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Chromosome Aberration, Cancer Mortality and Hormetic Phenomena among Inhabitants in Areas of High Background Radiation in China

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The respective average annual doses are about 330 and 110 mR/yr, in the high background radiation areas (HBRA) in Yangjiang County and the control areas (CA) in Enping and Taishan Counties. Both the HBRA and CA are in Guangdong Province which borders the South China Sea. The frequencies of chromosome aberration in circulating lymphocytes were examined for persons residing in the HBRA and CA. Those in the HBRA had increased frequencies of detectable abnormalities in stable aberrations (translocations and inversions) and unstable aberrations (dicentrics and rings). Previous reports have shown that when samples of circulating lymphocytes taken from inhabitants were tested *in vitro* for mitotic responses to phytohemagglutinin (PHA) and for the degree of unscheduled DNA synthesis (UDS) induced by UV-irradiation, there were higher responsiveness and UDS rates for those in the HBRA than in the CA. In contrast, mortality from all cancers and those from leukemia, breast and lung cancers that are inducible by radiation was not higher in the HBRA. Although the differences in the cancer mortality rates for the HBRA and CA are not significant, the findings are compatible with the assumption that the lower mortality from cancer in the HBRA is the result of the hormetic effects of the three-fold higher dose rate of background radiation in that areas. This assumption requires further study.

INTRODUCTION

At high doses, ionizing radiation increases the incidence of cancer, but the effects of low-dose of radiation have yet to be shown. The cancer risks associated with low level radiation therefore are estimated by extrapolation from high dose data on human cancers[1]. Information on the health status of general populations exposed to low doses of radiation at low rates over many years is scant. The high background radiation areas (HBRA) in Yangjiang County in South China, provide a unique opportunity to study the potential hazard from low-dose radiation delivered over a lifespan. In comparison with high background areas in India and Brazil[2,3], the HBRA in China is superior for an epidemiologic study because the population is homogeneous and very stable. The approximately 80,000 persons who reside in the HBRA are mostly farmers of Han extraction whose families have lived in the same place for many generations[4]. Since 1972, the High Background Radiation Research Group (HBRRG) has been studying the health status of inhabitants of the HBRA

and of neighboring control areas (CA) in Enping and Taishan Counties, Guandong Province, to assess whether the three-fold differences in background radiation between the HBR and control areas would give a detectable increase in detrimental effects[5].

We here summarize the results of studies on chromosome abnormality, cancer mortality and radiation hormesis-like phenomena[6–8].

SUBJECTS AND METHODS

Levels of background radiation[5]

The HBRA studies consist of two regions in Yangjiang County that cover about 500 km^2 in which thorium-containing monazites washed down from nearby heights have raised the level of background radiation to 2.9 times that of nearby control areas that are similar in altitude and population. The average annual whole body exposures from external radiation (primarily gamma rays) are 330 mR/yr in the HBRA and 110 mR/yr in the control areas (CA).

The thorium isotope content of human lung tissue and of Ra-226 and Ra-228 in human teeth and bone as well as the concentrations of thoron and its decay products in indoor and outdoor air in the HBRA are higher than those in the CA by factors of 3 to 8.

Cytogenetic studies

To determine the frequency of chromosome aberrations in circulating lymphocytes, examinees were randomly selected from inhabitants who had been born in the HBRA and CA and lived in the same place thereafter and who were non-smokers (see Table 1 for details).

Preparation of the whole blood microcultures and slides, and the aberration analysis

Table 1. The subjects and methods used for chromosome analysis

Subjects	Medical radiation exposure	Natural dose rate (mGy/yr)	Medium used	Time (hr) before adding colchicine	Method used to prepare specimens
Farmers 16–56 years old	None for past 6 months	2.00–2.19	Eagle's	46	Conventional^{+}
Farmers 16–56 years old	None for past 6 months	2.60–2.80	Eagle's	46	Conventional^{+}
Students 15–16 years old	Never experienced	2.40–2.59	RPMI-1640	44	M-1 Cell^{++}
Women 50–65 years old	Never experienced	1.80–2.80	RPMI-1640	0	M-1 Cell^{++}

$^{+}$ Studies done in 1982.

$^{++}$ Studies done in 1985 and 1990. Only cells at metaphase of the first *in vitro* mitosis were scored.

were done following the standard procedures recommended by WHO[9] (see Table 1 for details). Cells at metaphase were analyzed for all types of structural chromosomal aberration in coded samples taken from each examinee.

Epidemiologic studies of cancer mortality[5]

Data for the period 1970 to 1978 were obtained from a retrospective survey. In 1979, a registry system was established for the HBRA and CA, local physicians being required to report all cancer cases and cancer deaths. An expert group visited the HBRA and CA twice a year to check up and confirm reported diagnoses by interviewing the patients, their relatives or both. All available records of cancer cases were collected from regional and provincial hospitals.

PHA responsiveness of lymphocytes[6,7]

Samples of venous blood were taken from native-born inhabitants who were in good health. Whole blood microculture was used to assess the proliferative response of cultured lymphocytes to phytohemagglutinin (PHA) in the morphologic transformation of lymphocytes over a 72-hour culture period. Transformation into blasts was identified microscopically. The percentage of transformed cells per 200 lymphocytes was used as the standard of PHA responsiveness for each sample.

UDS in lymphocytes[7,8]

Native-born inhabitants were carefully selected so that only non-smokers, non-alcoholics and healthy persons were studied. Mononuclear leukocytes were obtained from heparinized venous blood by sedimentation over Ficoll-urografin. To assess the DNA repair capacity of the leukocytes obtained, we assayed unscheduled DNA synthesis (UDS) *in vitro* by measuring UV-induced ^{3}H-thymidine uptake in the cells' DNA[10].

RESULTS

Cytogenetic findings

Fragments were not included in the statistical analyses. In 1982, the frequencies of chromosome aberration in inhabitants 16–56 years old were analyzed by the conventional method (Table 1). As seen from Table 2, the frequencies of unstable aberrations (dicentrics and rings) were significantly higher in the HBRA than in the CA whereas those of stable aberrations (translocations and inversions) were not.

In 1985 and 1990, the M-1 cell technique that scores only cells at the first metaphase after PHA treatment was used (Table 1). The frequencies of both the stable and unstable aberrations were lower in students 15–16 years old, in both the HBRA and CA, than were those in women 50–65 years old (Table 3). In contrast, the frequencies of stable aberrations in HBRA inhabitants (young students and elderly women) were significantly higher than those in the CA; but, for unstable aberrations, there was a significant difference for the

Table 2. Chromosome aberrations in lymphocytes of HBRA and CA inhabitants 16–56 years old

Area	Dose rate (mGy/yr)	Subjects examined	Cells scored	Frequency (/1000 cells)	
				[dic+r]$^{+}$	[t+inv]$^{+}$
Control	0.70–0.79	104	20,778	0.05±0.05	0.00
High background	2.00–2.19	106	21,144	0.19±0.10	0.05±0.05
High background	2.60–2.80	120	24,000	0.33±0.12*	0.08±0.06

$^{+}$ [dic + r] = [dicentrics and rings], [t + inv] = [translocations and inversions]; frequencies with Poisson standard errors.
* $P<0.05$.

Table 3. Chromosome aberrations among inhabitants of different ages of HBRA and CA

Subjects	Area	No. of subjects	No. of cells scored	Frequency per 1000 cells	
				[dic+r]$^{+}$	[t+inv]
Students					
15–16 years old	HBRA	122	24,400	0.21±0.09	0.45±0.14
	CA	99	19,800	0.20±0.10	0.05±0.05
				P>0.90	P<0.01
Women^{++}					
50–65 years old	HBRA	85	8,500	1.76±0.46	2.35±0.53
	CA	76	7,600	0.66±0.29	0.92±0.35
				P<0.05	P<0.05

$^{+}$ See Table 1 for difference in time of the colchicine addition to the culture medium used in scoring cells at the first metaphase for the 15–16-year old and the 50–65-year old groups; [dic + r] = [dicentrics and rings]; [t + inv] = [translocations and inversions].
$^{++}$ This research was supported in part by United States Public Health Service Contract N01CP-61018. Data for subjects who had been exposed to X-rays are not included. Results of an independent review (at the Oak Ridge Associated Universities, TN., U.S.A.) of 50% of the metaphase aberration agreed with the original scoring. The values for metaphases with stable aberrations compared to the original values were 0.28 vs. 0.29 for the HBRA and 0.16 vs. 0.18 for the CA[11].

two areas only in elderly woman.

Cancer mortality[5]

The mortality from all cancers in the HBRA was lower than in the CA (Table 4). Leukemia, breast and lung cancers are recognized as malignancies apt to be induced by ionizing radiation. The rates of deaths from these three types of tumor in the HBRA were lower than those in the CA (Table 5); but, the difference in the two areas is not statistically significant.

Table 4. Cancer mortalities in the survey area 1970–1986[5)]

Sex	Area	No. of person. years	Adjusted mortality[+] (/10^5 person.yrs)	Relative risk	95% Confidence interval
Males					
	HBRA	530,952	58.6	0.93	0.81–1.06
	CA	504,458	63.3		P=0.65
Females					
	HBRA	477,817	37.3	0.96	0.81–1.14
	CA	490,612	39.0		P=0.35

[+] Adjusted for the combined population of the HBRA and CA.

Table 5. Adjusted mortalities (1970–86) in the survey areas from tumors susceptible to induction by ionizing radiation[5)]

Type of cancer	Mortalities (per 10^5 person-years)			
	Males		Females	
	HBRA	CA	HBRA	CA
Leukemia[++]	3.21 (17)[+]	3.70 (18)	2.80 (14)	3.06 (15)
Breast	none	none	1.60 (7)	2.51 (13)
Lung	3.36 (17)	3.39 (18)	1.82 (8)	3.23 (17)
Total	6.57 (34)	7.09 (36)	6.22 (29)	8.80 (45)

[+] Figures in parentheses are the numbers of cancer deaths.
[++] All cases diagnosed from histopathologic evidence.

PHA responsiveness of lymphocytes[6,7)]

PHA responsiveness of peripheral lymphocytes taken from inhabitants in the HBRA and CA were compared in 1982 and 1985. As shown in Table 6. The percent of lymphocytes that showed a proliferative response to PHA stimulation was significantly higher for young inhabitants of the HBRA than of the CA whereas the seemingly higher responsiveness of lymphocytes from elderly inhabitants of the HBRA was not statistically significant.

UDS in lymphocytes[7,8)]

Results of studies of UDS in lymphocytes done in 1985 and 1988 indicate that lymphocytes taken from inhabitants of the HBRA showed a slight increase in UDS when compared with samples from the CA but this increase was significant only for young men (Table 7).

Table 6. Mitotic responses of lymphocytes to PHA[6,7] in the survey areas.

Age (years)	HBRA		CA	
	No. of subjects	Responsive cells (%)	No. of subjects	Responsive cells (%)
15–16	121	76.3±1.2*	118	74.0±1.0
16–25	82	77.2±1.1**	84	71.3±1.3
45–55	66	72.2±1.4	64	69.8±1.6

* $P<0.05$, ** $P<0.01$.

Table 7. Comparison of UV-induced unscheduled DNA synthesis (UDS) in lymphocytes from HBRA and CA inhabitants[7,8]

Age (years)	Examinees	Subjects		Ratio of UDS$^+$ in HBRA to that in CA
		HBRA	CA	
16–25				
	Men and women	20	28	1.14
	Men	10	13	1.24
15–16				
	Men and women	15	23	1.19
	Men	10	18	1.34*

$^+$ UDS activities were measured by use of the radioactivities of ^{3}H-thymidines incorporated into 10^6 lymphocytes after UV irradiation.
* $P<0.05$.

DISCUSSION

Significant increases in the frequencies of stable and unstable chromosome aberrations in the circulating lymphocytes of HBRA inhabitants were found when compared with frequencies in the CA, the difference being more evident for elderly women than for young students (Table 3). The difference in the two areas was clear for unstable aberrations in elderly women (Table 3). On the assumption that continuous exposure to background radiation is a major cause of chromosome aberration in these inhabitants, the findings are explanable as follows: Even if stable and unstable aberrations are produced at rates representing the same order of magnitude, stable aberration frequencies would increase more rapidly with age than would unstable ones because unstable aberrations are more quickly lost, thereby accounting for the more evident increase in stable aberration frequencies in elderly women in the HBRA.

The overall frequencies of aberrations in elderly women in the HBRA and CA are

much lower than reported for the general populations of normal background areas in other countries[12,13]. This may be because of lower exposure of the inhabitants of the HBRA and CA in China to diagnostic x-rays and other mitogenic pollutants. The low background rates of chromosome abnormalities in the inhabitants of the HBRA and CA improve our ability to detect low-dose radiation effects.

Mortality from all cancers, and mortalities from leukemia, breast and lung cancers were lower in inhabitants of the HBRA than of the CA[5] (Tables 4 and 5). In contrast, high doses of radiation are known to increase the incidences of leukemia, thyroid tumor, and breast and lung cancers in atomic bomb survivors, the dose response relations being approximately linear for the inductions of leukemia, breast and lung cancers[14]. The reason for this contradiction has to be determined.

Radiation exposure and aging are both considered important factors that reduce human immune competence. Circulating lymphocytes taken from atomic bomb survivors exposed to 2 Gy and higher doses showed lower PHA responsiveness than those taken from controls, in all the age groups surveyed except the youngest. Among the controls, patients with histories of malignant tumors also showed decreases in PHA responsiveness[15]. In contrast, lymphocytes taken from survivors who had been exposed to about 0.5 Gy, and who subsequently had moved to the United States, showed higher PHA responses than controls.[16] Lymphocytes taken from inhabitants of the HBRA showed higher PHA responses than those taken from inhabitants of the CA[6,7] (Table 6). A close relation is considered to exist between a decrease in lymphocytic function and the development of malignant tumors.

Lymphocytes from inhabitants of the HBRA were better able to repair the injury to DNA induced by UV-irradiation than those from CA inhabitants[7,8] (Table 7). This supports observations obtained at the galleries of spas in Badgastein, Austria, where inhalation of Rn-222 and its daughters appeared to enhance the repair capacities of lymphocytes from persons exposed to natural alpha particles[17]. Whether the enhancement of UDS in lymphocytes is due to the *de novo* synthesis of repair enzymes has yet to be determined.

Although elevated levels of natural background radiation repeatedly have been shown to increase the frequency of chromosome aberration in circulating lymphocytes (Tables 2 and 3), no detectable increase in cancer mortality has been found[5] (Table 4). In contrast, lymphocytes of inhabitants of the HBRA had higher PHA responses and higher UDS rates than those of residents of the CA[6–8] (Tables 6 and 7), possibly due to the hormetic effects of low-level radiation. We speculate that under certain circumstances high natural background radiation may induce chromosome aberration with concomitant stimulation of immunologic activity and DNA repair. An internal linkage may exist among chromosome aberration, carcinogenesis and hormesis after chronic exposure to low-level radiation. If more information can be obtained on the health status of the inhabitants of the HBRA and CA, we may be able to better explain the reduced cancer mortality in the HBRA.

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