<td>0.103 ± 0.02</td>
<td>0.001</td>
</tr>
</tbody>
</table>

TAS: total antioxidant status; H\(_2\)O\(_2\): hydrogen peroxide; TOS: total oxidant status; OSI: oxidative stress index.

nuclear medicine uses radioactive materials for the diagnosis and treatment of diseases. Meaning of ionized radiation at the nuclear medicine is energy transition. It has an important role because of its follow-up, counting and determination capacity. Previous studies have suggested that ionizing radiation generated free radicals (Greenstock, 1993; Hall, 1998; Horrobin, 1991). It has been demonstrated in numerous studies that free radicals are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins and nucleic acids in tissues (Irmak et al., 2002; Koyu et al., 2009; Reiter et al., 1999). These free radicals increased peroxidation in plasma and tissues as well as decreased the activities of the antioxidant system in the organism. Besides, it is not known whether lipid peroxidation was caused by radiopharmaceuticals that are used in nuclear medicine in experiment animals. This is a unique study that investigates tissue damage after radiopharmaceutical material usage. The present study shows that the exposure to 99mTc sestamibi has significant effects on rat heart tissue and plasma, suggesting that ROS were generated under the experimental conditions employed.

Our results showed a significant increase in the level of MDA in the myocardial tissue indicating a noticeable increase in lipid peroxidation biomarker. The increase in MDA observed in the heart following the treatment with 99mTc sestamibi was probably ascribed to the excessive production of free radicals. The present study indicated that 99mTc sestamibi treatment induced oxidative stress as demonstrated by compromised antioxidant defenses and increased the lipid peroxidation in the heart, and the evaluation of cardiac impairment was performed using the estimation of MDA levels. Our results are in agreement with the studies that demonstrated a significant increase in MDA levels of human plasma by different radiopharmaceutical treatment (Cicek et al., 2006, 2007; Gurbuz et al., 2010; Kaya et al., 1999).
Ionized radiation is one of the most powerful free radical builders. 99mTc is also a radioactive material that combines with sestamibi. After the intravenous injection of 99mTc sestamibi, it arrives the heart tissue through blood circulation and accumulates in the mitochondria of myocardial tissue. It has been detected that the mitochondria (energy source of the cell) are the radiosensitive zones of the cytoplasm. As a result, antioxidant enzymes show protective effects against these pathologies in different levels related with the free radical formation period of 99mTc sestamibi in mitochondria.

Several antioxidant enzyme systems have been reported, which have different activities. Antioxidant enzymes such as SOD, CAT and GSH-Px are considered the primary defenses that prevent biological macromolecules from oxidative damage. We found statistically significant differences of the antioxidant enzyme activities among the sham and study groups.

In this study, decreased activity of the antioxidant enzyme, SOD, was found in the heart tissue of rats treated with 99mTc sestamibi. This result suggests that the increase in the lipid peroxidation in the heart of rats treated with 99mTc sestamibi may be related to the decrease in the activity of SOD, which scavenge hydroperoxides and lipid peroxides. SOD, the first line of defense against ROS, catalyzes the dismutation of the $O_2^-$ into $H_2O_2$. It protects the cell against the toxic effects of superoxide radicals (Oury et al., 1996). A significant decrease in the CAT activity in 99mTc sestamibi-treated group was observed. The decreased activities of CAT in the cardiac myocytes of rats treated with 99mTc sestamibi indicate the highly reduced capacity to scavenge $H_2O_2$ produced in the myocytes in response to acute stress (Halliwell and Gutteridge, 1999). $H_2O_2$ can then be transformed into water and molecular oxygen by CAT. Therefore, the decreased CAT activity in tissue indicates a high degree of oxidative stress resulting in the increased endogenous $H_2O_2$. The results of these studies concern the decrease in the two antioxidant enzymes support our findings (El-Habit et al., 2000; Gurbuz et al., 2010; Nikishkin et al., 1992; Sabitha and Shyamaladevi, 1999).

GSH-Px activity was significantly increased in 99mTc sestamibi-treated rats compared with the control rats. GSH-Px is an important antioxidant enzyme that plays a role in the elimination of $H_2O_2$ and lipid hydroperoxides and reduces peroxides using reduced glutathione as a hydrogen donor. The increased GSH-Px activity also reflects the increased production of $H_2O_2$, which agrees with the findings of observation in increased MDA. The elevated enzyme activities of GSH-Px in the 99mTc sestamibi group indirectly showed an increase in the number of free radicals and suggest that these enzymes play an important role in clearing excessive free radicals. These finding are similar to the results of Gurbuz et al. (2010), which studied antioxidant enzymes in relation to risk factors in subjects exposed to 99mTc methylendiphosphonate.

At the same time, TAS and TOS values were measured in plasma specimens after 60 min from 99mTc sestamibi injection. TAS values were found to be decreased and TOS values were found to be increased, but no meaningful significance was detected. Besides, OSI values significantly decreased. With these results, damage with 99mTc-MIBI on heart tissue was lower than plasma. According to our findings, a functional decrease is likely to occur in nonenzymatic antioxidants as a result of interaction with oxidants. Thus, we think that decrease in TAS levels can be attributed to the utilization of TAS during the neutralization process of the oxidants, which increases following the oxidant stress by various reactions.

In conclusion, these findings demonstrate that in vivo acute administration of 99mTc sestamibi results in the induction of lipid peroxidation and changes the activities of antioxidant enzymes in the heart tissue of rats. However, we also know that it is difficult to extrapolate effects from rodents to humans. In addition, further investigations will be needed about tissue changes related with radiopharmaceuticals.

Funding
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References


Koyu A, Ozguner F, Yilmaz HR, Uz E, Cesur G and Ozcelik N (2009) The protective effect of caffeic acid phenethyl ester (CAPE) on oxidative stress in rat liver exposed to the 900 MHz electromagnetic field. Toxicology and Industrial Health 25: 429–434.


