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The radiotracer ^{99m}Tc-MIBI is not genotoxic for human peripheral blood lymphocytes at diagnostic radioactive dose

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ABSTRACT. The radiotracer technetium-99m methoxyisobutyl isonitrile (^{99m}Tc-MIBI) has been widely used for myocardial blood flow imaging. We investigated the genotoxicity of ^{99m}Tc-MIBI in cultured human lymphocytes at the same concentration used in patients. Radioactivity doses were determined in whole blood at 5 min post-injection of 20 mCi ^{99m}Tc-MIBI in patients. Subsequently, whole blood of human volunteers was incubated with 1, 2.3, 4 or 8 μCi ^{99m}Tc-MIBI. After a 30-min incubation, the lymphocytes were stimulated with a mitogen to assay for micronuclei in cytokinesis-blocked binucleated cells. The frequency of micronuclei in samples treated with this radiopharmaceutical up to 2-fold (8 μCi) the concentration of ^{99m}Tc-MIBI normally found in the blood of patients was not more than in control lymphocyte cultures. We concluded that there is no increased induction of micronuclei in lymphocytes incubated with ^{99m}Tc-MIBI at the radioactivity doses used for diagnostic purposes.

Key words: Radiopharmaceutical; Genotoxicity; ^{99m}Tc-MIBI; Micronucleus

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INTRODUCTION

Radiopharmaceuticals are radioactive compounds that contain a radionuclide with a non-radioactive ligand; they are used for diagnostic and therapeutic purposes in patients. Radiopharmaceuticals are applied in trace amounts without pharmacological properties. The different localization of these radiotracers results in imaging of normal and abnormal tissues. Technetium-99m (^{99m}Tc) is widely used for the diagnosis of diseases in nuclear medicine, due to its ideal energy, half-life, and chelating ability. It forms chelates with different ligands and its complex is transferred to the target organ in order to make the image. ^{99m}Tc methoxyisobutyl isonitrile (^{99m}Tc-MIBI) is a synthetic, monovalent cation that is used as a myocardial perfusion agent. The cellular uptake of ^{99m}Tc-MIBI is related to the cell membrane potential, and passage through the membrane is thought to involve passive diffusion (Jones et al., 2008). This radiopharmaceutical is also used for parathyroid and thyroid imaging and evaluation of various tumors. ^{99m}Tc not only emits gamma rays (140.5 keV), but also low-energy Auger and conversion electrons. It has been established that ^{99m}Tc-MIBI is taken up by human peripheral blood lymphocytes, cells which are known to be highly radiosensitive (Mertz et al., 1986; Schwochau, 1994; Pomplun et al., 2006). Ionizing radiation passing through the cell can interact with water molecules and generate free radicals and reactive oxygen species. These toxic substances can interact with macromolecules such as DNA and induce damage. Taibi et al. (2006) showed that ^{99m}Tc-MIBI induced genotoxic effects and apoptosis at a dose of 0.1 Gy in human peripheral blood lymphocytes. The genotoxic effect depends on the radiation absorbed from a dose of radiopharmaceutical. Since the radiopharmaceutical dose of this agent is different with high dose (0.1 Gy), it is important to estimate the genotoxic effects of ^{99m}Tc-MIBI at diagnostic doses that are used in patients. With regard to the wide usage of this radiotracer in nuclear medicine and also the concern of patients and physicians about ionizing irradiation, the aim of this study was to determine the genotoxic effect of ^{99m}Tc-MIBI on human peripheral blood lymphocytes *in vitro*, at a dose at which this agent is used as a radiotracer in patients undergoing myocardial perfusion.

MATERIAL AND METHODS

Chemicals and preparation of ^{99m}Tc-MIBI

All chemicals were obtained from Merck and Sigma Company, and used without further purification. ^{99m}Tc, in the form of sodium pertechnetate (Na $^{99m}TcO_4$), was eluted from an in-house ^{99}Mo/^{99m}Tc generator system with normal saline. A commercial sestamibi kit (AEOI, Tehran, Iran) was used, and labeling and quality control procedures were performed according to manufacturer instructions.

Irradiation protocol

The study protocol was approved by the university Ethics Committee and all patients gave their written informed consent to participate in the study. Ten patients who were referred to the Research Institute for Nuclear Medicine (Tehran University of Medical Sciences) for myocardial perfusion imaging were randomly selected to be included in the study. All patients received a dose of 740-925 MBq (20-25 mCi) ^{99m}Tc-MIBI, and 2 mL

whole blood was collected from the patients by venipuncture, 5 min after radiopharmaceutical injection. The activity was determined with a dose calibrator (CRC 127, Capintec) for 2-mL blood samples. At this stage, the activity of ^{99m}Tc in human blood vessels was determined for the next step for the irradiation protocol.

After obtaining written informed consent, blood samples from three healthy, non-smoking male volunteers, aged 25-35 years, were collected in heparinized tubes. Twelve milliliters of blood was collected from each volunteer. The blood was divided into five 2-mL tubes representing five different treatment groups based on blood radioactivity level: control (zero), lowest, mean, highest, and two times highest. ^{99m}Tc-MIBI was added to blood tubes at 1, 2.3, 4, and 8 µCi. These tubes were incubated at 37°C. After 30 min, RPMI cell culture medium was added to each tube and the cells were centrifuged at 1200 rpm for 8 min. The supernatant was removed and the cells were washed with RPMI again, to remove ^{99m}Tc-MIBI from the blood cells. The blood cells were then submitted to the micronucleus assay.

Micronucleus assay

A volume of 0.5 mL of each sample (control and irradiated samples) was added to 4.5 mL RPMI 1640 culture medium (Gibco, USA), containing 10% fetal bovine serum, 0.1 mL/5 mL phytohemagglutinin (Gibco, USA), antibiotics (100 IU/mL penicillin, 100 µg/mL streptomycin), 2 mM glutamine (Sigma, USA). All cultures were set up in duplicate and incubated at 37 ± 1°C in a humidified atmosphere of 5% CO_2/95% air. Cytochalasin B (Fluka, final concentration: 30 µg/5 mL) was added after 44 h of culture. At the end of 72 h of incubation, the cells were collected by centrifugation and resuspended in cold 0.075 M potassium chloride for 8 min at 1000 rpm, and then immediately resuspended in fixative solution (methanol:acetic acid, 6:1). Fixed cells were dropped onto clean microscopic slides, air-dried and stained with Giemsa solution. All slides were coded by an individual other than the scorer, and evaluated at 400X magnification for the frequency of micronuclei in cytokinesis-blocked binucleated cells with well-preserved cytoplasm. Criteria for scoring of micronuclei were a diameter between 1/16th and 1/3rd the size of the main nuclei, non-refractile, not linked to main nuclei and not overlapping the main nuclei (Fenech, 2000). A total of 1000 binucleated cells for each duplicate culture for each volunteer at each radiation dose and control were examined to record the frequency of micronuclei in binucleated lymphocytes.

Statistical analysis

For each volunteer, at each level of radioactivity *in vitro*, the incidence of radiation-induced micronuclei was recorded. The data were analyzed by ANOVA with the Tukey HSD *post hoc* test.

RESULTS

A preliminary experiment was carried out to determine the radioactivity level of ^{99m}Tc-MIBI in the blood of patients undergoing myocardial perfusion scan. ^{99m}Tc-MIBI, 20-25 mCi, was administrated to patients. After 5 min, radioactivity levels of 1, 2.3, and 4 µCi were determined for the lowest, mean and highest levels of the radiopharmaceutical in 2 mL whole blood of patients.

The percentages of micronuclei in lymphocytes of volunteers at 1, 2.3, and 4 µCi were 1.2 ± 0.4, 1.04 ± 0.6 and 1.7 ± 0.8%, respectively. The activity of 8 µCi was selected to represent a level 2-fold higher than the highest activity found in whole blood of patients given the radiopharmaceutical, and the incidence of micronuclei in lymphocytes was 1.7 ± 0.7, compared to 1.6 ± 0.4% in non-treated control (Table 1). The frequencies of micronuclei found in the ^{99m}Tc-MIBI-treated groups were not significantly higher than those of the control group. No genotoxicity based on the micronucleus assay was observed in human lymphocyte cells exposed *in vitro* to ^{99m}Tc-MIBI at doses representing activity levels of this radiopharmaceutical in patients' blood. A typical image of binucleated cells with a micronucleus found in this study is shown in Figure 1.

Table 1. Percentages of micronuclei induced *in vitro* by different activity of ^{99m}Tc-MIBI in cultured blood lymphocytes from human volunteers**.**

	Binucleated cells with micronuclei (%)*				
	Control	1 µCi	2.3 µCi	4 µCi	8 µCi
Volunteer 1	2	1.2	0.8	1.9	2.4
Volunteer 2	1.2	0.8	0.6	0.7	1
Volunteer 3	1.8	1.6	1.7	2.4	1.6
Mean ± SD	1.6 ± 0.4	1.2 ± 0.4	1.04 ± 0.6	1.7 ± 0.8	1.7 ± 0.7

*1000 binucleated cells were examined in each culture.

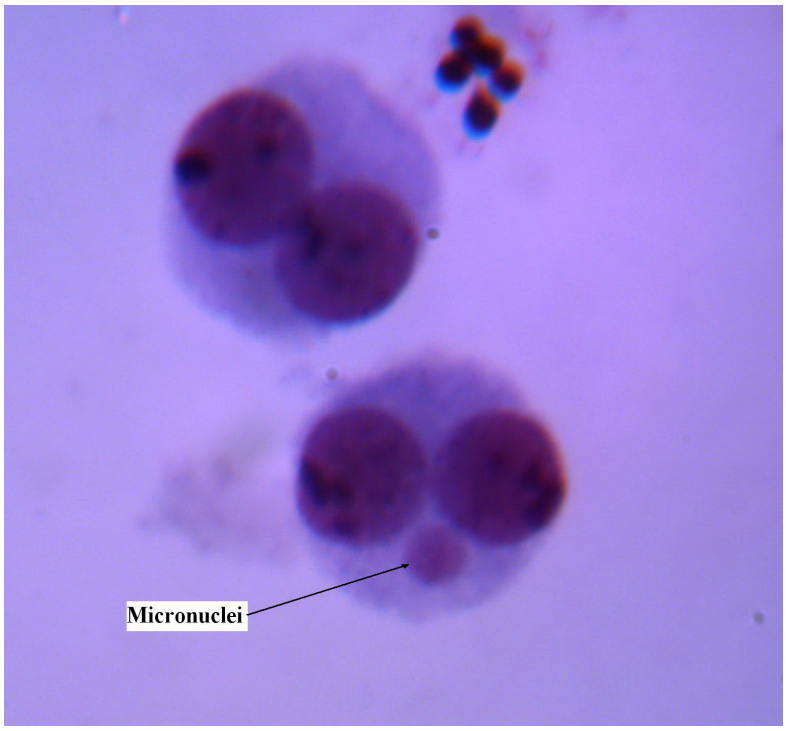

Figure 1. A typical binucleated lymphocyte with micronucleus in our study.

DISCUSSION

Genotoxicity evaluation has been used in risk estimation with regard to damage induced by ionizing radiation (Taibi et al., 2006; Hosseinimehr et al., 2009). An important biomarker for assessing chromosome damage induced by ionizing radiation is the presence of micronuclei in lymphocyte cells, which are chromosome fragments or whole chromosomes that are left behind during mitotic division and appear in the cytoplasm of divided cells, being smaller than the main nuclei (Fenech, 2000). Genotoxic agents may cause cancer, hereditary disorders, and abnormalities in the developing embryo (Lundqvist et al., 2007). In this study, the genotoxicity induced by ^{99m}Tc-MIBI in human lymphocytes was investigated by the micronucleus method. We did not observe any genotoxicity induced by ^{99m}Tc-MIBI in lymphocytes. The frequency of micronuclei did not differ significantly between human lymphocytes treated with ^{99m}Tc-MIBI and control groups. The induction of micronuclei by ^{99m}Tc-MIBI appeared to be dose independent at doses similar to those in whole blood of patients, implying that ^{99m}Tc-MIBI does not induce any additional genotoxicity in human lymphocytes with this protocol. There was no evidence of any genotoxicity induced by two times the radioactivity level of ^{99m}Tc-MIBI in whole blood of patients. Taibi et al. (2006) studied the effects of incubation of healthy human lymphocytes *in vitro* with increasing activities of ^{99m}Tc-MIBI corresponding to absorbed doses ranging from 1 µGy to 1 Gy. The frequencies of micronuclei were similar in control cultures and lymphocytes exposed to 10 µGy, 100 µGy and 1 cGy. A significantly higher frequency of micronuclei was observed after exposure to 10 cGy (corresponding to 0.25 mCi of ^{99m}Tc-MIBI) (Taibi et al., 2006). In that study, the authors exposed lymphocytes to ^{99m}Tc-MIBI but at radioactivity levels not adjusted to those in patients. Our findings can be considered similar to those of Taibi et al. (2006) with regard to the fact that we treated lymphocytes with a maximum radioactivity of 8 µCi and observed no genotoxicity.

^{99m}Tc-MIBI was chosen for the following reasons. First, over 80% of radiopharmaceuticals currently used in diagnostic nuclear medicine are labeled with ^{99m}Tc, whose nuclear properties are optimal with regard to low irradiation and effective detection of emitted gamma rays (Schwochau, 1994). Decaying ^{99m}Tc not only emits gamma rays, but also Auger electrons that cause subcellular side effects due to the extremely short range in the cell (Pomplun et al., 2006). Second, cardiac perfusion scintigraphy is a routine nuclear diagnostic test for heart disease. The induction of chromosome aberrations by ^{99m}Tc-hexamethylene diphosphonate (^{99m}Tc-HDP) was studied in patients undergoing bone scintigraphy (Guiraud-Vitaux et al., 2005). ^{99m}Tc-HDP did not cause any genotoxicity after intravenous administration of 20-25 mCi of this radiopharmaceutical. Radiopharmaceutical genotoxicity was evaluated by the incubation of human lymphocytes with ^{99m}Tc-MIBI, during labeling of lymphocyes with ^{99m}Tc; increasing concentrations of ^{99m}Tc resulted in proportional increases in micronuclei in lymphocytes at doses of 0.236, 0.435, and 0.580 mCi per 10^7 lymphocyte cells (Merz et al., 1986). The 0.580 mCi dose of ^{99m}Tc-MIBI corresponded to 1 Gy of 250 Kev X-ray, and the aim of that study was to determine if there was any genotoxicity induced by ^{99m}Tc-MIBI after labeling of lymphocytes. Patients exposed to clinical doses of ^{99m}Tc did not show any chromosome aberrations and mutations in peripheral blood lymphocytes from nuclear medicine (Kelsey et al., 1991). Internal radiation from different radionuclides as well as external radiation of various types can induce genotoxic effects that can be related to an increased risk of cancer. Of course, for radiolabeled compounds, it is possible to make fairly accurate estimates of the hazard and

risks to humans caused by ionizing radiation with dose response models, using extrapolation from high dose effects of ionizing irradiation in animals to humans (Lundqvist et al., 2007).

With regard to the wide usage of ^{99m}Tc-MIBI for diagnostic purposes, there are several studies to assess the genotoxicity induced by this agent in lymphocytes. Despite the demonstration of increasing numbers of micronuclei in lymphocytes after exposure to high radioactivity levels of ^{99m}Tc-MIBI, no genetic damage was observed at low doses of this radiotracer. Our study showed that incubation of whole blood cell with different doses of ^{99m}Tc-MIBI corresponding to the same blood radioactivity levels found in patients, did not cause any genotoxicity in lymphocytes.

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