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Intrachromosomal Distribution of Spontaneous and Induced Breaks in Human Lymphocytes

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Abstract. Spontaneous and induced breaks in the chromosomes of human lymphocytes *in vitro* are nonrandomly distributed. If one puts several alkylating agents and 5-fluorodeoxyuridine (FUDR) in one group and if one compares the pattern of the distribution of the collected breaks with the findings in another group formed by dimethylbenzanthracene, X-rays, and spontaneous breaks, the probability was far below 0.005, that the breaks in these two groups were distributed homogeneously within the chromosomal material. This indicates the presence of at least two different mechanisms in the origin of chromosomal breakage. The findings in the distributions of breaks induced by alkylating agents and FUDR, however, show that the distribution studies related here are no practicable cytological criteria for the detection of different breakage mechanisms.

Key Words
Chromosome breakage
Cytostatics
Karyotype
Lymphocytes

Many studies have shown that the distribution of breaks in human chromosomes is nonrandom. Table I includes some of these findings.

The present study was designed for a statistical analysis of results which were obtained in earlier experiments with X-rays [6] and some cytostatic agents [5, 7, 8, 11, 21]. The breaks following the treatment of the lymphocyte cultures with a carcinogenic hydrocarbon (7,12-dimethylbenzanthracene) [12] and the spontaneous aberrations in the cells of a proband with glutathione reductase deficiency [10] were collected from recent cultures.

Material and Methods

One hundred and twenty-six lymphocyte cultures were prepared and treated according to the method of MORRHEAD *et al.* [9] with slight modifications. The

Table I. Some earlier data on the distribution of breaks in human chromosomes

Authors	Material	Treatment
AYA *et al.* [1]	lymphocytes	herpes simplex virus
COHEN [2]	lymphocytes	streptonigrin
COHEN and SHAW [3]	lymphocytes	mitomycin C
GEBHART [4]	lymphocytes	myleran
HAMPEL and BALIG [6]	lymphocytes	X-rays
HAMPEL *et al.* [8]	lymphocytes	several cytostatic agents
HAMPEL and LEVAN [9]	fetal lung cells	low temperature
HAMPEL *et al.* [10]	lymphocytes	activated cyclophosphamide
HAMPEL *et al.* [12]	lymphocytes	dimethylbenzanthracene
KELLER and NORDÉN [13]	lymphocytes and bone marrow cells	patients with B_{12}-deficiency
KERKIS *et al.* [14]	lymphocytes	patients with acute viral hepatitis
KIHLMAN *et al.* [15]	lymphocytes	deoxyadenosine, cytosinearabinoside
KRONE *et al.* [16]	fibroblasts	hydroxylamine, bromodeoxyuridine
LUBS and SAMUELSON [18]	lymphocytes	untreated
NICHOLS *et al.* [20]	lymphocytes	deoxyadenosine, cytosinearabinoside, morbilli virus
STOPIK and HAMPEL [21]	lymphocytes	N-substituted cyclophosphamide derivatives

total time of incubation was 72 h at a temperature of 37.0±0.2 °C. These samples were exposed to different chromosome breaking agents 24 h before fixation [5].

The following agents were included in this study: (1) 2,3,5-tris-ethyleneimino-benzochinone (1,4) (trenimon); (2) 2,4,6-triethyleneimino-1,3,5-triazine (TEM); (3) N,N′,N″-triethylenethiophosphoamide (TESPA); (4) derivates of N,N-bis-(2-chloroethyl)-N′,O-propylene phosphoric acid ester diamide (cyclophosphamide, cytoxan) gained by incubation with rat liver slices [11]; (5) N,N-bis-(2-chloroethyl)-O-(3-amino-propyl)-phosphoric acid amide ester (A 2); (6) N,N,N′-tris-(2-chloroethyl)-N′,O-propylene phosphoric acid ester diamide (Z 4828); (7) N-(2-chloroethyl)-N′-(2-chloroethyl)-N′,O-propylene phosphoric acid ester diamide (Z 4942); (8) 5-fluorodeoxyuridine (FUDR); (9) 7,12-dimethylbenzanthracene; (10) X-rays 180 kV, 0.5 mm Cu, 65 R per min, 40 cm distance from the focus; (11) the distribution of spontaneous breaks was studied in the cells of a patient with glutathione reductase deficiency (NAD [P] H: glutathione oxidoreductase, EC 1.6.4.2) [10].

Chromatid and isochromatid breaks were collected from metaphases without visible translocations. Gaps and acentric fragments were not included in this study. The breaks were visually enumerated in the different chromosomes or chromosome groups 1, 2, 3, 4–5, 6–12, X, 13–15, 16, 17–18, 19–20, 21–22, and Y.

For the study of the intrachromosomal distribution of breaks, the long arms and short arms, where possible, were divided into 3 segments equal in size: *proximal, intermediate,* and *distal* from the centromere. If an arbitrary value of 1,000 units is taken for the total length of the chromosomes of a female or of a male karyotype the relative lengths of the chromosomal segments can be expressed in parts from 1,000. They were calculated from the data of LEVAN and NICHOLS [17], which are based on measurements of chromosomes in male and female karyotypes.

Results

Table II shows an abbreviated example for a distribution of dimethylbenzanthracene-induced breaks. The first column contains the symbols for some chromosome segments. A sample of 1,000 breaks was collected. If one assumes a random distribution of the breaks caused by this agent the values in column 2 and 3 must be equal. However, this null hypothesis of a mere random intrachromosomal distribution could here be abandoned with a probability of far below 0.001.

Table II. χ^2 test for randomness in distribution of breaks induced by 7,12-dimethylbenzanthracene in human chromosomes (abbreviated)

Chromosome segments	Relative length = expected breaks	Found breaks	χ^2
1 l d	15.25	30	14.26
i	15.25	12	0.69
p	15.25	21	2.16
s d	13.84	7	3.38
i	13.84	9	1.69
p	13.84	7	3.38
2 l d	16.57	32	14.36
⋮	⋮	⋮	⋮
21–22 Y l	34.14	15	10.73
s	8.77	–	–
	1,000.00	1,000	$\Sigma\chi^2$ 220.19
			df = 41
			$p \ll 0.001$

l = long arms, s = short arms, d = distal segment, i = intermediate segment, p = proximal segment.

Table III. List of $\Sigma\chi^2$ - values from earlier distribution studies

	$\Sigma\chi^2$ df=41	
Group I		
Trenimon	493.97	a,b $\bar{x}_{1,2,\ldots\ldots42}$
TEM	536.00	(values from all
Thio-tepa	734.31	chromosome segments in group I and II,
Cytoxan metabolites	616.49	respectively)
A2	567.99	$b/a=\xi=1$
Z4828 cytoxan derivatives	464.99	$a<b$
Z4942	486.88	
FUDR	826.45	
		df=41
Group II		$\hat{\chi}^2=72.91$
Dimethylbenzanthracene	220.19	$p<0.005$
X-irradiation	202.43	
Spontaneous breaks (glutathione reductase deficiency)	288.64	

One may compare the $\Sigma\chi^2$-values of the cytostatic agents placed in group I with the corresponding figures of group II containing one carcinogen, X-rays and spontaneous breaks (table III). The $\Sigma\chi^2$-values in group II were obviously lower than in group I. The next step was to test if the distribution of breaks in group I and II are homogeneous or not.

If the average number of breaks counted in the 42 chromosome segments in group I and II, respectively, are represented by the symbols a and b, a being less than b, the null hypothesis was b/a equal to ξ equal to 1. With a probability at a level of less than 0.005 this hypothesis could be rejected, too.

Discussion

In other words, the inhomogeneous intrachromosomal distribution of breaks in the 2 groups formed by several mutagenic agents indicates at least the presence of 2 different mechanisms in the origin of chromosomal breaks. It should be emphasized that the analyzed carcinogen and

the X-ray-induced aberrations can be placed in one group with chromosomal lesions that one may define as spontaneous breaks. The different action of FUDR and alkylating agents on chromosomal material was not accompanied by different findings in this study, although FUDR, for instance, unlike alkylating drugs, does not induce visible interchanges [7]. Therefore, no general conclusions can be drawn from the intrachromosomal distributions of breaks induced by different mutagenic agents concerning the mode of action of these agents on chromosomal material. Further studies are necessary, to support these findings here related with a theoretical basis.

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