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In Vivo Mutagenicity and Clastogenicity of Ionizing
Radiation in Nuclear Medicine

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I. Technical Progress Report

A. Overall Objectives and Specific Aims

The overall goal of our research remains to investigate the mutagenic and clastogenic effects of exposure to low levels of ionizing radiation to human lymphocytes.

Principally, we are studying hospital patients referred to a nuclear medicine department for diagnostic cardiac imaging and nuclear medicine technologists who administer radionuclides.

Methods for examining fresh human peripheral blood lymphocytes for the presence of structural chromosome aberrations induced by ionizing radiation have been in common use for many years. Recently, techniques have been developed which allow direct measurement of gene mutation in the same cells. Thus it has become possible to investigate these two distinct genetic changes in parallel in various human populations.

We are utilizing these endpoints to examine sets of individuals who have been exposed to ionizing radiation as a result of medical procedures. The cohorts we are studying include:

- 1) Nuclear medicine technicians who are exposed to a variety of sources of ionizing radiation through the preparation and administration of diagnostic radionuclides. Controls for this group are a set of physical therapists working in the same hospital environment.

2) Two sets of nuclear medicine patients, studied before and after cardiac imaging. One set receives Technetium-99m, which emits the majority of its energy as a gamma-ray. The second set receives Thallium-201, which emits a sizeable fraction of its energy as a shower of Auger electrons.

3) Sets of controls, matched 1:1 to exposed individuals for disease, smoking, age, gender and socio-economic status, since these factors may be associated with one or both laboratory endpoints.

4) A new group is a set of Hodgkins disease patients, who have been treated with high doses of ionizing radiation during the course of radiotherapy. This group has been included to serve as a positive control for the genetic effects of radiation.

For each individual included in the study, we are determining the frequency of hprt mutants as well as the frequency of chromosome aberrations in isolated peripheral blood lymphocytes. In cases where a significantly elevated mutant frequency is observed, we will isolate individual mutant colonies and characterize them with molecular biologic techniques to determine whether the mutants arose from independent mutations.

B. Progress Report for Year 02.

Significant progress has been made in accomplishing the objectives and aims summarized above. Emphasis in the first year, as described in the first progress report, was on optimization of the hprt mutation assay, measurement of mutant frequencies in patients imaged with thallium-201, and measurement of mutant frequencies in controls. Emphasis in the second year has been on measurements of 1) chromosome aberrations in patients imaged with thallium-201, 2) mutant frequencies in patients imaged with technetium-99, 3) mutant frequencies in nuclear medicine technicians and physical therapists, and 4) mutant frequencies in patients treated for Hodgkins disease with radiotherapy. The progress in these areas is described below in more detail.

1. Chromosome aberrations in patients imaged with thallium-201

In the first progress report, we presented data showing that there was no effect of thallium-201 imaging on the frequency of mutants at the hprt locus. We have now completed an analysis of chromosome aberrations in these same patients (Table 1). The mean aberration frequency in lymphocytes exposed for 24 hr to thallium-201 did not differ from the pre-exposure mean aberration frequency. In addition, there was no significant difference in aberration frequency in individuals restudied at 1 month post-imaging, although there was a suggestion of an increase at this time

TABLE 1. Frequency of Chromosome-type Aberrations in First-Division Metaphases Before and After Thallium-201 Exposure

Subject	<u>Before</u>		<u>After</u>		<u>1 Month After</u>	
	Metaphases Examined	Total Aberrations	Metaphases Examined	Total Aberrations	Metaphases Examined	Total Aberrations
1	200	1	200	1	200	1
2	200	2	200	2	200	1
3	200	1	200	1	200	1
4	200	1	200	0	200	3
5	200	0	200	1	200	0
6	200	1	200	0	200	1
7	200	0	200	1	200	1
8	200	0	200	2	200	4
11	200	0	200	1	200	2
12	200	0	200	1	200	1
Total	2000	6	2000	10	2000	15

point. However, since studies with numerous other agents predicts that the maximal expression of induced chromosome aberrations would be at the 24 hr time point, and since there were no significant increases in any particular individual, it seems unlikely that thallium-201 induced chromosomal damage in lymphocytes. All of the work with this agent is described in detail in the appended manuscript (Appendix 1), which has been submitted for publication.

2. Mutant frequencies in patients imaged with technetium-99

As reported previously, patients were enrolled in the program with the help of Dr. Kevin Donohoe of the Nuclear Medicine Division of the Beth Israel Hospital in Boston, MA. Blood samples were taken immediately before the imaging procedure, and 60 min later, before appreciable renal clearance had occurred. This second sample was held for 24 hr prior to processing, and is referred to as the 24 hr time point. We have also procured 1 month samples from as many patients as possible. However, some patients decided to not continue their participation in the study. Others had additional medical procedures (e.g., x-rays, chemotherapy) or new medications added which might be expected to contribute to elevated frequencies of mutations or chromosome aberrations; these patients were dropped from our study.

Table 2 summarizes results obtained with the hprt mutation assay in 34 patients imaged with technetium-99. In 12 of these patients, data has been obtained after 1 month.

There is a suggestion of an elevated mean mutant frequency after treatment with this radionuclide, especially at the 1 month time point. More work is required to substantiate this, and this will constitute a major effort in year 03.

Chromosome aberration analysis is well underway in this group of patients. Slides have been coded and are being analyzed on an ongoing basis. We are scoring slides for those patients who have complete MF data (before, 24 hour and 1 month time points), analyzing 500 metaphases per patient, per time point. We will decode the data upon completion of scoring.

TABLE 2. hprt mutant frequency after Tc-99m imaging

ID#	PRE MF ($\times 10^{-6}$)	24 hr MF ($\times 10^{-6}$)	1 mo MF ($\times 10^{-6}$)
AT1	0.6	1.2	1.5
FC1	1.6	1.1	5.3
PS1	4.1	3.4	3.0
VT1	4.2	3.6	2.1
HM1	2.3	1.8	7.5
BT1	0.8	2.4	9.3
JW1	1.1	1.9	2.4
AC1	1.9	0.9	0.2
WL1	1.3	1.6	6.2
KA1	2.5	3.1	3.6
HE1	3.5	4.7	1.9
FN1	3.4	2.9	1.1
CB1	0.5	0.6	
FC1	2.4	1.4	
EN1	2.0	2.6	
HM1	9.9	9.2	
JM1	3.2	2.4	
AS1	6.7	2.9	
AD1	8.4	8.9	
CB2	4.6	3.1	
AJ1	1.3	0.2	
JQ1	0.5	3.2	
WT1	4.1	0.4	
LG1	3.0	1.3	
PD1	3.4	17.4	
VK1	0.5	1.4	
AS1	0.5	1.7	
IL1	0.4	0.3	
TJ1	3.7	3.0	
MR1	0.6	6.7	
GC1	2.1	1.6	
HP1	2.9	4.3	
JL1	1.1	1.5	
RP1	7.3	1.1	
Overall Mean	2.8	3.1	3.7
+/- STD	2.3	3.3	2.8
Mean of Twelve	2.3	2.4	3.7
+/- STD	1.3	1.2	2.8

3. Mutant frequencies in nuclear medicine technicians and physical therapists

We have examined mutant frequencies in 9 nuclear medicine technicians and 10 physical therapists. As shown in Table 3, there is no evidence that handling radionuclides results in elevated mutant frequencies.

TABLE 3. Mutant frequencies in nuclear medicine technicians and physical therapists

<u>Nuclear Medicine Techs</u>		<u>Physical Therapists</u>	
MF x 10 ⁶	PE	MF x 10 ⁶	PE
8.7	0.016	3.1	0.028
4.9	0.083	2.4	0.179
2.7	0.100	2.0	0.044
2.2	0.061	4.8	0.075
0.7	0.174	6.3	0.083
4.5	0.057	3.3	0.026
6.4	0.263	0.9	0.094
1.7	0.515	4.9	0.107
2.6	0.402	2.5	0.138
		3.3	0.104
		6.4	0.068
MEAN	3.8	3.6	
STD	2.5	1.8	

MF is mutant frequency. PE is plating efficiency. STD is one standard deviation of the mean.

As a result of the data presented in Table 3, we feel that further work on mutation frequencies in nuclear medicine technicians is not warranted.

4. Mutant frequencies in patients with Hodgkins disease who received radiotherapy and/or chemotherapy.

Since, as is noted above and in Appendix 1, results from the study of thallium-201-exposed patients revealed no effect of low dose radiation exposure on mutant fraction, we felt it important to add a postive control group to our series of investigations. The major criteria for selection of a group for study as positive control included:

- i) The ability to complete this work within budgetary constraints.
- ii) Exposure to doses of radiation in excess of that which is established to be mutagenic in peripheral blood lymphocytes.
- iii) Comparability to groups previously studied by other investigators.
- iv) Access to a large number of individuals for study.
- v) Potential for expansion of the study, as data is generated, to investigate the relationship of somatic mutation to radiation exposure as well as to the risk of an adverse health outcome (cancer).

The first criteria was met through the addition of a graduate student to our research personnel. NIH training funds have been added to the support of this portion of the work.

The additional major selection criteria were considered and patients with Hodgkin's disease (HD) deemed most appropriate for study. These patients receive therapeutic x-ray doses similar to cancer patients previously studied by other groups (breast cancer patients studied by Messing and Bradley and a variety of patients receiving cancer therapy study by Albertini's group). Large numbers of Hodgkin's disease patients are treated and followed at the Harvard Joint Center for Radiation Therapy and we have a long history of collaboration with these researchers and clinicians. In addition, HD patients receive varied treatments, including radiation alone, radiation plus chemotherapuetics and chemotherapy alone. Hence, while we are most interested in radiation exposure, this work will provide a more general positive control for our work. An additional advantage of study of HD patients, is that they have a very high 5 year survival rate. They also have defined, elevated second cancer risks (which are attributable to the therapy).

In the past year, we have studied 86 HD patients and 71 controls in an effort to expand the scope of the work to include an investigation which serves as a positive control for the series of low dose exposure investigations. The results of the study are presented in full in the appended manuscript, currently being reviewed for publication (Appendix 2). Briefly, we found that 3 of the 18 MFs in newly diagnosed, untreated HD patients were elevated above

control. In the 45 patients who received radiation therapy alone, 14 had persistently elevated MFs. In 23 patients treated with both x-rays and chemotherapy, 10 were observed to have MFs above control levels. In controls, our range of MFs was comparable to those reported by other groups and smoking was associated with a dose-related rise in MF.

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