P-2b-134

Induction of chromosome aberrations in human lymphocytes by technetium-99m. *In vitro* and *in vivo* studies.

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INTRODUCTION

Research on the effects of low-dose exposure to ionizing radiation in human has been widely studied. In diagnostic Nuclear Medecine, calculated doses delivered to whole-body and organs were very low (1) and scintigraphies are considered as safe. Consequently only a few studies concerning the potential biological and genetic effects of radiopharmaceutical administration have been carried out (2, 3)

The aim of this study was to evaluate the potential cytogenetic effects of exposure to a clinical dose of technetium 99m (^{99m}Tc). The induction by ionizing radiations of unstable structural chromosome aberrations (dicentrics, ring and excess acentric fragments) in peripheral blood lymphocytes is considered to be a useful technique to complete physical dosimetry, and presently is the most sensitive biological method to monitor human radiation exposure (4). A specific relationships between activty of ^{99m}Tc and number of unstable chromosomal aberration was established *in vitro*. At our knowledge, such relationships have never been performed with this radioisotope. However, the scoring of chromosome aberrations in human lymphocytes varied with effects of radiation quality, dose and dose rate (5). In this context, whole blood samples from healthy donors were irradiated by external ^{99m}Tc source and frequency of unstable chromosomal aberrations versus activity of ^{99m}Tc was assessed using conventional cytogenetic technique. Unstable chromosome aberrations in peripherical blood lymphocytes from 5 patients exposed to a ^{99m}Tc for bone scintigraphy were also examinated. *In vitro* and *in vivo* results of unstable aberration scoring were compared. Stable aberration scoring (translocations) performed on the same *in vivo* and *in vitro* irradiated blood sample are actualy being carried out in our laboratory, to evaluate the potential genetic risk of ^{99m}Tc exposure. Primilary stable aberration scoring are also presented.

MATERIALS AND METHODS

In vitro external irradiation procedure:

Heparinized whole-blood samples from healthy donors were irradiated by ^{99m}Tc-microspheres (as external irradiation sources) labeled with increasing activities : 0, 17, 32, 141, 296, and 340 MBq for 3 hours at 37°C under gently agitation. After irradiation, labeled microspheres were removed by sedimentation. To invoid inter individual bias, lymphocyte irradiation protocol was performed on the same donor blood. Test of a Poisson distribution (μ test) was assessed to evaluate the homogeneity of the irradiation model .

In vivo irradiation

Protocol of blood samples collection was authorized by the local Ethic Committee (CCPPRB). Patients choosen were volunteer patients (18-60 years old), receiving no medication known to be mutagenetic or carcinogenic, scheduled for benign bone disease scintigraphy. Informed consents were obtained after the nature and possible consequences of the study were explained. The study has yet be carried out for 5 patients. Blood samples were obtained prior as a control, after 3 hours, time corresponding to the end of the medical exam or 6 hours (half-life of ^{99m}Tc) and/or 24 hours (optimal lymphocyte exposure to decaying ^{99m}Tc) after injection of 925 MBq of ^{99m}Tc labeled hydroxy methylene disphophonate (HDP)

Unstable chromosome aberrations scoring:

Control, *in vitro* and *in vivo* irradiated whole-blood sample cultures were carried out in duplicate for 48 hours according to standard cytogenetic procedures (6). Cellular division is stopped in metaphase stage by adding demecolcine for the 3 last hours of the culture. Chromosome slides were obtained after classical hypotonic traitement and lymphocyte metaphases were fixed. The slides were then stained with Fluorescence Plus Giemsa (FPG) allowing distinction between cells in first and cell in second division. Unstable chromosome aberrations, dicentrics, rings, and excess acentric fragments were scored only in first cycle complete metaphases.

Stable chromosome aberrations scoring

Translocation [complete reciprocal translocation (TRc), incomplete reciprocal translocations (TRi), terminal translocation (TT)] scoring was assessed after fluorescence in situ hybridation (FISH) painting. Chromosome painting was performed on metaphase according to Sorokine and coll. (7) and using human whole chromosome specific DNA probes for chromosome 2, 4 and 12 (Vysis, Voisins-Le-Bretonneux, France). Probes bound to chromosome 2 and 12 were detected by FITC fluorescein isothiocyane. Probes specific for chromosome 4 were revealed by rhodamine. Chromosomal DNA was counterstained with 4',6-diamidine-2'-phenylindole

dihyrochloride and metaphases were analysed under fluorescence microscope.

RESULTS

Unstable chromosome aberrations scoring: *In vitro* irradiation

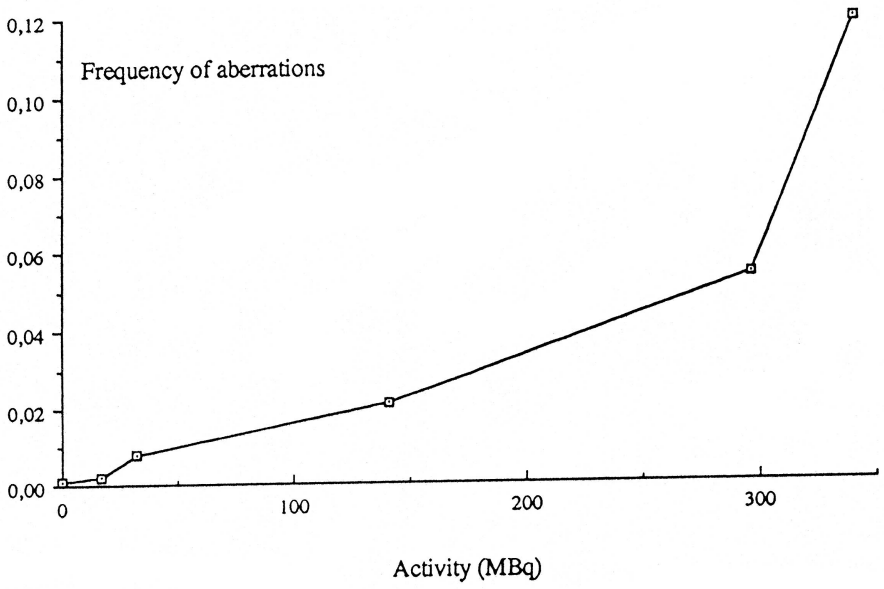

Figure 1. Actvity/frequency of unstable chromosomal aberration curve.

Calibration curve fitting was done using the method of iteratively reweighted least squares. The curve was well fitted following a linear-quadratic model.

$$Y = 1.29.10^{-3} + 7.63.10^{-5} A + 6.17.10^{-7} A^2$$

Calculation of μ values shows that the distribution of unstable chromosome aberration within lymphocytes and for each activities followed a Poisson distribution (μ values <1.96). Consequently *in vitro* irradation of lymphocytes using 99mTc labelled microspheres was homogeneous.

Unstable chromosome aberrations scoring: *In vivo* irradiation

Patients	Sample time	cell scoring	Observed instables aberration	Aberration number
1	H0 H3 H24	250 500 500	 Excess fragment	 2
2	H0 H3	250 500	- -	
3	H0 H3 H24	250 500 500	Excess fragment	1 -
4	H0 H6	250 509	 Excess fragment	 2
5	H0 H24	250 500		

Figure 2. Results of unstable aberration scoring after *in vivo* irradiation.
The number of analyzed metaphases, the number of unstable aberration for each patient and also for each sample time are indicated.

No cytogenetic effect was noted 3, 6, and 24 hours after an administration of ^{99m}Tc-HDP in the 5 patients studied.

Stable chromosome aberrations scoring: *in vitro* irradiation.

Activity (MBq)	Metaphase scoring	Total number of translocations	Freqency of aberration
0	590	1	0.0017
17	1041	4	0.0038
32	975	4	0.0041
296	281	7	0.0249

Figure 3. Primilary results of stable aberration scoring observed at 4 levels of ^{99m}Tc *in vitro* irradiation.

Stable chromosome aberrations scoring : *in vivo* irradiation.
Translocations scoring was performed only on 3 sample times (H0, H6, H24) corresponding to the patients 5 and 6. No ^{99m}Tc -induction of stable aberrations was observed at H6 and H24 versus H0.

DISCUSSION

The activity/effect curve, shows linear quadratic appearance of unstable chromosomal aberrations. The small positive background level of unstable chromosome aberrations, corresponding to spontaneous aberration frequency was 1. 10^{-3} which is in agreement with the literature (5). Analysis of this curve shows the actual appearance of unstable chromosomal aberrations induced by a high concentration of ^{99m}Tc (50 MBq/ml). In this present study, we observed no cytogenetic effect induced by clinical exposure to ^{99m}Tc 3, 6 and 24 hours after administration. As predicted by the *in vitro* curve, this result is consistent with low detectable blood concentrations of ^{99m}Tc after *in vivo* injection of ^{99m}Tc-RP. This result is also in agreement with previous works (3) showing no detectable increase in either chromosome damage or mutation at the hprt locus in peripherical lymphocytes after an *in vivo* low dose exposure to ^{99m}Tc. *In vivo* exposure using another radionuclide used in diagnostic Nuclear Medicine, thallium 201, reported similar conclusions (8). *In vivo* administration of ^{99m}Tc-RP does not appear to involve cytogenetic risks.

Stable chromosome aberrations are presently being carried out in our laboratory. Primilary results obtained after *in vitro* irradation show that ^{99m}Tc is able to produce such aberrations. No stable chromosomal aberrations seem to be induce after administration of ^{99m}Tc-HDP in the 2 patients studied.

However these primilary results need further confirmation.

REFERENCES

1. International Committee on radiation Protection. *Radiation dose to patients from radiopharmaceuticals.* ICRP. Publ. 53, Pergamon, Oxford, UK (1988)
2. C.de Labriolle-Vaylet, N.Colas-Linhart, M.Sala-Trepat, A.Petiet, P.Voisin and B.Bok. *Biological consequences of the heterogeneous irradiation of lymphocytes during technetium-99m hexamethylpropylene amine oxime white blood cell labelling*. Eur. J. Nucl. Med. 25, 1423-1428 (1998).
3. K.T.Kelsey, K.J.Donohoe, A.Memisoglu, B.Baxter, M.Caggana and H.LLiber. *In vivo exposure of human lymphocytes to technetium 99m in nuclear medicine patients does not induce detectable genetic effects*. Mutation Res. 260, 239-246 (1991).
4. M.T.Doloy, J.L.Malarbet, G.Guedeney, M.Bourguignon, A.Leroy and R.Masse. *Use of unstable chromosome aberration for biological dosimetry after the first post irradiation mitosis*. Radiat. Res. 125, 141-151 (1991)..
5. D.C.Lloyd, A.A.Edwards, J.S.Prosser and M.J.Corp. *The dose response relationship obtained at constant irradiation times for induction of chromosome aberrations in human lymphocytes by cobalt-60 gamma rays.* Radiat Environ. Biophys. 23, 179-189 (1984).
6. AIEA(Agence Internationale de l'Energie Atomique). *Biological dosimetry : chromosome aberration analysis for dose assessment*.Technical report series. 260 (1984)**.**
7. Sorokine-Dism*. Is FISH painting an appropriate biological marker for doses estimates of suspected accidental radiation overexposure ? A rewiew of cases investigated in France from 1995 to 1996.* Envir. Health Perspectives. 105 (6) (1996).
8. M.Bachand, A.M.Seifert and K.Messing. *Nuclear medicine patient do not have higher mutant frequencies after exposure to thallium-201*. Mutation Res. 262, 1-6 (1991).