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Chromosomal aberrations and *hprt* mutant frequencies in long-term American thorotrast survivors

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Abstract.
Purpose: Patients injected with thorotrast, a radiologic contrast medium used from the 1920s to early 1950s, received chronic internal exposure to thorium-232, an α-emitter. Epidemiologic studies have observed markedly elevated risks of death from hepatic and hematologic cancers and extensive chromosomal damage among these patients. Few investigations have correlated multiple measures of genetic damage to determine whether these have independent induction kinetics. The distribution of chromosomal aberrations (CA) and mutant frequencies (MF) at the hypoxanthine phosphoribosyltransferase (*hprt*) locus was evaluated in eight long-term thorotrast survivors (mean exposure time = 47.4 years) and five individuals who received a nonradioactive contrast medium during the same era.
Materials and methods: Peripheral blood lymphocytes were harvested from whole blood, CA were scored in 500 complete metaphases and a clonal assay was used to determine *hprt* MF. Symmetrical aberrations were not evaluated. Differences in frequencies and correlations between endpoints were assessed using nonparametric methods.
Results: Thorotrast-exposed individuals differed from the comparison group in total number of multicentrics and centric and acentric rings (per 500 cells [median, mean ± sd]: 11, 18.3 ± 23.1 vs 2, 2.4 ± 1.1, $p = 0.04$). There was no difference between the groups on *hprt* MF (12.6, 15.9 ± 13.5 vs 16.6, 14.0 ± 8.8[$\times 10^{-6}$]; $p = 1.0$). Among the exposed, *hprt* MF was moderately correlated with the frequency of asymmetrical chromosomal aberrations, although the association was not statistically significant.
Conclusion: Noting the limitations of small samples, long-term thorotrast survivors were observed to be at an increased risk for genetic damage.

1. Introduction

Patients injected with thorotrast, a contrast medium used worldwide from the 1920s to the early 1950s to enhance the quality of diagnostic radiographs, received chronic internal exposure to thorium-232, a naturally occurring α-emitter (Travis *et al.* 1992). Macrophages engulf and distribute thorotrast to organs of the reticuloendothelial system, including the liver, spleen and bone marrow (Kaul and Noffz 1978). These patients' cells experience ongoing exposure to α-particles leading to the continual development of chromosomal damage and cell killing.

Epidemiologic studies have observed a markedly elevated incidence of and mortality from hepatic and hematologic cancers among Thorotrast-exposed patients (da Motta *et al.* 1979, Mori *et al.* 1983, van Kaick *et al.* 1983, van Kaick *et al.* 1991, Andersson and Storm 1992, Andersson *et al.* 1994, Andersson *et al.* 1995, Goldman *et al.* 1997). Extensive chromosomal aberrations and sister-chromatid exchanges have been reported in peripheral blood lymphocytes from thorotrast survivors (Fischer *et al.* 1966, Buckton *et al.* 1967, Fischer and Golob 1967, Kemmer *et al.* 1971, Janower *et al.* 1972, Ishihara *et al.* 1978, Teixeira-Pinto and Azevedo e Silva 1979, Steinstrasser 1981a,b, Sasaki *et al.* 1987). Among 25 members of the American cohort who were examined in the early 1970s, the average number of chromosomal breaks (rings, dicentrics and rearrangements counted as two breaks and fragments as one break) was 34 per 100 cells (Janower *et al.* 1972). The proportion among the comparison group was 2 per 100 cells.

Characteristic of high linear-energy-transfer radiation, the extent of chromosomal damage per peripheral blood lymphocyte from thorotrast patients is greater than would be expected based on the Poisson distribution (Sasaki *et al.* 1987). Clear dose–response relationships between absorbed dose of radiation and

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number of chromosomal abnormalities generally have not been observed (Kemmer *et al.* 1979), possibly because of the complex relationship between chromosomal damage and cell killing. Whether various mechanisms of genetic damage in thorotrast-exposed individuals have independent induction kinetics has not been addressed.

Presented in this report is the distribution of chromosomal aberrations and mutant frequencies at the hypoxanthine phosphoribosyltransferase (*hprt*) locus in peripheral blood lymphocytes from eight long-term American thorotrast survivors (mean exposure time = 47.4 years) and five individuals who underwent similar diagnostic procedures with a nonradioactive contrast medium during the same era. In addition, correlations among specific types of chromosomal aberrations and *hprt* mutant frequency are assessed.

2. Materials and methods

2.1. *Study population*

Participants were identified from among members of the American thorotrast cohort. Cohort members were men and women who underwent cerebral angiography with intravascular administration of thorotrast for neurological indications from the late 1930s to the early 1950s at one of three hospitals and were originally identified in the 1960s through medical record review as described previously (Janower *et al.* 1972). Patients who underwent angiography with diodrast, a nonradioactive contrast medium, for similar indications in the late 1940s and early 1950s were also identified. A total of 723 thorotrast and 315 diodrast patients were ascertained.

With follow-up through 1995, current vital status was known for 92%; of these 90% of the thorotrast and 75% of the diodrast group were deceased (Goldman *et al.* 1997). Surviving cohort members were identified through motor vehicle registries, town censuses, and telephone books. As they were identified, surviving cohort members were contacted by mail or telephone to invite them to provide a blood sample for this substudy. Eight of 11 people with thorotrast exposure and five of six with diodrast exposure who were invited to participate agreed and successfully provided a blood sample between November 1993 and November 1994. Venous blood was drawn into 10-ml tubes containing sodium EDTA and was processed immediately for the *hprt* mutant frequency assay in the laboratory of Dr Kelsey or shipped by overnight courier at room temperature in an insulated biohazard container to the laboratory of Dr Wiencke for evaluation of aberrations.

2.2. *Chromosomal aberrations*

Within 36 h of blood drawing, whole blood (0.5 ml) was added to 4.5 ml of RPMI 1640 medium containing 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin, and 2% phytohemagglutinin M (Gibco). The blood was cultured at 37°C in 1-oz glass prescription bottles for 48 h. Colcemid (final concentration 2×10^{-7} M) was added to arrest cells in mitosis and cells were harvested 2 h later. Fixation was performed by standard cytological procedures; the cells were exposed to a 0.075 M KCl solution for 8 min to spread chromosomes and then fixed in a methanol–acetic acid solution (3:1). Cytological preparations were made by placing cells on wet slides and staining with Giemsa. Chromosomal aberrations were scored in 500 complete metaphases (300 for one participant). Aberration data were not available for one thorotrast participant. Scored were chromatid aberrations, including chromatid deletions and isochromatid deletions, and asymmetrical chromosome aberrations, including tricentrics, dicentrics, centric rings, acentric rings, interstitial deletions and terminal deletions. Chromatid gaps, defined as achromatic lesions less than the width of the chromatid were not recorded. To obtain an overall measure of unstable damage, the sum of centric rings, acentric rings, dicentrics and tricentrics, which were counted as two dicentrics each, were calculated. To calculate the number of terminal deletions that was not the result of multicentric formation, the number of dicentrics and twice the number of tricentrics were subtracted from the observed number of terminal deletions. If the number of multicentrics exceeded the number of terminal deletions, the remaining excess was subtracted from the number of interstitial deletions. The blood samples were received coded and the cells were scored without knowledge of the participant's exposure status.

2.3. *Hprt mutant frequency*

Mononuclear cells were isolated from peripheral blood by Ficoll-Hypaque separation medium. After washing the cells twice with Earle's balanced salt solution (EBSS), they were placed in a culture medium [RPMI 1640, 20% HL-1, 10% fetal bovine serum (Hyclone), penicillin (100 units/ml), and streptomycin (100 μg/ml)] along with 1 μg/ml of phytohemagglutinin (Burroughs–Wellcome). The cultures were kept in 5% CO_2 at 37°C for 40 h. The cells were counted on a hemacytometer and plated in a culture medium (same composition as above) with the addition of 0.125 μg/ml phytohemagglutinin

and 20% T-cell growth factor. The T-cell growth factor was derived from the supernatant of lymphokine-activated killer cells and was generously provided by Drs Timothy Eberlein and Deric D. Schoof (Brigham and Women's Hospital, Boston, MA, USA) and Dr Michael Atkins (Tufts University Medical School, Boston, MA, USA). Feeder cells, which were TK-6 human B-lymphoblastoid cells irradiated at 5000 cGy, were added at a concentration of 1×10^4 cells in culture medium to each microtiter well. Using serial dilution, 2×10^4 lymphocytes were plated into each well along with 6-thioguanine (20 μM) as the selective agent. 2, 5 and 10 lymphocytes per well were also plated without the selective agent to assess cloning efficiency. After 14 days, the plates were scored by one reader blinded to the participants' exposure status. The cloning efficiency was estimated assuming a Poisson distribution ($P_o = e^{-x}$): cloning efficiency $= -$ [ln(fraction negative cells)]/(number of cells per well). The mutant frequency was calculated as the ratio of the cloning efficiency with the selective agent to the cloning efficiency without the selective agent. *Hprt* mutant frequency was not available for two thorotrast participants due to poor cloning efficiency and technical problems.

2.4. *Statistical analysis*

Differences in the frequency of chromosomal aberrations expressed per 500 cells scored and *hprt* mutant frequency by thorotrast exposure status were evaluated using the nonparametric Wilcoxon rank sum test (Rosner 1986). Nonparametric Spearman correlation coefficients were calculated for the interrelations among the specific aberrations, *hprt* mutant frequency, volume of thorotrast, and duration of exposure to thorotrast among the thorotrast-exposed individuals (Rosner 1986). Using the χ^2 test for goodness of fit (Rosner 1986), we evaluated whether the observed distribution of unstable damage was consistent with the Poisson distribution or the 0-truncated Poisson distribution, which was previously reported to describe the overdispersion of the number of cells with multiple aberrations associated with internal exposure to thorotrast and α-particles (Sasaki *et al.* 1987). Analyses were conducted using SAS version 6.12 (SAS Institute, Cary, NC, USA) statistical software.

3. Results

Eight thorotrast and five diodrast survivors provided blood samples. The mean age at which the contrast media were administered was 22.9 ± 5.1 years for thorotrast patients and 24.6 ± 6.4 years for diodrast patients; the mean ages at which blood for these analyses was drawn were similar for the two groups (thorotrast: 70.2 ± 5.0 years; diodrast: 69.0 ± 7.8 years). The volume of thorotrast injected ranged from 5 ml to 40 ml among the six survivors for whom volume was recorded in their medical records. Demographic features and the distribution of asymmetrical chromosome and chromatid aberrations are summarized for each participant in table 1.

A total of 3300 and 2500 cells were scored for thorotrast and diodrast participants, respectively. Thorotrast survivors showed a higher frequency of asymmetrical chromosome, but not chromatid aberrations (table 2). In particular, the frequency of multicentrics (dicentrics plus tricentrics) was statistically significantly ($p = 0.03$) greater in the thorotrast group (median, mean ± sd: 11, 15.8 ± 19.0 per 500 cells) compared to the diodrast group (2, 1.6 ± 0.5 per 500 cells). Combining multicentrics and rings to represent unstable aberrations, the frequency differed between the two groups ($p = 0.04$) and ranged from 0.002 to 0.138 per cell (11, 18.3 ± 23.1 per 500 cells) among the thorotrast exposed and 0.002 to 0.008 per cell (2, 2.4 ± 1.1 per 500 cells) among the diodrast exposed. The frequency of multicentrics and rings were moderately correlated (Spearman $r = 0.69$, $p = 0.08$) in those with thorotrast exposure. Among the thorotrast-exposed participants, generally there was little evidence for positive correlations between asymmetrical chromosome aberrations and volume injected or duration of exposure. Across the volume range of 5–40 ml for the six Thorotrast-exposed individuals with known volume, the observed number of centric rings increased from 0 to 2 per 500 cells (Spearman $r = 0.9$, $p = 0.008$).

The distribution of multicentrics and rings among the thorotrast survivors was overdispersed relative to the Poisson distribution ($p = 0.03$), while the distribution among the diodrast group was not inconsistent with the Poisson distribution (table 3). The distribution of unstable aberrations among those exposed to thorotrast was not inconsistent with the 0-truncated Poisson distribution ($p = 0.5$). Possibly because of the small number of thorotrast survivors in the study, there was a deficit of cells with three aberrations and an excess of cells with four aberrations when compared to what would be expected under the 0-truncated Poisson distribution.

The *hprt* mutant frequency did not differ between the thorotrast and diodrast survivors ($p = 1.0$) (table 4). The cloning efficiency was somewhat lower among the thorotrast group, however, cloning efficiency and *hprt* mutant frequency were not correlated ($r = -0.05$, $p = 0.9$). Among five Thorotrast survivors with complete data, *hprt* mutant frequency was

Table 1. Participant characteristics and frequency of asymmetrical chromosome and chromatid aberrations among long-term American thorotrast ($n = 8$) and diodrast ($n = 5$) survivors.

ID	Volume of thorotrast (ml)	Age at injection (years)	Age at evaluation (years)	Sex	Cells (no.)	Aberrations							
						Chromosome				Chromatid			
						Tri	Dic	Rc	Ra	ID	TD	CD	Iso
1	16	20	64	F	500	2	13	1	1	15	10	11	0
2*	Unknown	29	77	F	—	—	—	—	—	—	—	—	—
3	10	18	63	M	500	0	1	0	0	0	8	14	6
4	14	23	71	M	300	0	5	0	0	8	0	13	6
5	24	25	74	F	500	0	3	2	0	0	12	4	0
6	40	17	69	M	500	1	11	2	0	6	0	4	0
7	Unknown	31	75	F	500	3	51	11	1	93	35	2	0
8	5	20	69	F	500	1	9	0	0	8	10	2	0
9	0	19	64	F	500	0	1	0	1	18	12	3	0
10	0	29	75	M	500	0	2	1	0	7	0	10	5†
11	0	17	59	M	500	0	2	0	2	5	7	4	0
12	0	26	69	M	500	0	2	0	0	1	14	3	0
13	0	32	78	F	500	0	1	0	0	0	2	4	0

Abbreviations: T = thorotrast, D = diodrast, F = female, M = male, Tri = tricentrics, Dic = dicentrics, Rc = centric rings, Ra = acentric rings, ID = interstitial deletions, TD = terminal deletions, CD = chromatid deletions and Iso = isochromatid deletions.

*Aberration data not available.

†Individual also had two chromatid exchanges.

Table 2. Mean number of asymmetrical chromosome and chromatid aberrations per 500 metaphases scored among long-term American thorotrast ($n = 7$) and diodrast ($n = 5$) survivors.

Aberration	Thorotrast			Diodrast			p*
	Median	Mean	SD	Median	Mean	SD	
Chromosome							
Tricentrics	1	1.0	1.2	0	0.0	0.0	0.07
Dicentrics	9	13.8	17.0	2	1.6	0.5	0.03
Centric rings	1	2.3	3.9	0	0.2	0.4	0.2
Acentric rings	0	0.3	0.5	0	0.6	0.9	0.6
Dicentrics plus rings†	11	18.3	23.1	2	2.4	1.1	0.04
Interstitial deletions	8	19.3	33.0	5	6.2	7.2	0.6
Terminal deletions	10	10.7	11.8	7	7.0	6.1	0.9
Total chromosome aberrations	22	47.4	65.5	16	15.6	10.7	0.2
Chromatid							
Chromatid deletions	4	8.4	7.5	4	4.8	2.9	0.6
Isochromatid deletions	0	2.3	4.1	0	1.0	2.2	0.7
Exchanges	0	0.0	0.0	0	0.4	0.9	0.3
Total chromatid aberrations	4	10.7	11.3	4	6.2	6.1	0.7

*From the nonparametric Wilcoxon rank sum test.

†Sum of centric rings, acentric rings, dicentrics and tricentrics (counted as two dicentrics each).

moderately, but statistically not significantly, positively correlated with multicentrics (Spearman $r = 0.60$, $p = 0.3$). *Hprt* mutant frequency was not correlated with rings (Spearman $r = -0.05$, $P = 0.9$).

4. Discussion

We evaluated the frequency of chromosomal aberrations and the *hprt* mutant frequency in a small sample of American men and women with 44–52 years of internal exposure to α-particles from the contrast medium thorotrast. Compared to similarly aged participants, who also underwent cerebral angiography with a nonradioactive contrast medium during the same era, the frequency of asymmetrical chromosome, but not chromatid, aberrations was elevated, in particular dicentrics and tricentrics. Thorotrast-exposed participants did not differ from the comparison group with regard to *hprt* mutant frequency.

Table 3. Number of cells with a given number of dicentrics and rings among long-term American thorotrast ($n = 7$) and diodrast ($n = 5$) survivors.

Contrast	Total cells	No. cells with given number of dicentrics and rings					Mean	Variance	Index of dispersion‡	p§
		0	1	2	3	4				
Thorotrast										
Observed	3300	3182	73	15	1	3	0.036	0.056	1.56	
Expected*		3184.1	113.9	2.0	0.02	0.0002				0.03
Expected†	226.9¶	134.9	70.1	18.2	3.2	0.4	0.52			0.5‖
Diodrast										
Observed	2500	2488	12	0	0	0	0.005	0.005	0.96	
Expected*		2488.0	11.9	0.03	0.0001	0				1.0

*The expected frequencies of cells with 0 or 1 or more aberrations was estimated by multiplying the number of cells scored by the probability of observing a given number of aberrations per cell predicted from the Poisson distribution; $\Pr(X = k) = \mu^k \exp(-\mu)/k!$, where μ = mean number of aberrations per cell and k = number of aberrations per cell.

†The mean number of aberrations among the exposed cells was estimated from $-\mu N - X\exp(-\mu) + X = 0$, where μ = mean number of aberrations among the exposed cells, N = the number of observed cells with at least one aberration and X is the total number of observed aberrations. The number of exposed cells was estimated from $N_x = N/(1 - \exp(-\mu))$, where N_x is the number of exposed cells, N = is the number of observed cells with at least one aberration and μ = mean number of aberrations per exposed cell. The expected frequency of exposed cells with 0 aberrations was estimated from $N_0 = N\exp(-\mu)/(1 - \exp(-\mu))$, where N_0 = the expected frequency of exposed cells with 0 aberrations, N = the number of observed cells with at least one aberration, and μ = the mean number of aberrations per exposed cell. The expected frequency of exposed cells with 1 or more aberrations was estimated by multiplying the observed number of cells with at least one aberration by the probability of observing a given number of aberrations per exposed cell predicted from the 0-truncated Poisson distribution; $\Pr(X = k) = \mu^k \exp(-\mu)/(1 - \exp(-\mu))k!$, where μ = mean number of aberrations per exposed cell and k = number of aberrations per exposed cell.

‡Variance/mean.

§From the χ^2 test for goodness of fit after collapsing over cells to obtain an expected of at least 5.

¶Expected number of exposed cells.

‖ Collapsing only over three and four aberrations per cell: $p = 0.4$.

Table 4. *Hprt* mutant frequency (MF) among long-term American thorotrast* ($n = 6$) and diodrast ($n = 5$) survivors.

ID	Contrast	Cloning efficiency (%)	MF ($\times 10^{-6}$)	Mutants (no.)
1	T	10	15.5	12
2	T	14	1.4	2
3	T	53	9.8	38
5	T	10	5.4	1
7	T	14	27	42
8	T	10	36.5	13
Median		12	12.6	12.5
Mean		18.4 ± 17.1	15.9 ± 13.5	18.0 ± 17.8
9	D	12	16.6	15
10	D	36	17.7	23
11	D	9	8.6	3
12	D	38	2.1	3
13	D	50	25.0	85
Median		36	16.6	15
Mean		29.0 ± 17.7	14.0 ± 8.8	25.8 ± 34.2
p†		0.6	1.0	0.6

*Not determined for two Thorotrast survivors because of poor cloning efficiency (4%) or technical problem.

†From the nonparametric Wilcoxon rank sum test.

An increased frequency of unstable chromosome aberrations in thorotrast survivors was noted. This class, which includes multicentrics and rings (Sasaki 1983), usually results in an unequal distribution of genetic material during mitosis and often yields daughter cells that are not viable. The marked increase in chromosomal abnormalities, reflected by the range of multicentrics and rings that were observed among the seven American thorotrast survivors (0.002–0.138 per cell), was similar to that

observed among 63 Japanese thorotrast survivors (0.013–0.355 per cell). The frequency of multicentrics and rings among the diodrast group (0.002–0.008 per cell) was similar to the mean background frequencies reported among an older general population sample in Japan (0.0015 per cell; Tonomura *et al.* 1983) and an adult sample in America (dicentrics 0.0016, rings 0.0002; Bender *et al.* 1988).

The distribution of chromosomal aberrations in lymphocytes of thorotrast-exposed individuals has been observed to be overdispersed relative to the Poisson distribution (Sasaki *et al.* 1987, Littlefield *et al.* 1997). In this small sample of thorotrast survivors we also observed a greater number of cells with two or more multicentrics or rings than expected based on the Poisson distribution; among the diodrast group no more than one chromosomal aberration was observed per cell. Others (Sasaki *et al.* 1987) have proposed that the distribution of chromosomal aberrations in peripheral blood lymphocytes from Thorotrast-exposed individuals is more consistent with the 0-truncated Poisson distribution because the examined cells consist of a mixture of exposed and unexposed cells. Assuming that the exposure is uniform in the pool of exposed cells, we found that the 0-truncated Poisson distribution better predicted the number of cells with one, two, or three or more unstable aberrations.

The *hprt* locus is frequently used to assess the extent of somatic mutations *in vivo* (Mendelsohn 1990). For a group of 48 normal adults, Davies *et al.* (1992) reported the *hprt* mutant frequency to range from 0.48 to 11.53 per million cells. Gender, smoking habits, alcohol, caffeine consumption, or X-ray exposure did not affect the frequency. The frequency, however, did increase with age ($0.09/10^6$ cells/year of age). No difference in *hprt* mutant frequency in peripheral lymphocytes between the thorotrast survivors and diodrast group was observed. The reason for the lack difference is unclear. However, high-LET radiation is relatively more efficient in inducing large chromosome-type lesions and these will not be efficiently detected in the lymphocyte cloning assays that use the *hprt* gene. The induction of point mutations by thorotrast is possible, but is likely to be smaller compared to the overall spectrum of damage. Consistent with this, among the thorotrast survivors, a positive correlation, although not statistically significant, was noted between the frequency of multicentrics, but not rings, and the *hprt* mutant frequency.

This study is limited by the small number of surviving thorotrast-exposed patients available to provide blood samples for this analysis. The statistical power to determine if the distribution of unstable chromosome aberrations was inconsistent with the 0-truncated Poisson distribution was limited. Only qualitative observations about differences in induction kinetics of different types of genetic damage could be made. We had little information on other endogenous and exogenous factors that might increase the burden of DNA damage independent of thorotrast exposure. Finally these participants who survived decades of internal α-particle exposure may differ from those patients who developed exposure-associated fatal cancers, blood dyscrasias, or liver cirrhosis. Sources of variability might include distribution of the contrast medium to target organs, radiation dose delivered to susceptible tissue and inherent factors, such as DNA-repair efficiency and age at first exposure. These factors would likely affect the nature and extent of genetic damage resulting from thorotrast exposure.

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