Radionuclide Angiography with Technetium-99m In Vivo Labeled Erythrocytes Does Not Lead to Induction of Mutations in the HPRT Gene of Human T-Lymphocytes

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Mutant frequencies were measured in T-lymphocytes of patients undergoing radionuclide angiography with erythrocytes labeled in vivo with technetium-99m. Blood from 13 patients was sampled before and after (8–120 days) an injection with 750 MBq technetium-99m. Frequencies of $HPRT^-$ mutants were measured with the T-cell cloning method. Results indicated that the mean frequency of mutants after treatment was significantly below that measured before exposure. Thus, in contrast to published data, our results do not support the conclusion that radionuclide angiography with technetium-99m induces $HPRT^-$ mutations. Further analysis of our data indicated that the decrease in mutant frequency after exposure can be accounted for by an effect of cloning efficiency.

J Nucl Med 1991; 32:814–818

Erythrocytes labeled in vivo with technetium-99m (^{99m}Tc) are often used for ventriculographic examinations of heart function. The effective dose equivalent due to this diagnostic test is reported to be 6.4 mSv (*1*), and it can be calculated that lymphocytes from patients undergoing this test receive, on average, a dose equivalent of about 14 mSv.

In 1987, Seifert et al. (*2*) reported that exposure of patients to a low dose of ionizing radiation from ^{99m}Tc is associated with an increase in hypoxanthine-guanine phosphoribosyl transferase ($HPRT^-$) mutant frequency in T-lymphocytes from peripheral blood. In view of this alarming finding and the frequent use of this diagnostic test, we considered it necessary to investigate whether or not the data of Seifert et al. could be confirmed.

In the present paper, results are presented of a study where mutants in the HPRT gene were measured in peripheral blood lymphocytes from 13 patients undergoing a diagnostic test for heart functions with ^{99m}Tc. From each patient, the first blood sample was taken immediately before administration of ^{99m}Tc and the second at 8–120 days thereafter.

Results of this study indicate that the mean mutant frequency in post-treatment samples is significantly below that for pre-treatment samples. This stands in contrast with results of Seifert et al. who found an increase.

MATERIALS AND METHODS

The procedure used for cloning of T-lymphocytes was basically the one described by O'Neill et al. (*3*) with the following modifications:

1. After their publication O'Neill and Albertini proposed the use of lymphokine-activated killer (LAK)-supernatant as a source of IL-2 (see below). In our own studies LAK-supernatant proved to be a very useful supplement to the cloning medium.
2. For improvement of colony growth β-mercaptoethanol was added to the medium (*4,5*).
3. In addition to the standard antibiotics (penicillin, streptomycin), amphotericin B (Fungizone) was used to avoid infection with fungi.

Media

The basic medium was RPMI 1640 (Gibco Life Technologies, Breda, The Netherlands) containing 25 m*M* HEPES. The pH was adjusted to 7.2 before addition of 2 g/l sodium bicarbonate.

A growth factor source was made from 'LAK-supernatant,' which was obtained from the Department of Clinical Oncology of the University Hospital Leiden. The supernatant is in fact a standard culture medium to which a large amount of recombinant IL-2 is added. Lymphocytes from cancer patients are cultured in this medium to stimulate proliferation of 'killer' cells. After culture, the LAK cells are transferred back to the patient (*6*). The remaining medium is centrifuged and stored frozen for our experiments. LAK supernatants from different sources were

Received Jul. 13, 1990; revision accepted Oct. 24, 1990.

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tested for their capacity to support clone formation by lymphocytes from one and the same donor. No substantial differences could be detected.

Lymphocyte Isolation and Storage

Approximately 40 ml samples of blood were collected from individuals in heparinized vacutainers (Becton Dickinson, Geiden, The Netherlands). The mononuclear cell fraction was immediately separated on Ficoll-Paque (Boehringer Mannheim BV, Almere, The Netherlands) and washed with phosphate-buffered saline (PBS) supplemented with 2% fetal bovine serum (FBS) (Hyclone, Greiner BV, The Netherlands, heat-inactivated). After viability counting, cells were suspended in priming medium (see below) or frozen by suspending the cells in 1.5 ml freezing medium (RPMI 1640 containing 40% FBS and 10% DMSO) and stored under liquid nitrogen.

Lymphocyte Priming

In some patients (marked in Table 1), freshly isolated lymphocytes were immediately suspended in priming medium to induce mitotic activity. In other donors, isolated lymphocytes were frozen and put in culture when both pre- and post-treatment samples of a particular patient were available. In the latter case, mutant frequencies could thus be determined under exactly the same culture conditions.

Frozen pre- and post-treatment samples of each patient were thawed simultaneously in a waterbath (37°C). The contents of each ampule were transferred to two 10-ml tubes, each containing 8 ml RPMI 1640 with 40% FBS and centrifuged (10 min at 350 g). After resuspending the pellet in RPMI 1640, cells were counted (viability usually greater than 90%), recentrifuged and seeded out in priming medium at a cell density of 2×10^6/ml, and incubated upright in 50-ml tissue culture flasks (Greiner, max. 15 ml suspension per flask) in humidified incubators in an atmosphere of 5%–6% CO_2 in air at 37°C. Phytohemagglutinin (PHA HA16, Wellcome Diagnostics, Weesp, The Netherlands) was added at a final concentration of 1 μg/ml.

Priming medium consisted of RPMI 1640 supplemented with 20% nutrient medium HL-1 (Ventrex, Biolab NV, Amersfoort, The Netherlands), 5% FBS, 5×10^{-5} *M* beta-mercaptoethanol, 2 m*M* L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin sulphate, and 2.5 μg/ml amphotericin B (Fungizone, Gibco).

Cloning

After incubation for 44 hr, primed lymphocytes were counted and plated in round-bottom 96 well microtiterplates (Nunc, Gibco) at 1, 2, 4, 8, or 16 cells per well in non-selection medium (−TG) and at 2×10^4 cells per well in selection medium (+TG). Each well contained 200 μl cloning medium.

TABLE 1
Frequencies of Patients Sampled Before and After Technetium Treatment

Patient no.	Age	%CE (cells/well) 2	4	8	16	Mean	Mutant selection (+TG) pos/total	Mf 10^{-6}	95% conf. lim.	Sample pre/post	Interval (days)
1	53.4	47.7	—	—	—	47.7	51/960	5.7	4.2 – 7.8	(pre)	8
		17.8	—	—	—	17.8	20/960	5.9	3.7 – 9.5	(post)	
2	67.6	3.5	—	—	—	3.5	15/960	22.5	11.9 – 42.5	(pre)	14
		4.5	—	—	—	4.5	16/953	18.8	10.4 – 34.2	(post)	
3	49.4	39.6	—	—	—	39.6	45/960	6.1	4.4 – 8.4	(pre)	14
		58.5	—	—	—	58.5	62/960	5.7	4.3 – 7.6	(post)	
4	52.3	48.5	37.6	—	—	39.5	24/456	6.18	4.5 – 10.4	(pre)	14
		—	31.3	—	—	31.3	67/958	11.6	8.6 – 15.3	(post)	
5F	51.4	0.9	1.3	0.6	0.8	0.9	1/136	40.0	5.6 – 293.7	(pre)	14
		5.8	6.7	7.9	10.0	7.9	16/480	22.4	13.5 – 37.1	(post)	
6	65.0	3.9	—	—	—	3.9	17/672	32.9	18.0 – 59.8	(pre)	15
		5.9	—	—	—	5.9	10/480	17.8	9.0 – 35.5	(post)	
7	58.3	23.5	—	—	—	23.5	13/224	12.7	7.2 – 22.4	(pre)	22
		1.7	—	—	—	1.7	4/960	12.1	4.0 – 37.2	(post)	
8F	53.3	2.9	3.3	2.0	1.1	2.4	0/56	<40	*	(pre)	29
		96.3	91.2	*	*	93.7	2/64	1.7	0.4 – 7.0	(post)	
9F	60.8	5.9	4.7	5.6	5.6	5.5	9/928	8.9	4.6 – 17.4	(pre)	9
		41.0	38.6	36.9	28.5	36.3	19/480	5.7	3.5 – 8.8	(post)	
10	59.5	4.2	—	—	—	4.2	24/960	30.2	17.7 – 51.5	(pre)	39
		22.7	26.7	22.0	21.7	23.3	39/864	9.9	7.2 – 13.8	(post)	
11F	62.1	*	*	1.9	1.8	1.9	5/192	71.5	28.8 – 177.2	(pre)	63
		11.8	10.1	11.8	12.5	11.6	48/960	22.2	16.4 – 30.0	(post)	
12F	53.5	10.4	10.5	12.6	13.3	11.7	13/632	9.0	5.2 – 15.7	(pre)	64
		48.4	55.3	*	*	51.8	9/384	2.3	1.2 – 4.6	(post)	
13	33.2	24.3	—	—	—	24.3	13/336	8.1	7.3 – 9.1	(pre)	120
		89.6	—	—	—	89.6	128/960	7.8	6.5 – 9.9	(post)	

Pre-treatment: Mean CE ± s.d. = 16.0 ± 16.9; mean Mf ± s.d. = $21.2 \pm 19.9 \times 10^{-6}$.
Post-treatment: Mean CE ± s.d. = 33.4 ± 31.4; mean Mf ± s.d. = $11.1 \pm 7.2 \times 10^{-6}$.
* = Not possible to calculate.
— = Not done.
F = both pre- and post-sample frozen before Mf determination.

To determine cloning efficiencies (CE,−TG), we initially used four plates with 2 cells/well. Due to occasional low cloning efficiencies (CEs), it was decided later on to use an extra two plates with 4 cells/well, two plates with 8 cells/well, and two plates with 16 cells/well. For selection, we used, when possible, 10 plates of 2×10^4 cells/well. Plates were incubated for 12–14 days under conditions as described above to allow colony growth before scoring under an inverted microscope.

Cloning medium (non-selection) consisted of RPMI 1640 supplemented with 20% HL-1, 5% FBS, 12.5% LAK-supernatant as a source of IL-2, 5×10^{-5} *M* beta-mercaptoethanol, 2 m*M* L-glutamine, 0.25 μg PHA/ml, antibiotics as described above, and 5×10^4 irradiated feeder cells/ml. Selection medium also contained 2.5 μg/ml 6-thioguanine (TG, Sigma Instruchemie, Hilversam, The Netherlands).

Feeder cells were TK6-derived HPRT-deficient lymphoblastoid cells obtained from R.J. Albertini, and grown in RPMI 1640 supplemented with 10% heat-inactivated newborn calf serum (defined bovine serum, Hyclone). Before use as allogenic stimulators, they were killed by exposure to 40 Gy of x-rays at a dose rate of 25 Gy/min (Enraf Nonius, Delft, The Netherlands, 100 kV, 12 mA).

Study Population

Individuals who were referred to the nuclear medicine department of the University Hospital Leiden for a diagnostic test of heart function were asked to volunteer for this study. To study low-dose radiation effects, it was important that the selected patients had not previously received radiotherapy, chemotherapy, or relevant radiologic examination. It was rather difficult to find volunteers that fulfilled these criteria.

Blood samples were collected just before the beginning of the nuclear medicine procedure and at 8 to 120 days thereafter.

For the radionuclide angiographic examination, erythrocytes of patients were labeled with 750 MBq ^{99m}Tc according to the in vivo method (*7*). Twenty minutes after the injection of 2 ml of a pyrophosphate solution containing 1.5 mg stannous chloride (TechneScan PYP, Mallinckrodt Diagnostica, Petten, The Netherlands) 750 MBq [^{99m}Tc]pertechnetate were administered intravenously. Immediately thereafter multiple-gated scintigraphy was performed.

Dosimetry

Technetium-99m was produced in a radionuclide generator as pertechnetate ($^{99m}TcO_4^-$; Ultratechnekow FM, Mallinckrodt Diagnostica). In the generator, molybdenum-99 (^{99}Mo; T½ = 66 hr) was transformed by beta-decay into either ^{99m}Tc (87.9%) or ^{99}Tc (12.1%). Technetium-99m (T½ = 6 hr) decayed by isomeric transition to ^{99}Tc and emitted partially converted gamma-photons of 142 keV or 140 plus 2 keV. The effective output of the 140-keV gamma radiation was 87.2%.

Radionuclide purity of the pertechnetate solution was high. The ^{99}Mo content was less than 0.001%. The labeling efficiency of the in vivo method used was better than 90%. The half-life of ^{99m}Tc in the human body, due to excretion and physical decay, was approximately 5 hr.

The effective dose equivalent due to the nuclear medicine procedure was reported to be 6.4 mSv (*1*). From this, it was calculated that lymphocytes receive, on average, a dose equivalent of about 14 mSv.

Calculation of Mutant Frequencies

CEs were calculated by means of the following formula:

$$CE = (-\ln P_o)/x,$$

where P_o = the fraction of negative wells and x = the average number of cells/well. The calculated mutant frequency (Mf) is the ratio of the CE in the presence and absence of the selective agent 6-thioguanine (*8*).

For experiments with two or more types of non-selection plates, the arithmetical mean CE was used to calculate the Mf. Ninety-five percent confidence intervals for Mfs were calculated according to Furth et al. (*9*).

Statistical Analysis

Mfs and CEs were not normally distributed and were therefore transformed with the ^{10}log function. Despite this, we report the means and standard deviations of the untransformed Mfs and CEs. The differences of ^{10}log(Mf) and ^{10}log(CE) in patients before and after treatment were tested with the paired t-test.

Covariance analysis for repeated measurements was used to test for the difference between ^{10}log(Mf) before and after treatment while controlling for differences in ^{10}log(CE) before and after treatment (*10*).

RESULTS

Mfs of patients before and after ^{99m}Tc administration are presented in Table 1. The CEs for pre-treatment samples range between 0.9% and 47.7% (16.0 ± 16.9) and for post-treatment samples between 1.7% and 93.7% (mean 33.4 ± 31.4). The increase in CE after diagnostic examination is not significant ($p < 0.08$).

For Patient 8, it was impossible to obtain mutant clones due to the low CE (2.4%) and the small number of lymphocytes available for mutant selection. The Mf for this donor ($<40 \times 10^{-6}$) was calculated by assuming that there was less than one positive well out of a total of 56 wells. For the statistical analysis of the data, a Mf of 40×10^{-6} was used for this patient.

Mfs before ^{99m}Tc treatment ranged between 5.7 and 71.5 $\times 10^{-6}$ (mean $21.2 \pm 19.9 \times 10^{-6}$) and after ^{99m}Tc exposure between 1.7 and 22.4×10^{-6} (mean $11.1 \pm 7.2 \times 10^{-6}$). The decrease in Mf after diagnostic examination proved to be statistically significant ($p < 0.031$).

Because the mean CE doubled after treatment, the question arose whether the decrease in Mf after treatment could perhaps be attributed to an effect of CE. With covariance analysis for repeated measurements, it appeared that the regression of ^{10}log(Mf) on ^{10}log(CE) was significant ($p < 0.036$). However, the difference between the ^{10}log(Mf) measured before and after treatment and corrected for the regression of ^{10}log(Mf) and ^{10}log(CE) was not significant ($p < 0.125$). Thus, the apparent decrease of the Mf after treatment can fully be accounted for by the observed increase in CE after treatment.

DISCUSSION

Several laboratories have used the HPRT clonal assay to measure Mfs in normal adult donors. Results are quite

consistent, with mean Mfs ranging from 3 to 10.1×10^{-6} (*2,8,12–16*).

In our studies in normal adult donors, a mean Mf of 9.8×10^{-6} was found for non-smokers and 10.7×10^{-6} for smokers (data to be published elsewhere). Inspection of Mfs in individual donors indicated considerable differences. The highest Mfs were usually found in donors with relatively low CEs. In the literature, it has been reported that Mfs are strongly but inversely correlated with CEs (*11*). Because Mfs are measured by calculating the ratio between CEs with and without 6-TG selection, Mfs tend to be high when CEs in non-selection plates are low. In our laboratory, we have used the HPRT clonal assay routinely for several years and have observed that CEs for some donors (not only patients) were low. Parallel experiments with "high CE" donors and "low CE" donors indicated that low CEs are by no means always due to technical factors such as sub-optimal culture conditions. Therefore, we do not favor the elimination of data from these low CE donors. In our opinion, Mfs should always be presented together with their CEs.

A striking feature of the mutation studies with technetium patients is the fact that the CEs in nonselective medium (−TG) were generally lower (mean CE patients: 16.3%) than in our control population (mean CE total data: 40.9%, data not shown). The fact that Mfs in patients and controls were determined with exactly the same culture methods and media seems to indicate that the lower CEs in patients must be attributed to other factors such as the higher mean age of the patients (mean age: 55.3 yr; mean age controls: 37.1 yr) and/or their health condition. We believe, however, that the lower CEs in this group of patients should have no influence on the outcome of the present study because pre- and post-treatment samples were always from the same donor.

It is of interest to note that CEs after ^{99m}Tc treatment were, on average, twice as high as before treatment, although the difference was not statistically significant ($p = 0.08$; two-sided test). The reason(s) for the observed difference in CEs is not known. Obvious differences between pre- and post-treatment samples are that: (1) pre-treatment samples were not exposed to irradiation and (2) pre-treatment samples have not been exposed to $SnCl_2$ prior to treatment with ^{99m}Tc. With respect to the first difference, it is difficult to believe that the low dose of ^{99m}Tc would kill lymphocytes and thereby stimulate production of new relatively undamaged cells with a better CE. It is also difficult to understand how exposure of lymphocytes to $SnCl_2$ could induce a better CE.

It has been reported that $SnCl_2$ is readily taken up by human lymphocytes in vitro and that it can cause damage to DNA as demonstrated with DNA unwinding techniques (*17*). Such damage is induced at concentrations of the chemical that are about six times higher than those in patients.

Furthermore, $SnCl_2$ can interfere with the ability of lymphocytes to be stimulated by the polyvalent mitogen Concanavalin A (but in our studies PHA has been used). Such an effect is only demonstrable in vitro at a concentration that is about four times higher than in blood from patients. This effect is in fact also in opposition to our findings; namely, a stimulation of mitotic activity. DNA damaging effects of $SnCl_2$ have furthermore been reported for Chinese hamster ovary cells but again at concentrations at least one order of magnitude above the concentration in human blood (*18*).

Our observation of a doubling of the mean CE value in post-treatment samples stands in contrast with that of Seifert et al. (*2*) who found in their patients a 50% reduction of the mean CE value in post-treatment samples. They explained this reduction by assuming that there had been a loss of activity of T-lymphocyte growth factor during the time interval between pre- and post-treatment samples. In our experiments, the same batch of growth factor was used for the pre- and post-treatment samples and, apparently this has not resulted in a decrease of the CE value in post-treatment samples, but rather in an increase.

Our observations and data from the literature suggest that Mfs are inversely correlated with CEs. Therefore, the question can be asked whether our finding of a higher mean Mf value before rather than after treatment with ^{99m}Tc should not be attributed to the fact that CEs for pre-treatment samples were often lower than those for post-treatment samples. We have analyzed this problem by correcting the difference between the $^{10}\log(Mf)$ measured before and after treatment for the regression of $^{10}\log(Mf)$ on $^{10}\log(CE)$. After this procedure, the decrease in Mf after treatment was no longer statistically significant ($p < 0.125$).

Our findings are at variance with those published by Seifert et al. (*2*) who reported an increase of Mfs in patients following exposure to a very low dose of ionizing radiation from ^{99m}Tc ($p < 0.007$, two-tailed paired t-test on transformed data). However, their data, like ours, show a significant regression between $^{10}\log(Mf)$ and $^{10}\log(CE)$ (correlation = −0.76, $p < 0.018$). If we take into account this regression, then the differences between $^{10}\log(Mf)$ before and after treatment are no longer statistically significant ($p < 0.268$). This means that the increase in Mfs that they observed after treatment with ^{99m}Tc can, in our opinion, also be attributed to an effect of CE.

We therefore conclude that nuclear angiography with ^{99m}Tc, which represents a frequently used diagnostic test involving a relatively high dose of irradiation in comparison to other diagnostic tests with radionuclides, does not constitute a genetic risk as measured by induction of $HPRT^-$ mutants in T-lymphocytes.

ACKNOWLEDGMENTS

The authors would like to thank Joyphi Thijssen, Huib van Mossel, and Henriëtte Schoemaker for their technical assistance.

This work was supported by the Euratom Radiation Protection Programme (Contract no.: B16-E-166-NL) and the J.A. Cohen Institute, Interuniversity Institute for Radiopathology and Radiation Protection, Leiden, The Netherlands.

REFERENCES

1. ICRP. *Radiation dose to patients from radiopharmaceuticals.* Publication 53. Oxford: Pergamon Press; 1988.
2. Seifert AM, Bradley WEC, Messing K. Exposure of nuclear medicine patients to ionizing radiation is associated with rises in HPRT-mutant frequency in peripheral T-lymphocytes. *Mutation Res* 1987;191:57–63.
3. O'Neill JP, McGinnis MJ, Berman JK, Sullivan LM, Nicklas JA, Albertini RJ. Refinement of a T-lymphocyte cloning assay to quantify the in vivo thioguanine-resistant mutant frequency in humans. *Mutagenesis* 1987;2:87–94.
4. Lacombe P, Kraus L, Fay M, Pocidalo JJ. Lymphocyte glutathione status in relation to their Con A proliferative response. *FEBS Lett* 1985;191:227–230.
5. Kraus L, Gougerot-Pocidalo MA, Lacombe P, Pocidalo JJ. Depression of ConA proliferative response of immune cells by in vitro hyperoxic exposure: protective effects of thiol. *Int J Immunopharmacol* 1985;7:753–760.
6. Rosenberg SA, Lotze MT, Muul LM, et al. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. *N Engl J Med* 1987;316:889–897.
7. Hegge FN, Hamilton GW, Larson SM. Cardiac chamber imaging: a comparison of red blood cells labelled with Tc-99m in vitro and in vivo. *J Nucl Med* 1978;19:129–134.
8. O'Neill JP, Sullivan JP, Booker JK, et al. Longitudinal study of the in vivo HPRT mutant frequency in human T-lymphocytes as determined by a cell cloning assay. *Environ and Molec Mutagenesis* 1989;13:289–293.
9. Furth EM, Thilly WG, Penham BA, Liber HL, Rand WM. Quantitative assay for mutation in diploid human lymphoblasts using microtiterplates. *Anal Biochem* 1981;110:1–8.
10. Winer BJ. *Statistical principles in experimental design*, second edition. New York: McGraw-Hill; 1971:796–809.
11. Albertini RJ. Somatic gene mutations in vivo as indicated by the 6-thioguanine-resistant T-lymphocytes in human blood. *Mutation Res* 1985;150:411–422.
12. Albertini RJ, Sullivan LM, Berman JK, et al. Mutagenicity monitoring in humans by autoradiographic assay for human T-lymphocytes. *Mutation Res* 1988;204:481–492.
13. Hakado M, Akiyama M, Kyoizumi S, Kobuke K, Awa AA, Yamakido M. Measurement of in vivo HGPRT-deficient mutant cell frequency using a modified method for cloning human peripheral blood T-lymphocytes. *Mutation Res* 1988;197:161–169.
14. Henderson L, Cole H, Cole J, James SE, Green MHL. Detection of somatic mutations in man: evaluation of the microtiter cloning assay for T-lymphocytes. *Mutagenesis* 1986;1:195–200.
15. Messing K, Seifert AM, Bradley WEC. In vivo mutant frequency of technicians professionally exposed to ionizing radiation. In: Sorsa M, Norppa H, eds. *Monitoring of occupational genotoxicants.* New York: Alan R. Liss; 1986:87–97.
16. Seshadri R, Kutlaca RJ, Trainor K, Matthews C, Morley AA. Mutation rate of normal and malignant human lymphocytes. *Cancer Res* 1987;47:407–409.
17. McLean JRN, Birnboim HC, Pontefact R, Kaplan JG. The effect of tin chloride on the structure and function of DNA in human white blood cells. *Chem Biol Interactions* 1983;46:189–200.
18. McLean JRN, Blakey DH, Douglas GR, Kaplan JG. The effect of stannous and stannic (tin) chloride on DNA in Chinese hamster ovary cells. *Mutation Res* 1983;119:195–201.