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

Nelly Jacquet¹, F. Guiraud-Vitaux², Anne Petiet¹, A. Leroy³, P. Voisin³, Nicole Colas-Linhart¹.

¹ Laboratoire de Chimie et Biophysique des Traceurs. Faculté de Médecine Xavier Bichat. 75018 Paris, France.
² Service des Isotopes, CHI de Le Raincy-Montfermeil. 93370 Montfermeil, France.
³ Laboratoire de Dosimétrie Biologique Multiparamétrique, IPSN, Fontenay-aux-Roses, France.

**INTRODUCTION**

Research on the effects of low-dose exposure to ionizing radiation in human has been widely studied. In diagnostic Nuclear Medicine, 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 (⁹⁹mTc). 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 activity of ⁹⁹mTc 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 ⁹⁹mTc source and frequency of unstable chromosomal aberrations versus activity of ⁹⁹mTc was assessed using conventional cytogenetic technique. Unstable chromosome aberrations in peripheral blood lymphocytes from 5 patients exposed to a ⁹⁹mTc for bone scintigraphy were also examinated. 

**MATERIALS AND METHODS**

*In vitro* external irradiation procedure:

Heparinized whole-blood samples from healthy donors were irradiated by ⁹⁹mTc-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 avoid 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 ⁹⁹mTc) and/or 24 hours (optimal lymphocyte exposure to decaying ⁹⁹mTc) after injection of 925 MBq of ⁹⁹mTc 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 treatment 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 isothiocyante. Probes specific for chromosome 4 were revealed by rhodamine. Chromosomal DNA was counterstained with 4’,6-diamidine-2’-phenylindole
dihydrochloride and metaphases were analysed under fluorescence microscope.

RESULTS

Unstable chromosome aberrations scoring: *In vitro* irradiation

![Graph showing frequency of aberrations vs activity (MBq)](image)

**Figure 1. Activity/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.

\[ Y = 1.29 \times 10^{-3} + 7.63 \times 10^{-5} A + 6.17 \times 10^{-7} A^2 \]

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* irradiation of lymphocytes using 99mTc labelled microspheres was homogeneous.

Unstable chromosome aberrations scoring: *In vivo* irradiation

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sample time</th>
<th>cell scoring</th>
<th>Observed instables aberration</th>
<th>Aberration number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H0</td>
<td>250</td>
<td>Excess fragment</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>H3</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H24</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>H0</td>
<td>250</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>H3</td>
<td>500</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>H0</td>
<td>250</td>
<td>Excess fragment</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>H3</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H24</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
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<td></td>
<td>H6</td>
<td>509</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H24</td>
<td>500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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 99mTc-HDP in the 5 patients studied.

Stable chromosome aberrations scoring: *in vitro* irradiation.
<table>
<thead>
<tr>
<th>Activity (MBq)</th>
<th>Metaphase scoring</th>
<th>Total number of translocations</th>
<th>Frequency of aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>590</td>
<td>1</td>
<td>0.0017</td>
</tr>
<tr>
<td>17</td>
<td>1041</td>
<td>4</td>
<td>0.0038</td>
</tr>
<tr>
<td>32</td>
<td>975</td>
<td>4</td>
<td>0.0041</td>
</tr>
<tr>
<td>296</td>
<td>281</td>
<td>7</td>
<td>0.0249</td>
</tr>
</tbody>
</table>

Figure 3. Primarily 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 peripheral 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. Primarily results obtained after in vitro irradiation show that $^{99m}$Tc is able to produce such aberrations. No stable chromosomal aberrations seem to be induced after administration of $^{99m}$Tc-HDP in the 2 patients studied.

However these primarily results need further confirmation.

REFERENCES