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Genotoxicity of Thallium-201 in Patients with Angina Pectoris Undergoing Myocardial Perfusion Study

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YILDIRIM, M., IKBAL, M., TOS, T., SEVEN, B., PIRIM, I. and VAROGLU, E. *Genotoxicity of Thallium-201 in Patients with Angina Pectoris Undergoing Myocardial Perfusion Study.* Tohoku J. Exp. Med., 2005, **206** (4), 299-304 ── Thallium-201 (^{201}Tl) has been widely used as a nuclear reagent for myocardial blood flow imaging. The purpose of this study was to investigate genotoxic effects of ^{201}Tl in patients with angina pectoris (n = 21), who had undergone myocardial perfusion imaging. Lymphocytes were isolated from each patient before, and 3, 30 and 90 days after ^{201}Tl administration (111 MBq, 3 mCi) and were analyzed for chromosomal aberrations, sister chromatid exchanges, mitotic index and replicative index. There were significant increases in chromosomal aberrations and sister chromatid exchanges 3 days after ^{201}Tl administration ($p < 0.001$), although no difference was noted in these values after 30 and 90 days ($p > 0.05$). Moreover, decreased mitotic index and replicative index were noted after 3 days of ^{201}Tl administration ($p < 0.001$). These results suggest that the administration of ^{201}Tl for myocardial blood flow imaging may induce genetic damage. ──── myocardial perfusion; thallium-201; sister chromatid exchange; chromosomal aberration; mitotic index; replicative index

Thallium-201 (^{201}Tl), thallous chloride, has been widely used for a variety of nuclear medicine imaging procedures (Howell 1992). ^{201}Tl is a myocardial imaging agent being regularly used for many years in all nuclear medicine departments undertaking cardiological studies (Short 1999). ^{201}Tl, which is clinically used to image myocardial blood flow, has the physical half-life of 3.08 days and the whole body biological half-life is about 10 days (Saha 1984).

^{201}Tl decays by electron capture, resulting in the creation of a vacancy in an inner anatomic shell. The series of anatomic transitions that follow result in the emission of numerous (~ 37) low-energy Auger electrons (Rao et al. 1995). The intracellular localization of ^{125}I emitting Auger electrons is important for the cytotoxic effects. Its decay is highly mutagenic in human lymphoblastoid cells (Liber et al. 1983; Whaley et al. 1990). Ionizing radiation of technetium has been reported to induce a significant increase in hypoxanthine guanine phosphoribosyl transferase mutations frequency in lymphocytes (Seifert et al. 1987).

Cytogenetic tests play an important role in the detection of biological effects of low doses of

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radiation in patients exposed to ionizing radiation (Kelsey et al. 1991; Vera et al. 1997; Sonmez et al. 1997). Radiation exposure increases the frequency of micronuclei, gene mutation, decreased colony forming ability and leads to morphological abnormalities, apoptosis and neoplastic transformation (Chang et al. 1992; Lambert et al. 1998).

Sister chromatid exchange (SCE) is cytogenetic indicators arising during replication of damage DNA templates from reciprocal DNA interchanges between sister chromatids in replication process (Tucker et al. 1996). Although the mechanism of SCE formation is not completely elucidated, DNA damage and DNA repair defects are involved in the formation of SCE (Perry et al. 1975; Nakanishi et al. 1979). SCEs can arise from DNA damage occurring before DNA replication (Renault et al. 1982). It can occur at certain rates normally, and as a consequence of ultraviolet light exposures, ionizing radiation; exposures to chemotherapeutic drugs, viral infections, chronic diseases, Psoralens Plus Ultraviolet A (PUVA) and malignancies have an effect on frequencies of SCE (Gebhart 1981; Hamurcu et al. 2002).

In order to investigate the in vivo genotoxicity of exposures to diagnostic radionuclides, we have studied chromosomal aberrations (CA), cellular divisions and the frequency of SCE in a cohort of nuclear medicine patients exposed to ^{201}Tl.

Materials and Method

Twenty-one patients (9 females and 12 males; 50.81 ± 2.09 years old) were included in the study. All patients undergoing stress-rest myocardial perfusion studies were injected about 111 MBq (3 mCi) of ^{201}Tl immediately after stress. All subjects were otherwise healthy, and reported that they had not previously received radiotherapy, chemotherapy, extensive x-rays, or diagnostic tests in the nuclear medicine service, nor were they taking medication that would affect lymphocytes.

All patients were their own controls and were nonsmokers. Venous blood samples were taken from all patients. CA, mitotic index (MI), replicative index (RI) and SCE frequencies obtained from peripheral lymphocytes of patients were evaluated before, and 3, 30 and 90 days after ^{201}Tl administration. Chosen controls for CA, MI, RI and SCE frequencies were obtained just before treatment.

Cytogenetic analyses were performed according to routine techniques (Gebhart 1981; Bauchinger 1995). Briefly, whole blood samples were cultured for 72 h at 37°C in RPMI-1640 (Biological Industries, Israel), 20% fetal calf serum (Biological Industries), 2% phytohemaglutinin (Biological Industries), 5,000 μg/ml streptomycin (Biological Industries) and 5,000 IU/ml penicillin (Biological Industries) containing medium. 0.1 μg/ml colcemide (Biological Industries) was added 2 h prior to harvesting to arrest the cells at metaphase. For SCE demonstration, 5-bromo 2'-deoxyuridine (BrdU: Sigma, St. Louis, MO, USA) at 8 μg/ml was added at the initiation of cultures. All cultures were maintained in total darkness to minimize photolysis of BrdU. An amount of 0.1 μg/ml colcemide was added 3 h prior to harvesting to arrest the cells at metaphase. Cells were collected by centrifugation (10 min; 1,000 rpm). For CA and SCE, resuspended in a pre-warmed hypotonic solution (0.075 M KCl) for 20 min and fixed three times in acetic acid: methanol (1 : 3 vol/vol). For CA, Air-dried preparations were stained with 6% Giemsa in phosphate buffer for 10 min. A total of 100 well-separated metaphases in the CA assay, aberrations such as gaps and breaks were scored. Two to three stained slides were processed for determination of the MI as the number of mitotic cells per at least 500 cells. Bromodeoxyuridine-incorporated metaphase chromosomes were stained by the fluorescence plus Giemsa technique as described by Wolff and Perry (1974). In the SCE studies, 100 metaphases per patient were also scored to determine the proportion of cells that undergo one, two and three divisions. The RI was calculated according to the formula RI = $(M_1 + 2M_2 + 3M_3) / N$, where M1, M2 and M3 indicate those metaphases corresponding to the first, second and third divisions and N the total number of metaphases scored.

Statistical analyses were performed by repeated measures variance analysis.

Results

When compared to the basal values, there were significant increases in chromatid and chromosome gaps ($p = 0.001$), chromatid breaks ($p = 0.001$), chromosome breaks ($p = 0.004$), dicentric chromosomes ($p = 0.016$), acentric chromosomes ($p = 0.008$), aberrant cells ($p = 0.001$) in lymphocyte cultures from patients after 3 days of ^{201}Tl administration (Fig. 1, Table 1). In the analysis of SCE frequency, we have observed that it was in-

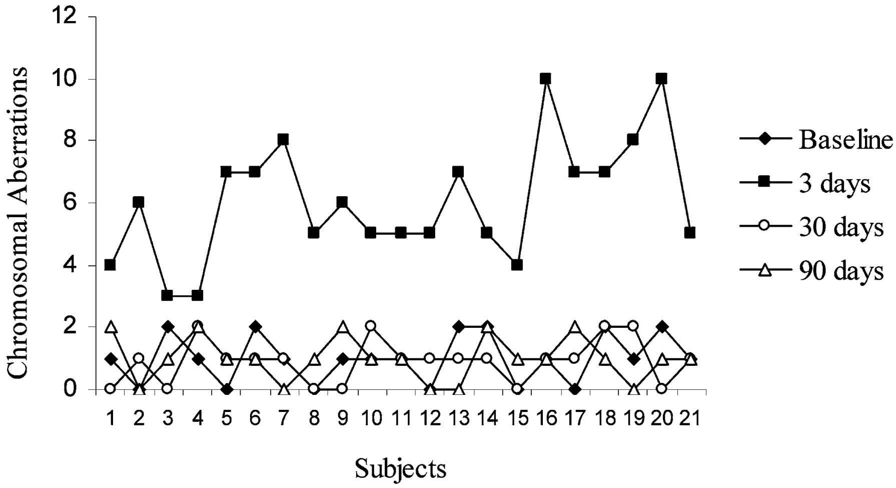

Fig. 1. The distribution of chromosomal aberration in all subjects depending on given times. The values obtained at the third day of ^{201}Tl administration displayed significantly increased CA as compared baseline values.

TABLE 1. *The distribution of chromosomal aberrations in patients with angina pectoris depending on time*

	Ctd. gaps (Mean ± S.E.)	Chr. gaps (Mean ± S.E.)	Crd. Brk. (Mean ± S.E.)	Chr. Brk. (Mean ± S.E.)	Dc. Chr. (Mean ± S.E.)	Ac. (Mean ± S.E.)	Abr. C. (Mean ± S.E.)
Before ^{201}Tl	0.48 ± 0.11	0.48 ± 0.11	0.19 ± 0.09	0.05 ± 0.05	0.05 ± 0.05	0.14 ± 0.08	1.00 ± 0.17
3 days after ^{201}Tl	2.71 ± 0.25[a]	2.19 ± 0.27[a]	1.29 ± 0.18[a]	0.52 ± 0.15[b]	0.50 ± 0.11[c]	0.67 ± 0.19[d]	6.05 ± 0.43[a]
30 days after ^{201}Tl	0.38 ± 0.11	0.29 ± 0.10	0.43 ± 0.11	0.10 ± 0.07	0.09 ± 0.07	0.14 ± 0.08	0.90 ± 0.15
90 days after ^{201}Tl	0.33 ± 0.13	0.33 ± 0.11	0.29 ± 0.10	0.05 ± 0.05	0.09 ± 0.07	0.10 ± 0.07	1.00 ± 0.15

Chromatid and chromosome gaps, chromatid and chromosome breaks, dicentric and acentric chromosomes, and aberrant cells values were higher at the third day of ^{201}Tl administration than those of the baseline values. ([a] $p = 0.001$, [b] $p = 0.004$, [c] $p = 0.016$, [d] $p = 0.008$)

Ctd. gaps, chromatid gaps; Chr. gaps, chromosome gaps; Crd. Brk., chromatid break; Chr. Brk., chromosome break; Dc. Chr, dicentric chromosome; Ac.Chr, acentric chromosome, Abr. C., aberrant cell; ± S.E., standard error of mean.

creased on the 3rd day after ^{201}Tl administration ($p = 0.001$; Fig. 2, Table 2). A significant decrease was observed in cellular division i.e., MI and RI after 3 days ($p = 0.001$; Figs. 3 and 4; Table 2). We also determined that the values related to CA, SCE, MI and RI obtained at 30 and 90 days after ^{201}Tl administration returned to the baseline levels. In the evaluation on the 3rd day, there was no significant difference in the genotoxicity of ^{201}Tl in terms of gender.

DISCUSSION

Most radionuclides used for diagnostic imaging emit Auger electrons, such as technetium-99m, iodine-131, indium-111, gallium-67 and ^{201}Tl (Kassis et al. 1983; Rao et al. 1988; Faraggi et al. 1994). Experimental studies have demonstrated that the radiobiological effects of Auger

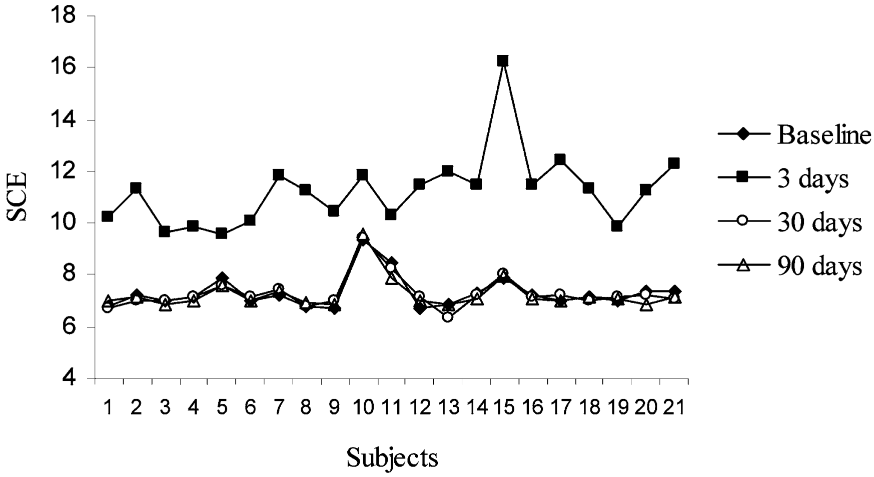

Fig. 2. The distribution of SCE in all subjects depending on given times. The values obtained at the third day of ^{201}Tl administration displayed significantly increased SCE as compared baseline values.

TABLE 2. *The distribution of MI, RI and SCE in patients with angina pectoris depending on time*

	SCE (Mean ± S.E.)	MI (Mean ± S.E.)	RI (Mean ± S.E.)
Before ^{201}Tl	7.29 ± 0.14	7.00 ± 0.24	2.09 ± 0.04
3 days after ^{201}Tl	11.25 ± 0.32*	5.40 ± 0.27*	1.73 ± 0.04*
30 days after ^{201}Tl	7.27 ± 0.14	6.84 ± 0.26	2.06 ± 0.04
90 days after ^{201}Tl	7.26 ± 0.14	6.94 ± 0.23	2.09 ± 0.04

There were significant increases in SCE but decreases in MI and RI in the third day measurements. ($^{*}p = 0.001$)

SCE, sister chromatid exchange; MI, mitotic index; RI, replicative index; ± S.E., standard error of mean.

emitters depend strongly on their subcellular localization (Makrigiorgos et al. 1990; Hofer 1996).

Bachand et al. (1991) reported no effect of exposure to 2 mCi of ^{201}Tl in their study. And also in that study, blood was sampled just before the first ^{201}Tl injection and a second time 1-3 months following the test. But in our study, venous blood samples were taken from all patients before and 3, 30 and 90 days after ^{201}Tl administration. The difference between our study and the previous study by Bachand et al. (1991) was probably due to a different time period of blood sampling.

Chromosome aberrations are known to be a very sensitive biological indicator of the radiation-produced damage to DNA, including DNA breakage (Vera et al. 1997). Induced chromosomal changes in human lymphocytes are well-established biomarkers of occupational or environmental exposure to genotoxic agents, and the most frequently used endpoints are CA and SCEs (Carrano et al. 1988). Alexey et al. (1999) recently reported that the thallium concentrations in blood in accidentally thallium-poisoned patients varied from 25 to 2,700 μg/l induced structural chromosome aberrations. In another study,

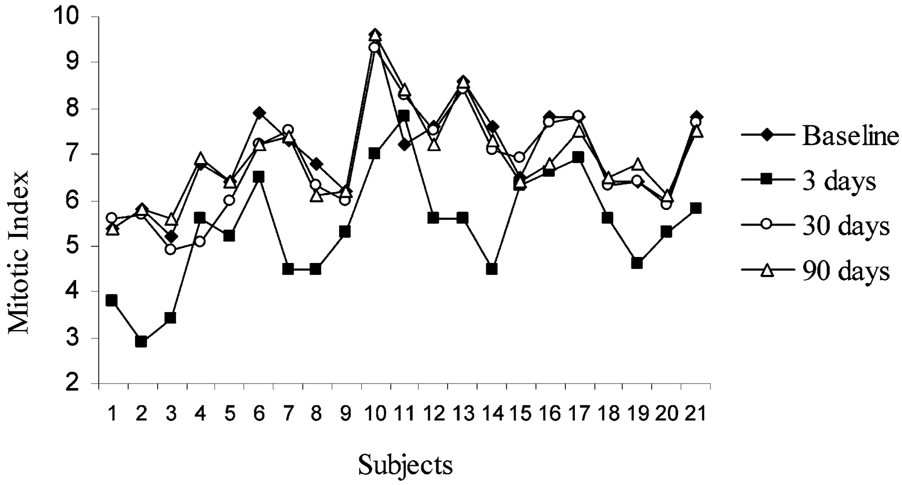

Fig. 3. The distribution of mitotic index in all subjects depending on given times. The values obtained at the third day of ^{201}Tl administration displayed significantly increased MI as compared baseline values.

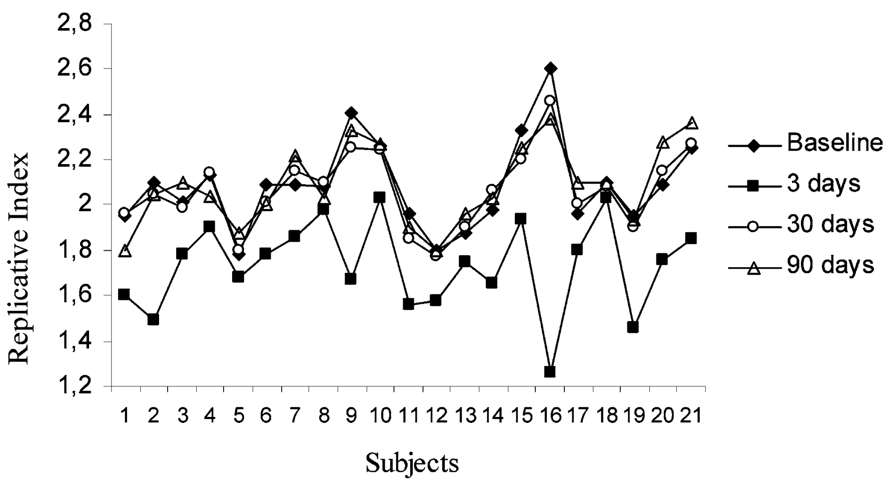

Fig. 4. The distribution of replicative index in all subjects depending on given times. The values obtained at the third day of ^{201}Tl administration displayed significantly increased RI as compared baseline values.

Migliore et al. (1999) reported that the blood of two young nonsmoking male donors administrated of Tl showed a statistically significant increase of micronuclei (MN) compared to controls. MN is formed during mitosis by the loss of whole chromosomes or portions of chromosomes from daughter nuclei and exists separately from the main nucleus of a cell (Tucker et al. 1996). We found that CA, SCE, MI and RI values were increased at the 3rd day measurements when compared to baseline levels, but the increases were not persistent because they were found to be decreased to baseline levels at the 30th and 90th days measurements. So, we can state that the increases in CA due to diagnostic ^{201}Tl administration are temporary.

SCEs are generally more sensitive indicators of genotoxic effects than structural aberrations, although unlike aberrations they do not indicate a mutagenic effect. The ease of analysis of SCEs has made them attractive for short-term assays, but their lack of specificity for the detection of mutagenic activity has led to their reduced used. We found an increased SCE frequency 3 days after ^{201}Tl administration. There is, however, no significant elevation in SCE 30 and 90 days after ^{201}Tl exposure. Indeed, it has been shown that SCE formation is brought about by DNA damage induced before DNA replication and as shown in the case of gamma irradiation SCE can arise as a result of two successive cell divisions.

In summary, ^{201}Tl is able to induce both cytotoxic and moderate genotoxic effects in human lymphocytes. For the evaluation of its real genotoxic hazard, further biomonitoring studies should be conducted in patients receiving with ^{201}Tl.

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